Chemical Research in Toxicology

Perspective

pubs.acs.org/crt

Thirdhand Smoke: New Evidence, Challenges, and Future Directions

Peyton Jacob, III,[*,†] Neal L. Benowitz,[‡,§] Hugo Destaillats,[∥] Lara Gundel,[∥] Bo Hang,[⊥] Manuela Martins-Green,[#] Georg E. Matt,[▽] Penelope J. E. Quintana,[○] Jonathan M. Samet,[◆] Suzaynn F. Schick,[¶] Prue Talbot,[#] Noel J. Aquilina,[††] Melbourne F. Hovell,[○] Jian-Hua Mao,[⊥] and Todd P. Whitehead[‡‡]

[†]Division of Clinical Pharmacology and Experimental Therapeutics, Departments Psychiatry and Medicine, University of California, San Francisco, California 94143, United States

[‡]Division of Clinical Pharmacology and Experimental Therapeutics, Medical Service, Departments of Medicine, and Bioengineering & Therapeutic Sciences, University of California, San Francisco, California 94143, United States

[§]Center for Tobacco Control Research and Education, University of California, San Francisco, California 94143, United States

[∥]Indoor Environment Group, Lawrence Berkeley National Laboratory, Berkeley, California 94720, United States

[⊥]Biological Systems & Engineering, Lawrence Berkeley National Laboratory, Berkeley, California 94720, United States

[#]Department of Cell Biology and Neuroscience, University of California Riverside 92521, United States

[▽]Department of Psychology, San Diego State University, San Diego, California 92182, United States

[○]Graduate School of Public Health, San Diego State University, San Diego, California 92182, United States

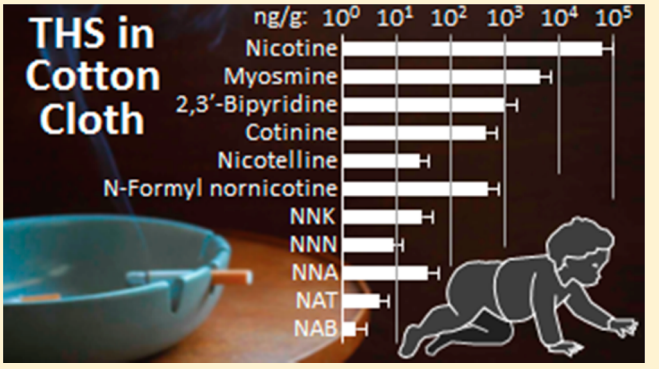
[◆]Department of Preventive Medicine, The Keck School of Medicine, University of Southern California, Los Angeles, California 90089, United States

[¶]Division of Occupational and Environmental Medicine, School of Medicine, University of California, San Francisco, California 94143, United States

[††]Department of Geosciences, University of Malta, Msida, MSD 2080, Malta

[‡‡]The Center for Integrative Research on Childhood Leukemia and the Environment, School of Public Health, University of California, Berkeley, 94704, United States

ABSTRACT: Thirdhand smoke (THS) is the contamination that persists after secondhand tobacco smoke has been emitted into air. It refers to the tobacco-related gases and particles that become embedded in materials, such as the carpet, walls, furniture, blankets, and toys. THS is not strictly smoke, but chemicals that adhere to surfaces from which they can be released back into the air undergo chemical transformations and/or accumulate. Currently, the hazards of THS are not as well documented as the hazards of secondhand smoke (SHS). In this Perspective, we describe the distribution and chemical changes that occur as SHS is transformed into THS, studies of environmental contamination by THS, human exposure studies, toxicology studies using animal models and *in vitro* systems, possible approaches for avoiding exposure, remediation of THS contamination, and priorities for further research.



CONTENTS

Special Issue: CRT30

Received: September 23, 2016
Published: November 30, 2016

DOI: 10.1021/acs.chemrestox.6b00343
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

1. INTRODUCTION

1.1. Definition. THS refers to tobacco residue and stale or aged secondhand smoke. THS is not strictly smoke but rather the residues left behind by smoking. It refers to the contamination of surfaces in contact with compounds emitted in SHS, the products generated by chemical transformations of these components, and the off-gassing of volatile components into the air.[1,2] The phrase "the four Rs" provides a working definition of THS: tobacco chemicals (some toxic) that remain, react, re-emit, and/or are resuspended long after active smoking ends. THS constituents may remain adsorbed to surfaces and dust particles, often penetrating deep into materials such as wallboard or upholstery; as they persist they may react with atmospheric oxidants to yield potentially harmful byproducts. Both precursors and byproducts may be re-emitted back to the gas phase, and airborne particles that initially deposited onto indoor surfaces may be resuspended.

1.2. Differences between THS and SHS. THS is conceptually distinct from SHS, which is the aerosol (particle-bound and gas phase constituents) present while smoking is taking place. Nonsmokers' exposures to SHS are associated with freshly emitted smoke. Hence, the primary pathway is inhalation, and the time scales for exposure are relatively short (minutes to a few hours). By contrast, exposure pathways for THS include not only inhalation but also dermal uptake from contact with contaminated surfaces (potentially including the clothing of smokers) and ingestion of THS that is on the hands or perhaps food. For toddlers, mouthing of objects in their environment is another route of potential oral exposure to THS. The time scale for the presence of THS indoors will generally be much longer than that for SHS and could stretch to months.

1.3. Why Study THS? Inhalation of tobacco smoke, both by those smoking actively and by nonsmokers involuntarily inhaling tobacco smoke, has been causally linked to a wide range of diseases and other adverse consequences.[3] Interest in THS accelerated after the results of internal research at Phillip Morris in the 1980s were made public through litigation settlements.[4] A researcher and coauthor of this paper (Schick) at the University of California San Francisco found records in Philip Morris papers showing that SHS can become more toxic as it ages. An analysis of unpublished results revealed that concentrations of carcinogenic tobacco-specific nitrosamines (TSNAs) increased over time in aging SHS. Soon after Schick's article appeared,[4] a laboratory study[5] showed that nicotine on surfaces can react with a common indoor pollutant to produce TSNAs under conditions that are commonly found in indoor environments. These high-impact discoveries directed attention to the concept that THS as a distinct entity poses health risks for children and adults. By 2011, both laboratory[5,6] and field studies[7,8] had produced sufficient evidence to warrant pursuing a programmatic research agenda to close gaps in our current understanding of the chemistry, exposure, toxicology, and health effects of THS, as well as its behavioral, economic, and sociocultural considerations and consequences.[2] The California Consortium on Thirdhand Smoke was launched in 2011 and renewed in 2014 to carry out the research agenda.

1.4. Objectives for This Perspective. This Perspective describes progress made by the Consortium and other investigators during the past five years, updating the review published in 2011.[2] This multidisciplinary Perspective covers THS chemistry, the occurrence of tobacco-derived substances in real world environments, including carcinogens, the toxicity of THS using *in vitro* and animal models, studies of human exposure using biomarkers, possible approaches to remediation of THS-contaminated environments, and how the results of research can influence public policy to reduce THS exposure. It is hoped that illuminating the toxic substance exposure potential of THS will encourage smoking cessation and tobacco control efforts.

1.5. Approach. The long-term goals of the California Consortium on Thirdhand Smoke are to identify the health effects of exposure to THS, develop environmental indicators and biomarkers of exposure to THS, and devise and disseminate evidence-based policies to prevent and remediate such exposures. The first three years (Phase I of the Consortium's collaborative multidisciplinary research) have led to sufficient understanding of exposure to and the mechanisms by which THS causes injury in order to lay the groundwork for more extensive investigation of its health effects and their policy implications. During the two years of Phase II, the Consortium has continued to use its highly successful collaborative structure to move the research toward addressing the question of how much harm THS causes to human health. The outcomes of the Consortium's research will be used to develop risk assessments as a basis for motivating and guiding policy development and implementation, particularly to those groups most likely to have the highest exposures.

DOI: 10.1021/acs.chemrestox.6b00343
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

2. BACKGROUND

2.1. Early History. Harmful emissions from combustion of tobacco in cigarettes and other tobacco products have been studied for decades dating back to at least the mid 1930s when Roffo in Argentina identified benzo[*a*]pyrene in cigarette tar and showed that tar induced cancer in mice.[9] Some of the earliest studies to link smoking and lung cancer appeared in 1939.[10,11] The US Surgeon General Report of 1964[12] led to widespread recognition that smoking tobacco was harmful, and it described some of the mechanisms by which smoking causes disease. The Surgeon General Report of 1986[13] documented the health effects of secondhand smoke, focusing on inhalation by nonsmokers (passive smoking). The indoor pollution described in the first article to quantify nicotine in dust of smokers' homes[14] would now be recognized as THS. By then studies of the indoor dynamic behavior of SHS had shown that many constituents of tobacco smoke sorb onto and desorb from indoor materials, based on their volatility and affinity for surfaces. Researchers had also found that rooms with THS-contaminated materials can exude nicotine and other compounds for long periods of time, long after smoking has ended.

2.2. Evidence of Human Exposure. The presence and amount of THS can be assessed through environmental sampling in air, in dust, and on surfaces. The study mentioned above[14] found elevated levels of nicotine in dust collected in the homes of Danish smokers, and the researchers observed a strong positive association of nicotine with smoking level. They concluded that nonsmokers inhale nicotine and other tobacco smoke constituents from respirable dust, even if smoking does not occur while the nonsmokers are present. Matt et al.[7] used a standardized dust sampling protocol in homes of smoking mothers of infants with and without indoor smoking bans.[7] In addition to dust, they also observed elevated levels of nicotine on household surfaces (e.g., coffee table in living room, bedframe where the infant slept), and on the hands of the smoking mother. Compared to infants in homes where no smoking was allowed, concentrations of cotinine (a biomarker for exposure to nicotine) in the urine were much higher in infants whose parents smoked indoors. If the parents only smoked outdoors, the infants had lower cotinine levels, but still many times higher than infants of nonsmoking parents. By the time the California Consortium began functioning in 2011, Matt's group had also documented THS levels in nonsmokers' homes that had been recently occupied by smokers[15] and in used cars.[16,17] Since then, nicotine and other THS constituents have been found in virtually any indoor environment in which tobacco has been smoked regularly, as well as in nonsmoking indoor environments that are near areas frequented by smokers, which will be discussed in subsequent sections of this Perspective.

2.3. Dynamic Behavior of Tobacco Smoke Pollutants in Indoor Environments. Mechanical or natural ventilation is the main process by which harmful pollutant concentrations can be kept at acceptable levels.[8] Typical ventilation (air exchange) rates in US residential and commercial buildings remove most airborne indoor pollutants over just a few hours by introducing cleaner outdoor air. However, ventilation alone cannot achieve acceptable indoor air quality if there is smoking.[18] The residence time of many airborne SHS constituents in indoor air is usually short. By contrast, surface-bound THS constituents can remain in contact with indoor air for days, weeks, and months, thus providing ample time for chemical transformations to take place as THS on surfaces interact with reactive pollutants in indoor air. The reactive atmospheric species of outdoor origin that can drive these reactions are significantly depleted during the outdoor-to-indoor transit; e.g., indoor ozone levels are often 20−70% of the outdoor concentration measured simultaneously, and OH radicals can be reduced by more than an order magnitude compared with outdoor air. However, these compounds are not completely removed from indoor air and often drive indoor chemistry.[19−21] Indoor combustion sources, such as gas stoves, may generate other reactive species, including nitrous acid (HONO), hydrogen peroxide (H_2O_2), and free radicals. Even though direct sunlight is absent from many indoor settings, recent evidence indicates that the role of direct photolysis in the generation of indoor OH and NO_3 radicals is more significant than originally thought.[22,23] Thus, oxygen- and nitrogen-containing radicals, oxidants, and nitrosating species can be present at levels that can support reactions of THS compounds with indoor pollutants. With the long residence times observed for surface-bound THS constituents, there is potential for these constituents to be slowly transformed into various byproducts as they age.

Nicotine is one of the most prevalent constituents in tobacco smoke, and it is a critically important constituent in THS chemistry because of its high emission rate and its high concentrations and persistence on indoor surfaces.[24,25] In contact with ozone, nicotine oxidizes, yielding numerous volatile and semivolatile species, as well as new ultrafine particles.[26] Laboratory studies have revealed that several of the identified oxidation byproducts are multifunctional carbonyls, amides, *N*-oxides, and carboxylic acids that have an asthma hazard index higher than that of nicotine, indicating that oxidative aging may lead to more harmful residues in THS. In addition, reactive oxygen species were detected in secondary organic aerosol (SOA) formed by ozonation of nicotine.[27,28]

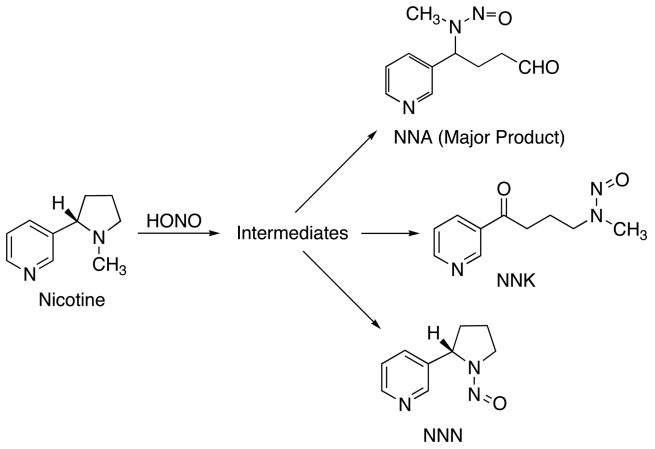
While ozone- and OH radical-driven oxidation is a major pathway for indoor chemistry, other reactions also lead to the formation of harmful byproducts. The nitrosation of nicotine by HONO emitted from combustion sources (including smoking) produced tobacco-specific nitrosamines (TSNAs) on indoor surfaces.[5] These TSNAs included *N*′-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and 4-(methylnitrosamino)-4-(3-pyridyl)butanal (NNA), a TSNA that is specific to THS as it is not commonly found in fresh smoke (Scheme 1) and must be formed by reaction with HONO from the combustion sources. The mechanisms of nitrosamine formation are similar to those described for gas phase formation of volatile nitrosamines and formation of TSNAs in aqueous media.[29,30] These studies replicated and extended unpublished research performed by Philip Morris in the 1980s, which revealed that TSNA concentrations increase over time and that secondhand smoke becomes more toxic as it ages.[4] Interest in THS accelerated after these findings were uncovered because some TSNAs, in particular NNK and NNN, are highly carcinogenic.

3. PROGRESS AND NEW EVIDENCE

3.1. Chemistry of THS and Approaches to Exposure Assessment. *3.1.1. THS Chemistry.* Initial THS studies focused on oxidation and nitrosation processes that lead to the formation of semivolatile or nonvolatile byproducts that likely remain on indoor surfaces, as described above. More

DOI: 10.1021/acs.chemrestox.6b00343
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

Scheme 1. Formation of TSNAs from the Reaction of Nicotine and Nitrous Acid



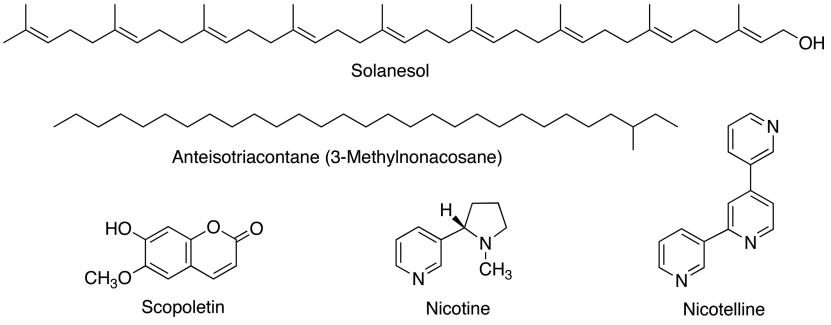
recently, the inhalable fraction of THS was also characterized in laboratory and field studies to provide more insights on inhalation exposure that remains highly relevant long after smoking has ended.[31] More than 50 volatile organic compounds (VOCs) in THS were identified in a room-sized chamber operating at an air exchange rate of 0.14 h^{-1} over 18 h after smoking, including aliphatic and aromatic hydrocarbons, furans, carbonyls, terpenoids, and nitriles. However, amines, including nicotine, the tracer 3-ethenylpyridine (3EP), and several others, were quickly removed from air during the initial 2 h, probably through strong adsorption to surfaces. Three of the persistent VOCs exceeded levels considered harmful for 8-h exposures by the State of California during all or most of the 18-h period: acrolein, methacrolein, and acrylonitrile. Other compounds such as acetonitrile, 2-methylfuran, and 2,5-dimethylfuran are potentially useful candidates for THS tracers due to their persistence. Using a risk assessment approach, the concentration data were used to estimate the disability-adjusted life years (DALYs) lost by nonsmokers due to long-term exposure to THS, in order to assess the integrated health impacts. The assessment showed that particulate matter emitted during smoking and that stayed airborne over several hours (indexed by $PM_{2.5}$) contributed the majority of the THS-associated disease burden, while acrolein, furan, acrylonitrile, and 1,3-butadiene were the most harmful VOCs among those for which epidemiological and/or toxicological data were available. It should be kept in mind that this approach carries a significant level of uncertainty, partly due to the fact that there are not enough data to inform the effects of hundreds of compounds present in THS. In addition, the disease burden of particulate matter is predicted from an integrated dose–response model that does not account for contributions of individual constituents. A time-resolved analysis for comparing SHS and THS contributions to DALYs was used to explore their relative impact. The analysis led to a finding that THS could be responsible for 5% to 60% of the predicted total disease burden, depending on where the arbitrary SHS/THS temporal transition is placed.[31]

3.1.2. Tracers for Tobacco Smoke-Derived Particulate Matter. Tobacco smoke is a complex mixture of particles and gases, which distribute themselves differently in indoor environments. The particles are small and distribute themselves widely as the air is mixed; more volatile and reactive gases are adsorbed onto surfaces. Numerous toxic substances are carried on the particulate matter (PM), but a tobacco-specific, environmentally stable tracer for tobacco smoke-derived particulate matter has been lacking. Since a model (discussed above) predicted that most of the toxicity of THS is due to fine particulate matter ($PM_{2.5}$), a marker that could differentiate tobacco smoke-derived PM from PM derived from other sources, and could be used for source apportionment, would be valuable in THS studies.

Solanesol[32] and scopoletin[33,34] have been used as environmental tracers for tobacco smoke indoors (Chart 1), but both have nontobacco sources[35] and stability issues. Solanesol is susceptible to oxidation by ozone or UV-induced decomposition.[2,36,37] Scopoletin is a phenolic coumarin derivative that likewise would be expected to be susceptible to oxidation under environmental conditions, and it is present in numerous plant species. Long-chain hydrocarbons present in tobacco and its smoke, iso- and anteisoalkanes (C_{29}–C_{34}), have also been used as environmental tracers for tobacco smoke.[38] These hydrocarbons have the advantage of stability but have nontobacco sources.[39,40] Nicotine has been utilized as a tracer for the PM derived from tobacco smoke,[33,34,41,42] but nicotine is volatile (calculated Log $p = -1.52$) and exists mainly in the gas phase of tobacco smoke collected indoors.[41,43,44] If smoking at relatively constant levels has resulted in a steady state for its partitioning among the PM, the gas phase, and the surfaces to which it may be adsorbed, nicotine may perform well as a tracer for tobacco smoke-derived PM.[33] This may not be the case in places where smoking is sporadic, as particles have different removal processes than gas phase nicotine. Ventilation, characteristics of the indoor space, such as composition furnishing and surfaces, could affect the partitioning of nicotine between the surfaces and the atmosphere.[45,46]

The tripyridine alkaloid nicotelline (Chart 1) has low volatility (calculated Log $p = -6.05$), is found almost entirely in the particulate matter ($f_p = 0.998$), and its mass in aged

Chart 1. Tracers of Tobacco Smoke Particulate Matter



DOI: 10.1021/acs.chemrestox.6b00343
Chem. Res. Toxicol. XXXX, XXX, XXX–XXX

cigarette smoke is highly correlated with the mass of PM (r^2 = 0.95) (Figure 1). For these reasons, nicotelline has been

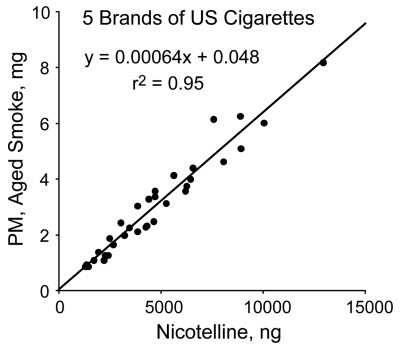


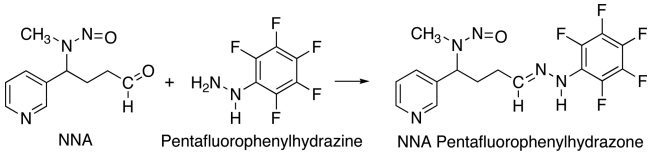
Figure 1. Correlation of nicotelline mass with PM mass in aged cigarette smoke from 5 US Brands. Smoke was generated from multiple cigarettes for each brand for times ranging from 2 to 6 h. Reproduced from ref 35. Copyright 2013 American Chemical Society.

proposed as a tracer for tobacco smoke-derived PM and should be applicable to the condensed phase in general (aerosols and stagnant surfaces).[35] Nicotelline concentrations have been measured in a variety of materials, such as those being used in THS toxicity studies described elsewhere in this Perspective, in real-world samples, including house dust as described above, and in PM collected outdoors, as discussed below. Approaches for using nicotelline in PM source apportionment have been proposed.[35]

3.1.3. Biomarkers to Distinguish THS from SHS Exposure: NNA Metabolites. A major challenge is developing a biomarker of exposure to distinguish THS exposure from SHS exposure. Ideally, for policy and mitigation purposes, this would be a substance unique to THS that is not present in significant amounts in SHS, one that is formed during the aging process as SHS is transformed into THS. In the pioneering studies by Sleiman et al., the major TSNA formed from the reaction of nicotine with nitrous acid, under conditions that modeled indoor environments, was 4-methylnitrosamino-4-(3-pyridyl)-butanal (NNA), as discussed above[5] (Scheme 1). However, NNA is rarely detected in mainstream or sidestream tobacco smoke, probably because being an aldehyde, it is too reactive to survive during the combustion process. Therefore, NNA is a likely candidate for an environmental tracer and biomarker for THS that has reacted with HONO. To evaluate this possibility, an analytical method for the determination of NNA in THS samples produced in the laboratory and in environmental samples was developed, and NNA was administered to mice, and their urine was analyzed for metabolites that might serve as biomarkers.

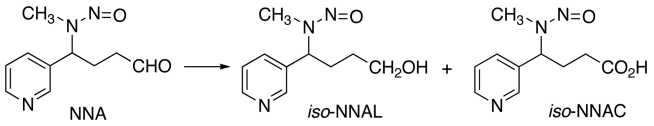
Presumably due to its chemical reactivity, attempts to measure NNA directly in THS samples led to erratic results, and concentrations in aqueous solution declined over time during storage. In addition, chromatographic separation of NNA from its isomer NNK may be challenging, especially if using GC-based methods.[47] Analytical methods for aldehydes often involve forming carbonyl adducts as derivatives to enhance stability. Conversion of NNA to its pentafluorophenylhydrazone derivative led to a satisfactory LC-MS/MS method that has been applied to THS extracts and to settled house dust[48] (Scheme 2). Since mammalian metabolites of NNA had not been reported, NNA was administered to mice, and urine was collected to analyze for two likely metabolites

Scheme 2. Conversion of NNA to Its Pentafluorophenylhydrazone Derivative



resulting from the reduction or oxidation of the aldehyde moiety, 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol (*iso*-NNAL) and the carboxylic acid 4-(methylnitrosamino)-4-(3-pyridyl)butyric acid (*iso*-NNAC), respectively[49] (Scheme 3).

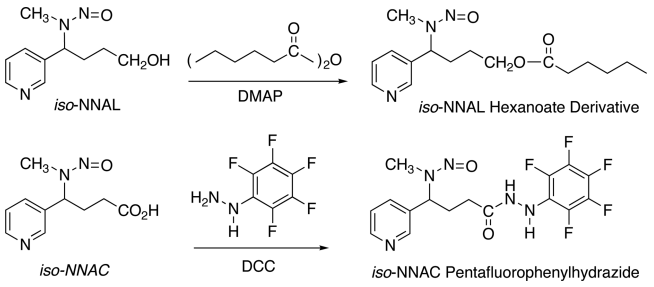
Scheme 3. Metabolism of NNA to *iso*-NNAL and *iso*-NNAC



The NNA was administered by application to the skin because another objective was to determine if transdermal absorption could occur since skin contact with THS-contaminated surfaces is a possible route of exposure to toxic substances present in THS. To minimize the possibility of exposure through the oral route, NNA was applied to shaved skin behind the head, and animals were housed individually to prevent cross-exposure among animals. The amount applied was 350 ng/cm^2, resulting in a total dose of 1.4 μg per mouse.

LC-MS/MS methods for quantifying *iso*-NNAL and *iso*-NNAC in urine were developed. Determination of *iso*-NNAL was analogous to a previously reported method for determination of the isomeric compound NNAL, which is a metabolite/biomarker of the tobacco-specific lung carcinogen 4-methylnitrosamino-1-(3-pyridyl)-1-butanone (NNK).[50] Determination of *iso*-NNAC was also performed by LC-MS/MS, following conversion to the pentafluorophenylhydrazide derivative (Scheme 4). Applying these methods to urine from

Scheme 4. Derivatization of NNA Metabolites for LC-MS/MS Analysis



6 mice, both metabolites were detected and quantified in urine, demonstrating that dermal absorption of NNA had occurred and that the two predicted metabolites were indeed formed. *iso*-NNAC concentrations exceeded *iso*-NNAL concentrations by 2 to 3 orders of magnitude, which suggests that *iso*-NNAC would be the preferred biomarker of exposure[49] (Table 1). The carcinogenic TSNA NNK was also administered to mice in similar fashion, and likewise, its metabolite NNAL was measured in urine, which demonstrated that both genotoxic TSNAs are absorbed through the skin.

DOI: 10.1021/acs.chemrestox.6b00343
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

Table 1. NNA Metabolites in Mouse Urine

	unpublished data[49]	
sample	*iso*-NNAL (ng/mL)	*iso*-NNAC (ng/mL)
M-1	3.94	389
M-2	4.16	496
M-3	4.90	286
M-4	6.05	367
M-5	2.26	206
M-6	4.00	150

However, developing NNA metabolites into biomarkers of exposure is a challenge. The dose administered to the mice was much higher than one would expect from human exposure in the real world. In settled house dust from smokers' homes analyzed for various tobacco alkaloids and TSNA, including NNA, measurable concentrations of NNA were found in house dust from 4 of the 6 homes sampled; the median NNA concentration was 0.46 ng/g.[48] NNA was not detected in any dust samples from 20 nonsmokers' homes that were also analyzed for tobacco alkaloids and TSNA. This finding, along with the high recovery of the administered dose in mice suggests that *iso*-NNAC would be a useful biomarker of exposure in humans if an analytical method of sufficient sensitivity could be developed. However, even if a suitable method were developed, it would be necessary to demonstrate that SHS exposure does not result in excretion of *iso*-NNAC. In order to evaluate the possibility that *iso*-NNAC could be developed into a human biomarker, Consortium studies are in progress to (1) determine whether *iso*-NNAC is an *in vitro* human metabolite and (2) develop an analytical method with the anticipated sensitivity needed, low pg/mL or even subpg/mL sensitivity as was accomplished for NNAL.[50]

3.1.3.1. NNAL/Cotinine Ratio. As discussed above, a major driving force in generating interest in THS was the discovery that as SHS ages, TSNA concentrations increase[4] and that this is likely due to the reaction of nicotine with ambient oxidant gases and nitrous acid.[5] Furthermore, nicotine is considerably more volatile than the TSNAs. After smoking no longer takes place in an indoor environment, over time it would be expected that nicotine remaining on surfaces or incorporated into house dust would be removed by ventilation at a greater rate than the TSNAs. Therefore, as SHS ages to become THS, the ratio of TSNA/nicotine should increase, due to *de novo* formation of TSNA and faster removal of nicotine than the TSNAs. In people exposed to THS, it would be expected that the exposure to TSNAs relative to nicotine would be greater than that in people exposed to SHS. Well-validated biomarkers exist for both nicotine and the TSNA NNK, their metabolites cotinine[51] and NNAL,[52] respectively. In addition, highly sensitive methods for the determination of cotinine[53,54] and NNAL[50] in human urine are available. If indeed the ratio of NNK/nicotine in the environment increased over time, then the ratio of the biomarkers NNAL/cotinine might serve as a biomarker to assess the relative exposure to THS compared to SHS.

There are data consistent with this hypothesis. The NNAL/cotinine ratio in urine was significantly higher for passive smokers when compared with that for active smokers, which would be expected on the basis of increasing NNK/nicotine ratio as SHS ages.[55] In a real-world environment, people exposed to SHS are generally exposed to THS as well. Young children, especially toddlers with parents who are smokers, would be expected to have relatively more THS exposure than adults living with smokers because they spend more time playing on the floor, may ingest house dust, tend to put objects in their mouths, and are likely to come in contact with parents' clothes. On this basis, it would be expected that the NNAL/cotinine ratio would be higher than that of adult nonsmokers exposed to SHS. Indeed, the NNAL/cotinine ratio of toddlers 6 months to 4 years in age was higher than that of adult nonsmokers exposed to SHS[56,57] (Figure 2).

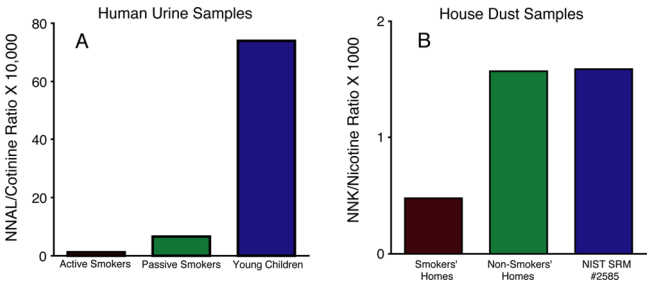


Figure 2. Possible approach to distinguishing THS exposure from SHS exposure: NNAL/cotinine ratio in urine. Panel A shows a higher ratio in smokers as compared to SHS-exposed nonsmokers, and a still higher ratio in infants and toddlers under the age of 2. Panel B shows a higher ratio of NNK/nicotine in nonsmokers' homes as compared to smokers' homes. These data are consistent with the loss of nicotine due to ventilation, and formation of NNK as smoke ages and SHS is transformed into THS. Smokers are exposed mainly to fresh smoke via inhalation. Nonsmokers are generally exposed to a relatively larger fraction of SHS/THS than are smokers, and toddlers are likely to be exposed to a relatively larger fraction of THS than adults due to hand to mouth behavior and contact with THS contaminated surfaces. Data are from Hovell et al.,[56] Jacob et al.,[57] and Whitehead et al.[48]

There are also real-world data suggesting that as tobacco smoke residues age, the NNK/nicotine ratio increases. Because of widespread contamination of the environment by tobacco smoke, tobacco alkaloids and TSNA can be detected in homes of nonsmokers and in the outdoor environment.[48,58] In homes of nonsmokers, where nicotine and TSNAs come primarily from air and dust from outdoors, and from clothing of occupants exposed to smoke elsewhere, one would expect that the THS would have aged more than that in homes of smokers in which input of fresh smoke containing nicotine occurs on a regular basis. In a study in which nicotine and TSNAs were measured in homes of smokers and nonsmokers,[48] the ratio of NNK/nicotine was higher in the homes of nonsmokers[57] (Figure 2).

Consortium studies are in progress to further evaluate the NNAL/cotinine ratio as a biomarker in both field studies and in a laboratory study in which human subjects are being exposed to clothing impregnated with THS. Future studies will also explore the possibility that DNA adducts of NNA might serve as biomarkers, as proposed in the discussion on toxicology below.

3.1.4. Application of Conventional Tobacco Smoke Tracers and Biomarkers. In the absence of validated tracers and biomarkers that have specificity for THS, the current best approach in exposure assessment studies is to use conventional tracers, such as tobacco alkaloids and TSNAs, and biomarkers, such as nicotine, other tobacco alkaloids, and TSNA metabolites. Self-reports from study subjects might be used to provide an estimate their relative exposures to SHS and THS. Studies using conventional tracers and biomarkers are discussed in subsequent sections. An ongoing human laboratory study at

DOI: 10.1021/acs.chemrestox.6b00343
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

the University of California, San Francisco will accomplish pure THS exposure, at least over a short time frame, by exposing the subjects to THS-contaminated clothing and measuring cotinine and NNAL excreted in urine.

3.2. Toxicology. *3.2.1. Generation of THS Samples for Toxicology Studies.* Generation and characterization of THS samples for toxicological studies has been a major endeavor of the California Consortium on Thirdhand Smoke. Different laboratories have successfully used a variety of methods for generating THS samples under controlled conditions.[5,59,60] These test samples consist primarily of materials (fabrics or paper) that have been impregnated with cigarette smoke, either sidestream, or a mixture of sidestream and mainstream. Most methods employ flow cells, in which cigarette smoke flows through a chamber containing substrates. The important factors to consider when generating standardized THS samples include smoke concentration, flow rate, time, substrate, and storage conditions. Smoking machines generate either sidestream smoke or a mixture of sidestream and mainstream smoke, and they provide more consistent smoke than human smokers and can operate continuously. For exposure to materials, the machine-generated SS or SS+MS is diluted with particle free ambient air before exposure to materials. Flow rates can be set to match air exchange rates in homes and public buildings so that the air volume in the chamber turns over between 2 and 0.5 times per hour.[61−64] The quantity of smoke can be tracked by simply counting cigarettes, or, more accurately, by measuring the change in concentration of aged SS for each episode of SS exposure, as measured by gravimetry. THS deposition to a sample of material will depend on the surface chemistry of the material and the total amount of THS PM that entered the exposure chamber in a long series of smoking episodes before the material was removed from the chamber. This method was used to derive the estimates of surface loading of THS (in $\mu g \cdot cm^{-2}$) that are included in the studies discussed in the genotoxicity and cytotoxicity sections of this Perspective.

Because chemical change is a defining characteristic of THS, time is a critical factor in generating THS samples. The duration of exposure and the elapsed time between the last smoke exposure and storage should be known. Experiments have shown that some of the toxins in THS on paper degrade when the samples are stored at room temperature.[65] Some of the compounds in THS are volatile, labile, and/or sensitive to UV degradation, so samples should be protected from light and packed in containers impermeable to volatile organic compounds. Storage at −20 °C is recommended, but well-packaged specimens can be shipped at room temperature.

For *in vitro* studies with cell and tissue cultures, many investigators have extracted THS from paper or cloth samples by agitating the paper or cloth in cell culture media, squeezing out the liquid, and centrifuging to remove fibers. These aqueous extracts of THS contain only the subset of THS chemicals that are soluble in the media, which typically contain salts, nutrients, and proteins. For studies in animals, THS-exposed materials can be put into animal cages, or the entire cage and bedding can be exposed to cigarette smoke and then used to house the animals, as described below. Concentrations of selected tobacco smoke constituents, including nicotine, other tobacco alkaloids, and TSNAs are used as a measure of THS loading on fabrics or potency of extracts.

3.2.2. Genotoxicity. THS and its specific constituents, such as TSNAs, can cause significant molecular and cellular changes *in vitro* and *in vivo* at concentrations that are relevant to real world exposures. Sleiman et al.[5] estimated the levels of TSNAs found on indoor surfaces: NNA, 2.2−3500 ng/m^2, and NNK, 0.31−500 ng/m^2, depending on the indoor matrix used for testing. Whereas in our studies using cell cultures the amounts of NNA added to the cells were at concentrations of 0.39−1.82 ng/mL, and for NNK 0.51−7.2 ng/mL, these values calculated on a per mL basis are comparable to the total amounts of NNA and NNK deposited per square meter of surface area and perhaps on the low end of the estimated range for indoor surface concentrations. Research on detection and identification of adducts and strand breaks in THS-treated DNA has revealed the genotoxic potential of THS exposure. Specific adduct(s) identified from the reactions with THS may prove useful as molecular biomarkers of exposure to THS.

3.2.2.1. THS-Induced Formation of DNA Strand Breaks. The genotoxic potential of THS and its known constituents was assessed in human cell lines using two *in vitro* assays.[66] THS cellulose paper substrates were generated in both Lawrence Berkeley National Laboratory (LBNL) and the University of California, San Francisco (UCSF) using systems that simulated short (acute)- and long (chronic)-term exposures. The acute THS sample papers were exposed to cigarette smoke (SS + MS) for 1 day, followed by 32 h of aging in the smoking chamber. The estimated THS surface loading was <500 $\mu g \cdot cm^{-2}$. The chronic THS sample papers were exposed to cigarette smoke (SS + MS) for 258 h over 192 days, leading to an estimated THS loading of 3 $\mu g \cdot cm^{-2}$.[60] The acute and chronic paper substrates were then extracted in cell culture medium. Twenty-four hour exposure of human HepG2 cells to samples either acutely exposed or chronically exposed to THS resulted in significant increases in DNA strand breaks in the alkaline Comet assay[66] (Figure 3). Cell cultures exposed to NNA alone showed significantly higher levels of DNA strand breaks than controls in the same assay, similar to NNK in parallel experiments. Most recently, a phospho-H2AX (γ-H2AX) and p53BP1 colocalization approach was utilized to

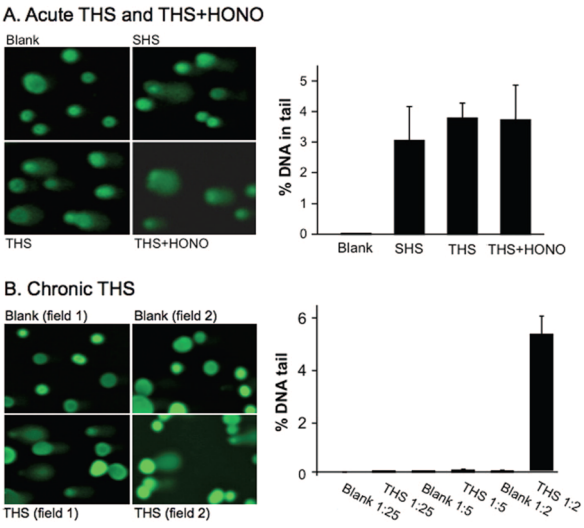


Figure 3. (A) Effect of acute THS and THS+HONO. HepG2 cells were exposed to samples at 37 °C for 24 h. The extent of DNA damage was analyzed by % DNA in the tail to total DNA from 90 cells. (B) HepG2 cells were exposed to chronic THS at varying dilutions under identical conditions as described above. HONO = nitrous acid. Reproduced with permission from ref 66. Copyright 2013 Bo Hang and Oxford University Press.

DOI: 10.1021/acs.chemrestox.6b00343
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

confirm the formation of double-strand breaks (DSBs) in cultured human BEAS-2B cells following exposure to THS exposure.[67] Histone H2AX is specifically phosphorylated at the sites of DSBs, and γ-H2AX foci detection has been used as a very efficient method to demonstrate colocalization of other damage responsive proteins to DSBs, such as p53BP1. DSBs are the most harmful type of DNA damage, as both strands of the DNA duplex are compromised.

3.2.2.2. THS-Induced Oxidative DNA Damage. Using the long amplicon−qPCR (LA-qPCR) assay, aqueous extracts of THS on paper caused significantly higher levels of oxidative DNA damage in both *HPRT* and *POLB* genes in cultured human lung BEAS-2B cells than controls. LA-qPCR is highly sensitive to oxidative DNA damage when coupled with the repair enzyme formamidopyrimidine DNA glycosylase (Fpg) that is specific for incision of oxidized purine lesions. These results suggest that THS exposure may cause oxidative damage in DNA that could be an important contributing factor in THS-mediated cellular toxicity.[66]

To further confirm the effect of THS on the accumulation of oxidative DNA lesions, the oxidative stress-induced DNA damage in mouse skin wounds exposed to THS was measured using the LA-qPCR assay.[68] THS exposure caused increased levels of oxidative DNA damage in mouse *Polβ* and *β-Globin* genes in the DNA samples from the THS-exposed skin wounds. This finding was in agreement with two other important observations in the same samples: (1) a significant increase in the levels of malondialdehyde (MDA), a marker for lipid peroxidation, and (2) high levels of 8-oxo-dG, a major oxidation product in DNA that causes G:C to T:A transversion and is associated with many disease mechanisms (Figure 4). Overall, these findings suggest that THS exposure causes oxidative DNA damage in both *in vitro* and *in vivo* systems. Oxidative DNA damage can lead to mutations, which can in turn lead to cancer.

3.2.2.3. NNA-Induced Formation of DNA Adducts. It is well accepted that formation of DNA adducts, especially bulky adducts, plays a central role in smoking-induced mutagenesis and carcinogenesis. If DNA adducts are not repaired, they can cause miscoding during DNA replication, thus leading to mutations.[69−71] With the use of LC-ESI-MS/MS and 2D NMR, several adducts from the *in vitro* reaction of NNA with deoxyguanosine (dG) were identified (Figure 5).[67] In addition to N^1-, O^6-methyl-dG and 8-oxo-dG, two modifications that are novel in structure were identified. (1) 1,N^2-NNA-dG: On HPLC chromatography, this adduct was the major adduct (peak 34.264) (Figure 5). The UV spectrum showed λ_{max}270 nm, 285 nm, and λ_{min} 250 nm. ES-MS/MS revealed a product of m/z 455.17 for $(M+1)^+$, which appears to result from the condensation of NNA and dG with the elimination of H_2O and oxidative removal of hydrogen atoms occurring after addition of neutral $C_{10}H_9N_3O$ to dG. The chemical structure of the adduct proposed in Figure 5 is based on ESI-MS/MS and 2D NMR experiments.[67] Given that NNA is highly selective for THS, this bulky covalent adduct would be a good candidate biomarker of THS exposure (see below). (2) 5′,3′-dimethyl-dG: NNA also causes novel sugar damage (Figure 5), which would lead to the breakage of the DNA backbone if this lesion were formed in THS-exposed cells. NNA also reacts with deoxycytosine (dC) *in vitro* as well, forming several products on C18-HPLC (unpublished data).

3.2.2.4. THS-Induced Metabolome Changes in Vitro. Exposure to THS extracts in two rodent male reproductive

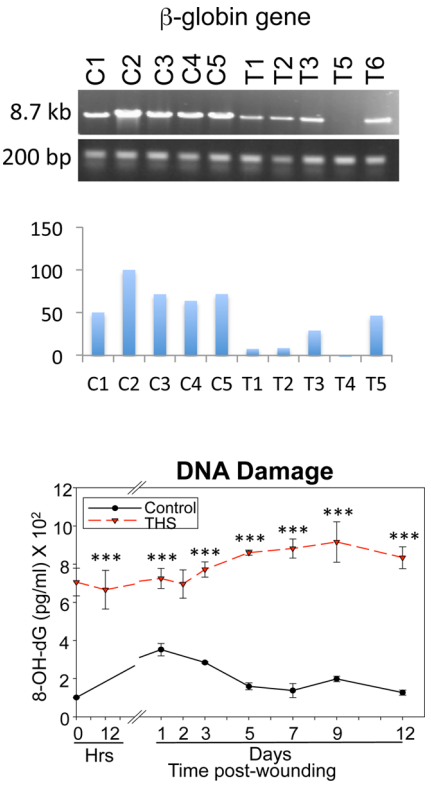


Figure 4. Oxidative DNA lesions induced by THS. Above: Formation of oxidative DNA damage in two mouse b-globin genes, as detected by LA-QPCR. Below: 8-oxo-dG detection using an 8-oxo-dG DNA damage assay. Adapted from ref 68. Copyright 2016 Elsevier.

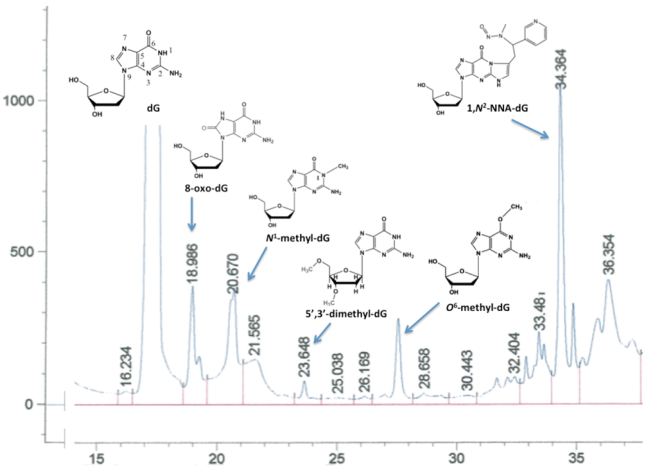


Figure 5. NNA reaction product with dG was separated by reverse phase (C18) chromatography and analyzed by UV spectroscopy, ESI-MS/MS and 2D-NMR. Data are from Hang et al.[67]

cell lines, GC-2 and TM-4, caused significant alterations in the metabolome.[72] At low THS concentrations that yielded normal cell viability, cell cycle, apoptosis, and ROS production, glutathione metabolism in GC-2 cells and nucleic acid and ammonia metabolism in TM-4 cells were changed significantly. RT-PCR analyses of mRNAs for enzyme genes showed changes in the expression levels of genes that encode enzymes involved in glutathione, nucleic acid, and ammonia metabolism. A metabolomic approach could help identify biomarkers for exposure and risk assessment in THS-related research.

DOI: 10.1021/acs.chemrestox.6b00343
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

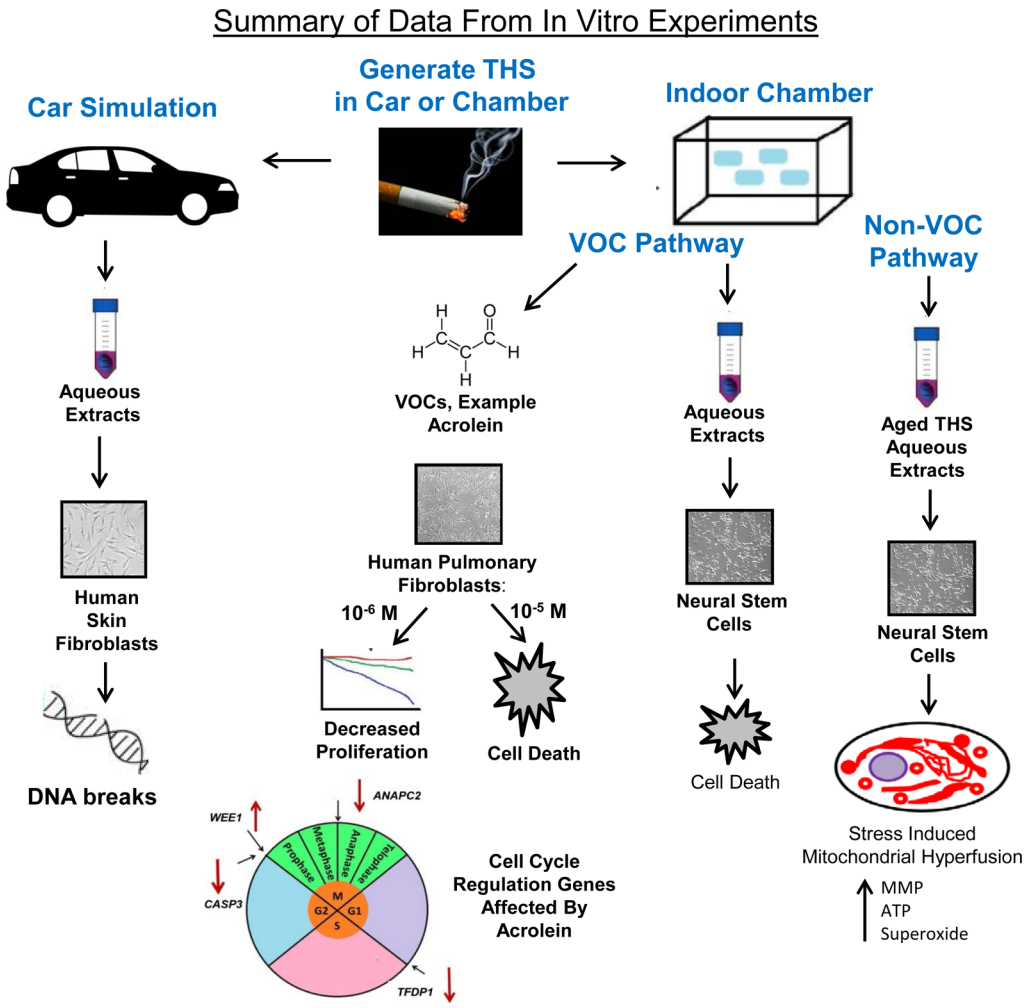


Figure 6. Summary of the effects of THS on cultured stem cells. In a simulated car experiment, THS extracts caused cell death and DNA strand breaks. In the VOC pathway, whole THS extracts and phenol, DMF, and acrolein killed various cell types at relatively high concentrations. Acrolein was the most potent of the three VOCs. Low concentrations of acrolein affected the expression of genes involved in the cell cycle in hPF resulting in decreased proliferation. In the non-VOC pathway, THS caused SIMH accompanied by downregulation of Fis1 and genes that decrease MMP. SIMH was accompanied by increase in MMP, ATP, and superoxide.

3.2.3. Studies in Cultured Cells: What Studies with Cultured Cells Can Tell Us about Human Health. Cells in culture can be used to study how environmental chemicals, such as THS, affect cellular processes, such as stress and survival, and can lead to a better understanding of how THS affects human health.[73−75] Numerous assays can be done *in vitro* with different cell types, using different concentrations of THS, and different experimental conditions. Assays can be acute or chronic, end points can be single or multiplexed, and designs can be low- or high-throughput.[76] Stem cells and differentiated cells from any organ can be compared, and THS can be studied independently from SHS. *In vitro* work can either establish a foundation upon which *in vivo* work with animals and humans can be based or it can provide a means to study results obtained *in vivo* in more depth.

There are relatively few *in vitro* studies on THS, in contrast to the vast literature dealing with mainstream (MS) and sidestream (SS) cigarette smoke.[77] Most *in vitro* work has been done with extracts of THS from fabrics or other matrices exposed in controlled laboratory conditions or in some cases using samples that came from simulated field sites.[66,78−81] It can be hypothesized that some of the effects that are well established for MS and SS smoke may also occur with exposure to THS.

3.2.3.1. Testing the Effect of THS on Cell Health Using Laboratory Controlled Conditions. Controlled laboratory experiments have been done to test the cytotoxicity of THS that was created and aged in a simulated car parked outdoors and in an exposure chamber that models an indoor space (such as an office) without windows.[78] In the car experiment, both car seat cover fabric and floor carpeting were tested. Using live cell imaging, THS extracts inhibited cell proliferation. When mouse neural stem cells (mNSC) and human pulmonary fibroblasts (hPF) were tested in the Comet assay, which measures DNA strand breaks, both the seat cover and carpet extracts of THS increased the percentage of cells with DNA damage (Figure 6). Similar data have also been shown in HepG2 cells exposed to THS extracted from terry cloth in cell culture media.[66]

In an experimental chamber designed for exposures to cigarette smoke,[60] terry cloth was exposed to cigarette smoke over a period of 16 months. The surface loading of THS on the terrycloth was 1.2 $\mu g \cdot cm^{-2}$. The effects of THS accumulation were assessed in the MTT assay, which measures mitochondrial reductase activity and can be used to assess cell survival and

DOI: 10.1021/acs.chemrestox.6b00343
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

health (Figure 6).[82] Toxicity was observed in extracts taken from terry cloth exposed to the equivalent of 54 cigarettes over an 8-month period. The surface loading of THS on the terry cloth was 0.7 $\mu g \cdot cm^{-2}$. This was considered a low dose of THS compared to the 4800 cigarettes that would be consumed by a pack-a-day smoker over an 8-month period. Toxicity returned to control values when smoking was stopped for several months, suggesting that the toxicity was due to volatile organic compounds (VOCs). However, when fabrics were extracted with culture medium containing serum protein, toxicity increased significantly, even in extracts that had been exposed to THS for only 4 months. Protein apparently removed a toxicant(s) that was not volatile. This idea was supported by the observation that extracts made with protein in the culture medium did not lose their toxicity when preincubated at 37 °C without cells for up to 72 h, which would be sufficient time for VOCs to escape. Fabrics that were aged for 2 months in the chamber without smoking had reduced cytotoxicity even when the extraction medium contained protein, suggesting that longer periods of aging do result in the loss of some of the protein extractable toxicants.

Similar results have been obtained using rat HepG2 cells exposed to THS extracts from laboratory experiments (1, 3, 5, 10, 15, or 20 cigarettes in a 27.6 L acrylic chamber) and samples collected from a smoker's home (60 cigarettes smoked over 3 days).[81] Both cotton and paper samples were tested. The MTT assay, the neutral red uptake (NRU) assay, and trypan blue staining were used to assess cell viability when treated with aqueous THS extracts. Effects were observed in all assays indicating damage to the mitochondria (MTT), lysosomal compartment (NRU), and plasma membrane (trypan blue). Both laboratory-generated THS and samples collected from smokers' homes showed toxicity in these assays. Together, these results show that even low levels of THS contain volatile and semivolatile toxicants that build up on surfaces and that these toxicants can inhibit cell proliferation and impair cell survival in a dose dependent manner.

3.2.3.2. Identification of Volatile Organic Compounds in THS That Are Toxic to Cultured Cells. Cigarette smoke contains numerous volatile chemicals that cause harm to cells and interfere with cellular processes.[83−86] Because VOCs desorb from sites of THS deposition[28] and form from chemical reactions of THS on surfaces, they could induce harm in humans occupying indoor spaces where VOCs are being emitted. To test this possibility, pieces of terry cloth were exposed to THS from the equivalent of 133 cigarettes over an 11-month period (Figure 6).[79] The estimated surface loading was 1.1 $\mu g \cdot cm^{-2}$. The fabric was aged 11 months in a sealed bag, then extracted and tested for cytoxicity using mouse neural stem cells (mNSC) in the MTT assay. Conditions were first optimized to extract THS-exposed cotton fabric (terry cloth). Extracts lost potency when the headspace of the extraction vessel was large or when extracts were allowed to age before testing, suggesting that VOCs were responsible for the observed cytotoxicity.

To understand how THS affects cells, live cell imaging experiments were done on cultured cells undergoing THS exposure. Analysis of time-lapse videos revealed that THS caused a concentration-dependent inhibition of cell growth, fragmentation of cells, vesiculation, and impaired motility. The effects on motility correlated with depolymerization of the actin filaments and microtubules by THS. This effect was lost when extracts of THS were aged before testing, again suggesting that VOCs were producing the effect. Cells were then screened for cytotoxicity using a library of 26 authentic standards of VOCs known to be present in cigarette smoke or THS (Figure 6).[31,87] In the MTT assay, only three of the 26 chemicals in the screen (phenol, 2,5-dimethylfuran, and acrolein) showed significant cytotoxicity when tested with mNSC, human pulmonary fibroblasts (hPF), and human lung epithelial cells (A549). Toxicity was not increased when media containing test chemicals were replaced every 4 h for 24 h, suggesting that the toxic effects are exerted early in exposure and do not increase with addition of fresh test chemical. Since THS chemicals are normally presented to an exposed individual as a mixture, not as individual chemicals, the three toxic VOCs were tested in combination. The MTT dose−response curves were shifted to the left, indicating increased cytotoxicity when acrolein, phenol, and 2,5-dimethylfuran were tested together. These results further demonstrated the importance of testing mixtures of THS chemicals when evaluating cytotoxicity.

Acrolein, which was the most potent of the toxic VOCs, was further tested in a live cell imaging assay (Figure 6). Like THS, acrolein caused cell death at high concentrations (10^{-5} M) and inhibited proliferation at low concentrations (10^{-6} M). Taking into account the rapid removal of free of acrolein by its binding to proteins in the culture medium, the effective concentration of acrolein *in vitro* would have been 10 to 100 lower than 10^{-6} M and would bracket the concentration of acrolein emitted from THS exposed materials.[31] However, acrolein did not cause blebbing, fragmentation, vesiculation, or inhibition of motility, as was the case for THS, suggesting that there are additional, as yet unidentified, chemicals in THS that alter these processes.

Gene expression arrays were used to determine how acrolein slowed proliferation of cultured cells (Figure 6). Low concentrations of acrolein (10^{-6} M) inhibited expression of *TFDP1*, which functions in the transition from the G1 to S phase of mitosis. In addition, *Casp3*, which plays a role in transitioning of cells from G2 into the M phase of the cell cycle, was down regulated. *Wee1* expression increased, which would inhibit transition from G2 into the M phase of the cycle, and *AnaPC2* was down regulated, which would inhibit movement of chromosomes into anaphase during the M phase of division. Taken together, these data show that acrolein can target multiple steps in the cell cycle in a manner that would slow cell proliferation.

3.2.3.3. Stress-Induced Mitochondrial Hyperfusion. Mitochondria are vital organelles that perform numerous functions in cells. They have unique regenerative properties and can maintain their homeostasis by undergoing rounds of fission and fusion that enable unhealthy mitochondria to either be revived or targeted for mitophagy (destruction).[88,89] Maintenance of a healthy pool of mitochondria is essential for cell health, and many diseases are due to mitochondrial malfunctioning.[90] Recent work has shown that mNSC undergo a process called "stress-induced mitochondrial hyperfusion" (SIMH) in response to THS exposure.[80] SIMH is characterized by the fusion of small round mitochondria into tubes, networks, and loops (Figure 6). The fused mitochondria differ from untreated controls in having an increased mitochondrial membrane potential (MMP), which can be visualized by labeling cells with Mitotracker Red (Figure 7). This effect is concentration dependent and can be observed when cells are treated with THS extracts from cloth that was exposed to as few as 11 cigarettes.[80]

DOI: 10.1021/acs.chemrestox.6b00343
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

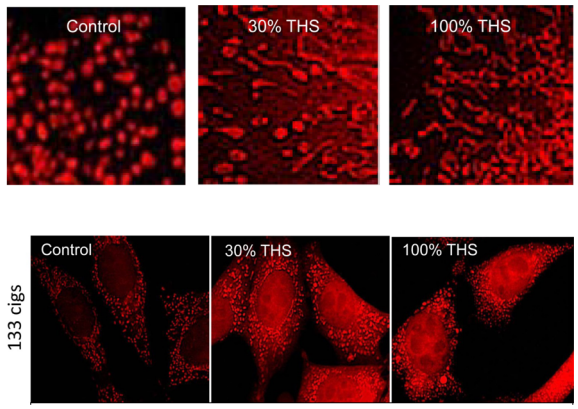


Figure 7. THS induces mitochondrial fusion. (Top panel) In controls, mNSC mitochondria are small and round in shape. In THS treatment groups (30 and 100%), mitochondria fuse together to form tubes, networks, and loops. THS induces an increase in mitochondrial membrane potential (MMP) (lower panel). mNSC treated with THS then incubated in Mitotracker Red show increased fluorescence relative to untreated controls, indicative of an increase in MMP. Reproduced with permission from ref 80. Copyright 2016 Oxford University Press.

The increase in MMP is accompanied by an increase in ATP production, the molecular fuel that provides energy for cellular processes (Figure 6).[80] This increase in ATP may be necessary to cope with the stress induced by THS exposure. The increase in ATP was accompanied by an increase in reactive oxygen species (specifically superoxide), which are potentially dangerous and can damage cellular molecules. The Mitotimer protein, which localizes in mitochondria and fluoresces green in newly synthesized mitochondrial proteins but changes to red fluorescence as oxidation of protein increases was used to show that SIMH is accompanied by an increase in oxidation of mitochondrial proteins. While this does not immediately kill cells, it does signal that mitochondria are unhealthy and that they are slowly being damaged by THS exposure.

When cells were exposed to a relatively low dose of THS for 15 days, they maintained a strong MMP and increased their rate of cell proliferation.[80] However, by 30 days of exposure, the same cells had lost their MMP, indicative of nonfunctional mitochondria, and cell proliferation had slowed. Gene expression data suggested that SIMH was brought about by the downregulation of (1) the *Fis1* gene which is needed for mitochondrial fission; lack of sufficient *Fis1* would favor mitochondrial fusion; (2) *Ucp* genes that function in reducing the MMP; down regulation of this group of genes would favor an increase in MMP; and (3) pro-apoptotic genes, such as *Tspo* and *Bid*, which would decrease the probably of apoptosis occurring. Suppression of these genes by THS treatment is consistent with the observed results of increased mitochondrial fusion, increased MMP, and decreased apoptosis during 15 days of treatment. All of these alterations in gene expression support the idea that SIMH is a pro-survival mechanism.

3.2.3.4. Relationship between in Vitro Studies and Human Health. *In vitro* studies on THS extracts have shown a dose–response relationship between exposure and response. The main responses observed to date at doses that do not kill cells have included the inhibition of proliferation, damage to DNA, alteration of the cytoskeleton and inhibition of motility, and induction of SIMH. Any of these responses could have effects on cell, organ, and human health. These effects may not be immediate, for example, SIMH may lead to cell death if THS stress is chronic; however, cells are not killed outright by SIMH, so effects on human health may take time to develop, as is also the case with conventional cigarette smoking. Any of these reported effects may contribute to morbidity which may worsen with longer exposures. Of particular concern would be changes to DNA, which if not properly repaired could eventually lead to cancer, one of the hallmarks of cigarette smoking.[3] The accumulation of carcinogens, such as TSNAs, is also a concern and could promote unwanted changes in DNA. One study has shown that a toddler mouthing a small piece of cloth exposed to THS from about 133 cigarettes would receive a TSNA exposure about 16-fold higher than the inhalation exposure of a passive smoker.[66] This model is based on the combined levels of NNK, NNN, and NNA, which may not be equally carcinogenic, which may change in concentration during aging, and which may be affected by ingestion as a mixture, rather than as isolated TSNAs.

In vitro studies have been done with various cell types, including stem cells, which were often more sensitive to THS exposure than differentiated cells. While very preliminary in nature, this observation should be pursued in the future as damage to stem cell populations may compromise health. Developing organisms are often more sensitive to environmental chemicals than adults, making studies on the prenatal and early postnatal periods of life particularly important.[91] In considering how *in vitro* studies relate to human health, it is important to consider dosage, and much more evidence is needed on THS exposure in real-world settings. We do know that responses to THS in cell cultures are dose-dependent. In most *in vitro* work to date, concentrations of THS extracts have been quite low. For example, the aqueous extract of cloth exposed to the equivalent of 11 cigarettes produced cytotoxic effects even when diluted. The assays with acrolein produced effects at a 10^{-6} M concentration, and combining toxicants in THS increased the potency. Future *in vitro* studies will continue to establish a foundation upon which our understanding of THS can expand and will provide models for examining the molecular effects of THS in greater depth.

3.2.4. Animal Toxicity Studies. Separating THS exposure from SHS exposure is a challenge in both animal and human studies. An exposure system for mice that mimics exposure of humans to THS in homes of smokers has been developed (Figure 8). Using a smoking machine (Teague Enterprises, Inc., Woodland, CA[92]) designed for exposing rodents to cigarette smoke, common household fabrics are placed in empty mouse cages and subjected to SHS exposure.[59] Cages contain materials commonly present in homes; 10 g of curtain material (cotton), 10 g of upholstery (cotton and fiber), and two 16 in^2 pieces of carpet (fiber) to maintain equal exposure levels across experimental groups. Two packs of 3R4F research cigarettes were smoked each day, 5 days/week. All cigarettes were smoked and stored in accordance with the Federal Trade Commission (FTC) smoking regimen.[93] Smoke was routed to a mixing compartment and distributed between two exposure chambers, each containing 4 cages with the materials. The materials were always exposed to the same level of SHS by adjusting the machine to deliver the same total particulate matter (TPM) to the chambers containing the cages with the materials. The levels of TPM were adjusted to fall within those found by the EPA in the homes of smokers (in the homes of smokers, 15–35 $\mu g/m^3$; in our machine, 30± 5 $\mu g/m^3$).

DOI: 10.1021/acs.chemrestox.6b00343
Chem. Res. Toxicol. XXXX, XXX, XXX–XXX

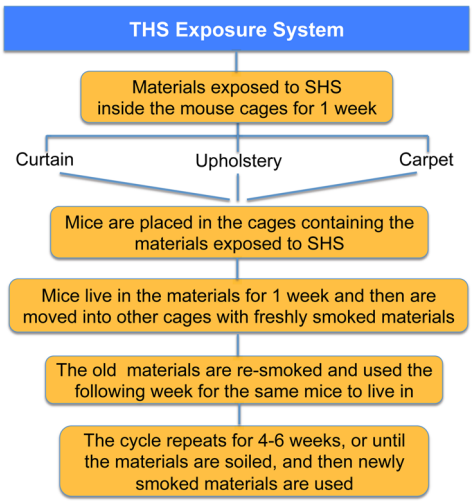


Figure 8. THS exposure system for studies in mice.

At the end of each week, cages were removed from the exposure chamber, bagged, and transported to the vivarium where mice were placed into the cages. For the next week, an identical set of cages and fabric was then prepared and exposed to smoke in the same way as that described above. Using two sets of cages and material, each of which was exposed on alternate weeks, ensured that mice always inhabited cages containing fabric that had been aged and with fresh THS at any given week (Figure 8). Throughout the exposure period, hair was removed weekly from the backs of the mice to mimic the bare skin of humans. This was done to mimic human skin that has very little hair. The back, rather than the belly was chosen because the belly is difficult to shave, and because mice burrow into the THS-exposed material, which is placed in the corner of the cage, there should be little effect on exposure. The experimental group was exposed to THS from right after weaning (3 weeks of age) to 24 weeks; the control group was never exposed to THS. The mice were fed a standard chow diet (percent calories: 58% carbohydrate, 28.5% protein, and 13.5% fat).

Using this system, the median NNAL level in the urine of THS-exposed mice was similar to that of a cohort of 50 infants/toddlers aged 0.5 to 4 years exposed to SHS, which suggests that the exposure system mimics exposure of children in the homes of smokers reasonably well.[59] However, the possibility of differences in metabolism of NNK to NNAL in children as compared to adults is a factor that cannot be ruled out. When examining the effects of THS exposure on the mice under these conditions, several physiological functions were altered.[59]

3.2.4.1. THS Effects on Skin. It has long been known that smokers' wounds heal poorly.[94] This is of particular concern for postsurgical wound healing. As a consequence, surgeons commonly recommend or require cessation of smoking for at least 4 weeks prior to surgery. The early effects of smoking that cause constriction of blood vessels are reversible in less than an hour after smoking, whereas the deficiencies in the inflammatory response do not return to normal until approximately 4 weeks after cessation,[94] and it is not known how long it takes for the damage to cells to be reversed. The wounds of mice exposed to THS took longer to heal and showed characteristics that are conducive to reopening, such as heavy keratinization of the epithelium.[59] The expression of numerous genes for keratins and keratin-associated proteins that are normally produced for hair and nails was increased. Also, the level of fibrillar collagen was greatly decreased in THS-exposed animals; the majority of collagen is not fibrillar and appears to be degraded, an observation consistent with gene array analysis showing a decrease in expression of tissue-inhibitor metalloproteinase 1 (TIMP1), an inhibitor of matrix metalloproteinases. The delay in wound closure is accompanied by decreased amounts of fibrillar collagen in the healing tissue and marked reduction of strength of wound tissue. This effect, in conjunction with the presence of keratins that convey rigidity to the epithelium and cells rich in contractile filaments, could cause or contribute to reopening of surgical wounds in smokers and, potentially, for those exposed to SHS and THS.

3.2.4.2. THS Effects on Lung. With THS exposure, the walls of the alveoli of the mice were disrupted and the alveoli contained secretions.[59] Some areas of the respiratory bronchioles, the alveoli of the THS-exposed mice showed leukocyte infiltration, in particular macrophages, indicating inflammation. In the interstitial tissue, excessive disorganized collagen fibers suggesting fibrosis were observed.[59] The elevated level of interstitial collagen, the thickened walls of some alveoli, the presence of macrophages in the walls of those alveoli and the increase in pro-inflammatory cytokines all suggest the possibility of increased risk for development of lung fibrosis in people who have been exposed to THS for prolonged periods of time. It is also possible that THS-exposed people have an increased susceptibility to toxicity from drugs that induce lung fibrosis.[95−97]

3.2.4.3. THS Effects on Liver. THS stimulates accumulation of fat in the hepatocytes (steatosis), giving the liver a pale red color compared to the deep red in normal liver, which was seen in 30% of the animals. The affected liver tissue contained large lipid droplets, whereas the droplets in control animals were very small. THS-exposed animals had greater amount of lipid with greater increase of triglycerides.[59] Lipid elevation of more than 5% above normal fat indicates that steatosis has progressed to nonalcoholic fatty liver disease (NAFLD), a condition that, with prolonged exposure, can lead to fibrosis, cirrhosis, and cancer in humans. The blood of animals exposed to THS showed significantly elevated levels of triglycerides and low-density lipoprotein (LDL, bad cholesterol), whereas levels of high-density lipoprotein (HDL, good cholesterol) were significantly decreased.[59] These changes in liver metabolism have potential implications for cardiovascular disease and stroke.[98−103] It is also possible that THS-exposure may aggravate drug-induced damage (e.g., by acetaminophen) at doses that normally would not be damaging.

3.2.4.4. THS Effects on Metabolism. THS-exposed animals had elevated fasting glucose levels that would be classified as prediabetic, and they were significantly less efficient than control animals in using insulin to bring down blood glucose levels when an insulin tolerance test was performed.[59,104] Similarly, glucose tolerance testing showed that THS-exposed mice handle the introduced glucose much less effectively than controls.[59,104] The elevated triglycerides, increased LDL, decreased HDL and defects in insulin metabolism are elements of "metabolic syndrome," a condition that predisposes humans to stroke, coronary artery disease and type 2 diabetes.[99−103] These results are consistent with findings that show that tobacco smoke exposure and active smoking contribute to insulin resistance and could be associated with metabolic syndrome in US adolescent children.[104]

DOI: 10.1021/acs.chemrestox.6b00343
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

Subsequent studies were performed to examine the insulin signaling pathway that brings glucose into cells. Reduced levels of the insulin receptor, phosphoinositide 3-kinase (PI3K) and AKT (also known as protein kinase B), all important molecules in insulin signaling and glucose uptake by cells were observed.[104] The inhibition of this signaling pathway results in the Glut4 (glucose) transporter remaining in the cytosol instead of being transported to the plasma membrane to allow the entrance of glucose into the cell. This will result in accumulation of glucose in the bloodstream (hyperglycemia). The effects on THS-induced insulin resistance were determined to be due to oxidative stress that causes damage to proteins, lipids, and DNA, key molecules in cellular function[104] (Figure 9). To confirm that oxidative stress is important in the THS-induced insulin resistance, mice exposed to THS were treated with the antioxidants *N*-acetyl cysteine (NAC) and alpha-tocopherol (α-toc), which significantly reversed oxidative stress, molecular damage, and insulin resistance. Conversely, feeding the mice a western diet while exposing them to THS increased oxidative stress and aggravated hyperglycemia and hyperinsulinemia. These results indicate that THS exposure results in insulin resistance similar to nonobese type II diabetes (NODII) through oxidative stress.[104]

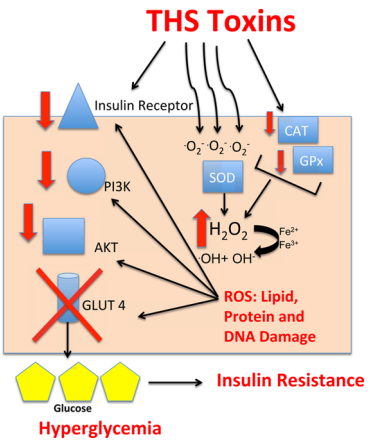


Figure 9. THS-induced insulin resistance.

3.2.4.5. THS Effects on Coagulation and Thrombosis. It is well-known that FHS and SHS increase the risk of coronary thrombosis.[3] However, it is not known whether exposure to THS has similar effects. We found that mice exposed to THS as described above have enhanced platelet aggregation and secretion responses. Furthermore, THS increases the speed of coagulation and cause of thrombosis suggesting that this form of smoke increases the risk of thrombosis-related disease.[105]

3.2.4.6. THS Effects on Behavior. In initial studies, THS-exposed animals appeared hyperactive.[59] To examine this in more detail, the mice were subjected to the Open Field test. Individual mice were placed in the Open Field, and walking, stationary, and rearing behaviors were assessed, as well as the frequency of transition from one of these behaviors to another. THS-exposed mice spent significantly more time walking, much less time standing still, and more time rearing than control mice. The frequency of transitions between these behaviors showed a similar pattern. In particular, THS-exposed mice were almost constantly in motion, whereas control mice were stationary for a considerable fraction of the time. Other exposed and nonexposed mice were studied in the Open Field test using Ethovision 7.1 video tracking software to track mice individually for an hour[59] (Figure 10). Again, the THS-exposed mice covered longer distances at higher velocities and spent significantly more time in the periphery of the field. The difference in behavior between the two groups was particularly striking in the first 2 min during which the THS-exposed mice moved on average at high but decreasing velocity and the last 10 min of the hour in which the control mice showed on average little activity, whereas the THS-exposed mice remained very active. These studies indicate that THS-exposed mice are hyperactive.[59]

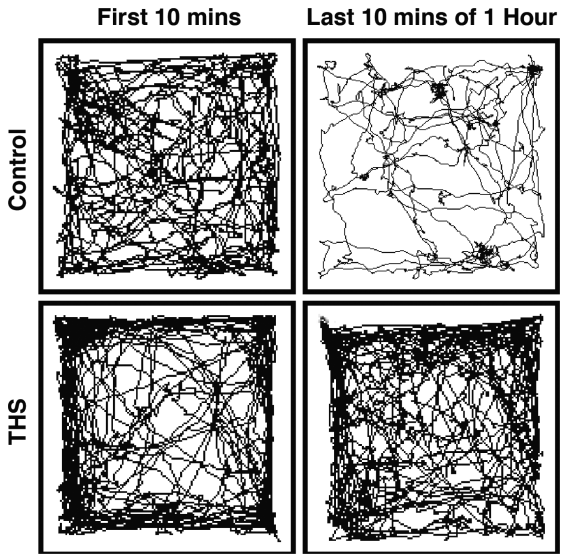


Figure 10. Effect of THS on mouse activity.

3.2.4.7. THS Effects on the Weight of Mice Exposed during Development and Weaning. The effects of THS exposure on the weight of mice exposed during pregnancy through weaning (postnatal day 21) and from birth until weaning were studied.[106] In both cases, the THS-exposed mice weighed less than the nonexposed mice. This was seen in both males and females. When the mice were removed to a nonexposed setting and followed over time, the THS-exposed mice regained the weight to be similar to that of nonexposed mice. Furthermore, at 17 weeks of age, both males and females exposed to THS during the first 3 weeks of life had altered white blood counts.[106] The eosinophil number was significantly higher in both genders, together with increased basophils in male mice and increased neutrophils in female mice. FACS analysis showed that early exposure to THS caused a significantly increased percentage of B-cells and T-suppressor cells, with decreased percentage of myeloid cells in adult mice. Equally remarkable is that exposure at the very young ages altered the white blood cell compartment leading to altered cell numbers in circulation. These results indicate that there is a window of susceptibility for some forms of cellular damage induced by THS-exposure. Damage that occurs during the very early stages of life can persist into adulthood. The results described in this section on the potential effects of THS exposure on human health are summarized in Figure 11.

3.3. Human Exposure and Risk Assessment.

3.3.1. Real-World Environmental Contamination. To evaluate the magnitude of the risks of THS to the general population, studies of the extent of environmental contami-

DOI: 10.1021/acs.chemrestox.6b00343
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

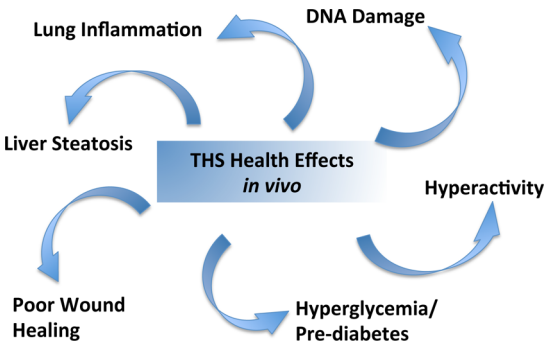


Figure 11. Potential health effects of THS exposure using a mouse model of exposure that mimics exposure of humans in their homes.

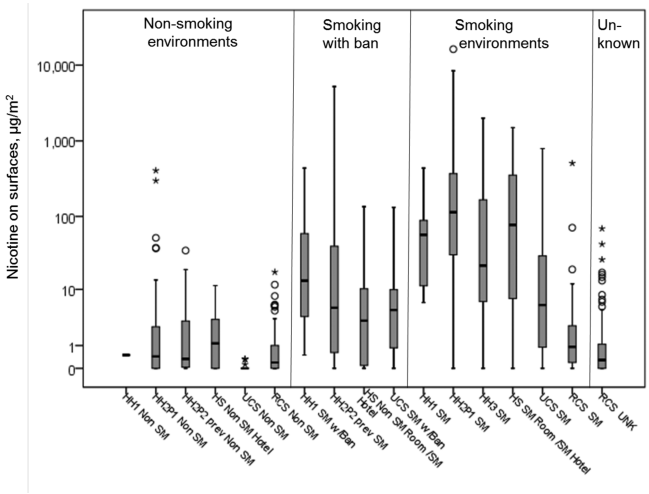


Figure 12. Surface nicotine measurements (μg nicotine/m^2) from homes, cars and hotels, by type of environment. Abbreviations: SM, smoking environment; non-SM, nonsmoking environment; w/Ban, with a ban on smoking in the environment. Studies: HH1, Healthy Homes I, Matt et al., 2004. In homes of women with infants, HH1 examined smoking behavior of mother and effects of home smoking ban on protecting infant from exposure through tobacco smoke pollution on air dust and surfaces. HH2, Healthy Homes II, Matt et al.[12] and Hoh et al.[109] HH2 examined persistence of tobacco smoke contamination in homes of smokers who moved out and contamination and exposure in new occupants. HH2P1, Part 1 if HH2, before the occupants moved out. HH2P2, Part 2 of HH2, after cleaning and reoccupancy of same home.

nation by toxic substances present in THS are required, along with an understanding of the prevalence and magnitude of THS exposures to key populations. After the first reports of nicotine contamination in house dust by Hein et al.[14] and of nicotine in house dust, surfaces, and on hands by Matt et al.,[7] THS contamination has been measured in many public and private environments, private homes, and cars, hotels; casinos and rental cars; taxis, and even in neonatal intensive care units (NICUs).[7,15,16,107−110] However, a systematic investigation of the percent of various environments such as homes and hospitality venues contaminated by THS has not been undertaken, as has been done for SHS. A striking feature of THS exposure is that exposure can be not only involuntary but also unknown, such as when an apartment has turnover of occupants and a new occupant moves into a THS contaminated space. Nicotine was found at significantly higher levels in dust and on surfaces from homes formerly occupied by smokers, even after being cleaned and occupied by nonsmokers a median of 62 days later.[15] A study of THS contamination in homes of former smokers found that THS levels declined after smoking ceased but contamination was still higher than that in homes of nonsmokers after 6 months.[111]

3.3.1.1. THS in Air. Air measures of THS in real world environments to date have mainly quantified nicotine as a marker, though chamber studies have shown that other toxic volatile chemicals such as the irritating and toxic VOC acrolein are present during the aging of THS.[31] Air samples collected in a large casino after a smoking ban showed measurable concentrations of nicotine months after the smoking stopped, demonstrating the magnitude of the reservoir of THS pollutants.[112]

3.3.1.2. THS on Surfaces. THS has also been measured on surfaces in homes, private and rental cars, hotels, and other public spaces (Figure 12).[113] Nicotine and other semivolatile compounds from THS can rapidly sorb into and onto furnishings, walls, and other surfaces, which can then act as reservoirs, releasing the chemicals back into the environment over months and years.[114] Nicotine levels can be as high on surfaces as in dust.[15] Highly toxic and mutagenic TSNAs can also be found contaminating surfaces in homes of smokers.[115] An investigation of THS contamination of surfaces, in Nanjing, China, revealed widespread nicotine contamination in public places and in public transportation.[116] Levels were very high compared to those of smoking rooms of hotels measured in California.[117] Interestingly, levels in nonsmoking environments in Nanjing were also much higher than those in studies in California, where many of the first studies have been performed.[113] Countries with high smoking rates probably have higher exposures and risks, and it is very important to perform field studies of THS in countries with high smoking rates.

3.3.1.3. THS in Indoor Dust. House dust from homes of smokers contains significantly higher levels of toxic contaminants including nicotine, PAHs, and TSNAs. The homes of smokers had higher nicotine concentrations per gram of dust and more dust loading (amount per surface area).[15,111] This finding held, even in homes with young children where parents did not smoke in the presence of their children.[7] Dust in cars in which smoking has taken place can also be highly contaminated with nicotine, indicating the presence of additional toxic THS compounds.[16,17] PAHs form during combustion and some PAHs are known human carcinogens. Cigarette smoke contains PAHs, and THS can present an additional exposure risk. In homes of smokers, both the total PAH in dust and individual PAH loading were significantly greater than those in dust from nonsmokers' homes and were correlated with nicotine levels in the same sample, further implicating the role of smoking in elevating exposures to these ubiquitous and toxic pollutants.[117]

3.3.1.4. Effect of Smoking Bans on THS Contamination. Matt et al. examined private-party used cars for sales in San Diego, California.[17] Cars offered for sale by smokers had significantly higher levels of nicotine (dust nicotine19.5 ug/g; surface nicotine 8.6 ug/m^2) than cars for sale by nonsmokers (3.4 ug/g; 0.1 ug/m^2), even when the smokers did not permit smoking in their cars (5.1 ug/g; 11.6 ug/m^2). Thirdhand smoke is a particularly important problem in indoor settings that experience high occupancy turn over, like apartments, rental cars, and hotel rooms. Because smoking prevalence ranges from about 10−25% across the states, it is very probable that most indoor environments have been occupied by a smoker within

DOI: 10.1021/acs.chemrestox.6b00343
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

Table 2. TSNAs in House Dust (ng/g)

	smokers					nonsmokers		
TSNA	study 1[a] (N = 2)	study 2[b] (N = 22)	study 3[c] (N = 6)	study 4[d] (N = 22)	study 4[e] (N = 13)	study 1[a] (N = 5)	study 2[b] (N = 24)	study 3[c] N = 20)
NNN	35	20	2.9	2.5	1.5	1.3	4	BLQ
NNK	84	540	5.8	8.9	11.2	3.2	40	0.51
NAB		510	0.18	0.9	BLQ		0	BLQ
NAT		70	BLQ	2.0	3.4		10	BLQ
NNA			0.6					BLQ

[a]Jacob et al.,[35] means. [b]Ramírez et al.,[118] medians. [c]Whitehead et al.,[48] medians, second sampling round, 2010. [d]Matt et al.,[107] geometric means, before smoking cessation. [e]Matt et al.,[108] geometric means, 1 week after verified smoking cessation; BLQ = below limit of quantitation.

Chart 2. Tobacco Alkaloids and TSNAs Measured in House Dust

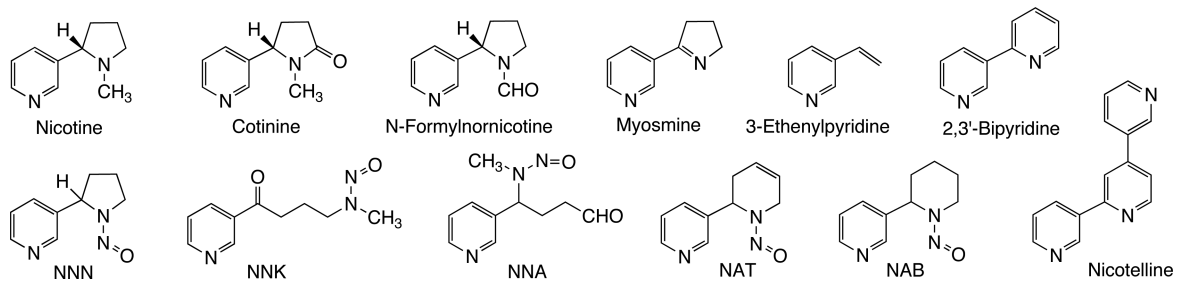


Table 3. Detection Frequencies and Median Concentrations (ng/g) of Tobacco Constituents in Vacuum Dust Collected from Homes Participating in the California Childhood Leukemia Study during the First and Second Sampling Rounds, by Tobacco Use at the Index Home[a]

	first sampling round (2002–2007)						second sampling round (2010)					
	smokeless tobacco users, N = 6		active smokers, N = 6		tobacco-free homes, N = 20		smokeless tobacco users, N = 5		active smokers, N = 6		tobacco-free homes, N = 20	
tobacco constituent	% det	median	% det	median	% det	median	% det	median	% det	median	% det	median
nicotine	100	14,000a	100	7,000a	90	520	100	15,000a	100	7,800a	95	510
Tobacco-Specific Nitrosamines												
NNN	67	5.7[b]	67	1.6[b]	5	<1.4b	80	4.3[b]	83	2.9[b]	10	<1.4[c]
NNK	100	6.3[b]	100	3.7[b]	40	<0.45b	100	3.4[b]	100	5.8[b]	65	0.51
NNA	50	1.2[b]	67	0.46a	0	<0.45b	40	<0.45[b,c]	50	0.60[b]	5	<0.45[c]
NAB	50	0.28	33	<0.15[c]	20	<0.15b	60	0.29[b]	50	0.18[b]	10	<0. Fifteen[c]
NAT	50	3.6[b]	0	<4.2[c]	0	<4.2b	40	<4.2b	0	<4.2[c]	5	<4.2[c]
Minor Tobacco Alkaloids												
cotinine	100	680[b]	100	430[b]	80	54	100	450[b]	100	460[b]	70	26
myosmine	100	310[b]	100	440[b]	85	54	100	140[b]	100	700[b]	80	45
N-formylnornicotine	100	370[b]	100	480[b]	90	43	100	200[b]	100	660[b]	75	30
nicotelline	100	5.5	100	8.0[b]	95	1.0	100	2.9[b]	100	7.1[b]	100	0.62
2,3′-bipyridine	83	76[b]	100	74[b]	60	6.2	100	52[b]	100	72[b]	75	5.9

[a]Reproduced from ref 48. Copyright 2015 American Chemical Society. % Det, detection frequency; NNN, *N*′-nitrosonornicotine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNA, 4-(methylnitrosamino)-4-(3-pyridyl)butanal; NAB, *N*′-nitrosoanabasine; NAT, *N*′-nitrosoanatabine. [b]Significantly greater than concentrations of the tobacco constituents in dust samples collected during the same sampling round from tobacco-free homes, using the Wilcoxon two-sample Z-test, two-sided $p < 0.05$. [c]Lower limit of quantitation, determined as the lowest calibration standard for which back-calculated values were within ±20% of the expected concentration.

the past few years. Matt et al. examined rental cars offered by national and local companies in San Diego, California.[107] They found that regardless of their designation by rental companies as nonsmoking or smoking allowed, dust collected in the car cabins showed elevated levels of nicotine, with means ranging from 9.2 ug/g (designated nonsmoker cars rented from national companies) to 33.7ug/m^2 (designated smoker cars rented from local companies). Matt et al. examined hotels with complete smoking bans and hotels that allowed smoking in some rooms.[108] They found that compared with hotels with complete smoking bans, surface nicotine and air 3-ethenylpyridine were elevated in both nonsmoking and smoking rooms of the hotels that allowed smoking. Air nicotine levels in smoking rooms were significantly higher than those in nonsmoking rooms of hotels with and without complete smoking bans. Hallway surfaces outside of smoking rooms also showed higher levels of nicotine than those outside of nonsmoking rooms. Matt et al. examined homes of smokers after they moved out and nonsmokers moved in.[15] They found that while dust surface and air nicotine levels decreased after change of occupancy, dust and surfaces continued to show higher levels compared to those of former nonsmokers homes. Most

DOI: 10.1021/acs.chemrestox.6b00343
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

recently, Matt et al. measured nicotine and NNK in the homes of smokers who quit.[111] The amount of nicotine on surfaces declined (baseline, 22.2 $\mu g/m^2$; 1 week after cessation, 10.8 $\mu g/m^2$), and the nicotine on fingers of nonsmoking residents declined (baseline, 29.1 ng/wipe; 1 week after cessation, 9.1 ng/wipe). However, there were no further decreases in the samples collected 1 and 3 months later. Concentrations of nicotine and NNK in dust did not change and remained near baseline levels after cessation (nicotine near 5 $\mu g/g$; NNK near 10 ng/g). Dust nicotine and NNK loadings, i.e., mass normalized to surface area, significantly increased immediately following cessation (nicotine baseline, 5.0 $\mu g/m^2$; week 1 after cessation, 9.3 $\mu g/m^2$; NNK baseline, 11.6 ng/m^2; week 1 after cessation, 36.3 ng/m^2) before returning to and remaining at near baseline levels.

3.3.1.5. THS Contamination of Smoke-Free Homes and Homes of Smokeless Tobacco Users. Carcinogenic tobacco-specific nitrosamines and various tobacco alkaloids have been found in dust from homes of nonsmokers as well as smokers, as discussed above.[35,48,118] Results from 4 studies are listed in Table 2. Summary measures of TSNA concentrations varied considerably among the studies, likely due to the small number of observations and to differences in smoking practices between populations. For example, the relatively low TSNA concentrations in study 3 may be in part attributable to the fact that 5 of the 6 smokers' homes in study 3 had indoor smoking bans. The one home with indoor smoking had NNN and NNK concentrations of 8.43 ng/g and 19.4 ng/g, respectively. In addition, studies 1, 3, and 4 were conducted in California, whereas study 2 was conducted in Tarragona, in eastern Spain. In general, smoking prevalence is lower in California than in Spain, which results in a lower TSNA background. Other factors that may differ greatly between populations, such as home size and carpet coverage, might also affect the accumulation of dust/smoke or the dilution of TSNAs in the home. As expected, concentrations were higher in smokers' homes than in nonsmokers' homes. However, the fact that readily measurable concentrations of TSNAs were found in nonsmokers' homes indicates that tobacco smoke contamination is pervasive. A contributing factor to the presence of TSNA in homes of nonsmokers could be de novo formation from the reaction of HONO with nicotine and other tobacco alkaloids, as discussed above, in addition to transport into the homes from the outdoor environment. Tobacco alkaloids and TSNA have been measured in outdoor venues; their presence and implications will be discussed in a subsequent section.

The study by Whitehead et al. found that house dust in homes of smokeless tobacco (oral snuff or chewing tobacco) users contained tobacco alkaloids and TSNAs (Chart 2), in concentrations statistically indistinguishable from homes of cigarette smokers and significantly higher than that in homes of nonsmokers (Table 3).[48] These findings indicate that (1) living with smokeless tobacco users may result in exposure to carcinogenic tobacco-specific nitrosamines, as would living with smokers, and (2) high concentrations of tobacco alkaloids and TSNAs in homes indicate tobacco use in general, not just the use of combusted products. Whitehead et al. also found that the ratio of the tobacco alkaloids myosmine/nicotine can be used as an indicator of the source of tobacco contamination, distinguishing between the use of smokeless tobacco products and tobacco smoking (Figure 13). Concentrations of myosmine relative to nicotine were much higher in smokers' homes, presumably due to its formation from nicotine and nornicotine during combustion.

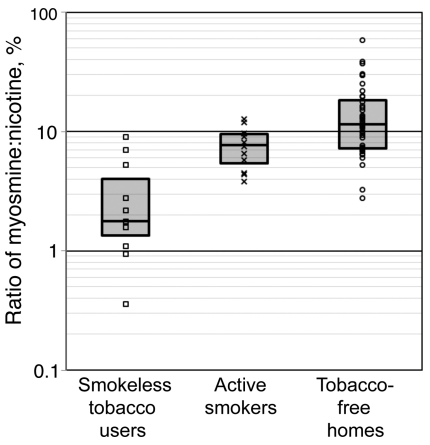


Figure 13. Myosmine-to-nicotine ratios (% nicotine) in vacuum dust samples collected from homes participating in the California Childhood Leukemia Study during the first (2002–2007) and second (2010) sampling rounds, by tobacco-use category. The vertical axis is shown on a logarithmic scale. Box plots represent the 25th, 50th, and 75th percentiles. Reproduced from ref 48. Copyright 2015 American Chemical Society.

3.3.2. THS Contamination Outdoors. About 6 trillion cigarettes are smoked worldwide each year.[119] The sidestream smoke, i.e., the smoke from a smoldering cigarette not inhaled by the smoker, releases 10.5–34.4 mg of particulate matter ("tar") and 1.9–5.3 mg of nicotine per cigarette into the environment.[120] On the basis of the mean values of those ranges, cigarette smoking releases about 135 million kilograms of particulate matter (PM) and about 22 million kilograms of nicotine into the environment each year worldwide. Despite these rather large amounts, very little data have been published on concentrations of tobacco-derived substances in the outdoor environment. Nicotine concentrations have been measured in outdoor locations[121,122] (Table 4), and increased levels of respirable fine particles ($PM_{2.5}$) have been measured outdoors in the proximity of smokers.[122,123] Using hydrocarbons present in cigarette smoke as tracers, it was reported that 1.0–1.3% of the fine particle mass concentration in the outdoor air in Los Angeles in 1982 was derived from cigarette smoke.[38] In a recent study, tobacco-specific nitrosamines were detected in particulate matter collected outdoors in London (Table 4).[58]

Table 4. Tobacco Alkaloids, TSNAs, and Benzo[*a*]pyrene in Outdoor Air (pg/m^3)

analyte	study 1[a]	study 2[b] (% RSD)	study 3[c] (% RSD)	study 4[d]
nicotine	17,000–48,000		3,390 (13)	
nicotelline			7.0 (17)	
NNN		200 (7.8)	0.17 (21)	
NNK		290 (14)	0.63 (12)	
benzo[*a*] pyrene			23 (78)	15–21

[a]Rome, 4 locations in February, 2011, Cecinato et al.[121] [b]London, July–August, 2012, Farren et al.[58] [c]San Francisco, CA, July–August, 2016, Aquilina, N., Havel, C., and Jacob, P., unpublished data. [d]Pallas, Finland, 1996–1998, Boström et al.[126]

DOI: 10.1021/acs.chemrestox.6b00343
Chem. Res. Toxicol. XXXX, XXX, XXX–XXX

There are two main reasons for interest in outdoor contamination by tobacco smoke. One is to establish baseline levels of exposure. There is clear evidence that tobacco smoke-derived substances in the outdoor environment are transported indoors leading to readily detectible concentrations on surfaces and in house dust. Published studies have reported nicotine, other tobacco alkaloids, and TSNA in homes of nonsmokers, as discussed above.[35,48,113,118] As a nonsmoker whose home tested positive for nicotine and TSNAs said: "No one has ever smoked in my home. My neighbors don't smoke. Where is it coming from?" Outdoor air and dust is one source, as well as tobacco smoke-contaminated clothing and other articles brought into the home.[124]

The second reason is to determine whether exposure outdoors poses a health risk. For people in the vicinity of smokers, it is reasonable to believe that exposure levels in air could be high enough to be unhealthful. St. Helen et al.[125] reported exposure to secondhand smoke outside of a bar and a restaurant, measured by the biomarkers cotinine and NNAL, which were elevated in nonsmokers after outdoor exposure. It is not yet known whether exposure to THS-contaminated dust, air, or surfaces in outdoor microenvironments is a significant health risk. This will require further study.

Studies of atmospheric contamination by tobacco smoke constituents outdoors have been initiated at the University of California, San Francisco. The chemistry of outdoor THS would be expected to differ considerably from indoor THS due to more intense UV radiation, mixing with atmospheric constituents, and higher concentrations of reactive species outdoors such as ozone, other oxidants, and reactive nitrogen compounds. As a first step, airborne particulate matter (TPM) was collected on the roof of a building at a large urban hospital in San Francisco using a high-volume sampler, well away from active smokers. Airborne nicotine was also collected, using a sodium bisulfate treated filter, since nicotine is present largely in the gas phase.[41,43,44] Filters were extracted and analyzed for nicotine, nicotelline, NNN, and NNK.[48] Benzo[*a*]pyrene concentrations were also measured because it is a thoroughly studied, potent carcinogen that is ubiquitous in the environment and serves to put concentrations of the TSNA carcinogens, NNN and NNK, into perspective in terms of concentrations and possible health risk. Preliminary results are presented in Table 4. Outdoor nicotine concentrations were lower, by a factor of 5−10, than those reported in Rome. NNN and NNK concentrations were 3 orders of magnitude lower than those reported in London. These differences could be due to differences in smoking prevalence or to different atmospheric conditions (concentrations of reactive species, wind velocity, or humidity) that could affect decomposition and dispersion. Concentrations of nicotelline, a proposed tracer for tobacco smoke derived particulate matter,[35] were about 7 pg/m^3 and detectable in all samples analyzed. Benzo[α]pyrene concentrations averaged 23 pg/m^3, which is lower than those generally found in large cities. Table 4 includes B[a]P data from Pallas, Finland,[126] which like San Francisco has relatively low PAH levels in outdoor air.

Characterizing the extent of outdoor contamination has both public health and policy implications. Homes and motor vehicles owned by smokers sell for lower prices than those owned by nonsmokers, as discussed in section 3.4.1 below. If measuring tobacco-derived substances in homes and motor vehicles becomes an established method for assessing tobacco smoke contamination, cutpoints to distinguish contamination by smoking inside from contamination by outdoor sources will need to be established. Clearly, additional research on outdoor contamination by tobacco smoke is warranted.

3.3.3. Human Exposure Studies. The first study with the objective of measuring THS exposure in humans was reported by Matt et al. in 2004 who examined infants of smoking mothers with and without smoking bans in their homes.[7] They compared cotinine levels in urine and nicotine and cotinine levels in the hair of infants. Average urinary cotinine levels for the infants were 15.5 ng/mL in those with smoking mothers in homes without smoking bans, 2.3 ng/mL in homes of smoking mothers with strict smoking bans, and 0.33 ng/mL in homes of nonsmoking mothers with strict smoking bans. For hair nicotine levels, the corresponding average nicotine concentrations were 5.9, 2.7, and 0.53 ng/g, respectively. Their findings showed that strict smoking bans and the absence of SHS exposure reduced but did not eliminate the exposure to tobacco smoke toxicants. The study also showed that cotinine levels in urine were associated with living room and bedroom nicotine levels in dust and on surfaces, as well as bedroom air nicotine levels. In their study of the persistence of THS in homes occupied by smokers, Matt et al.[15] found that nonsmokers living in homes previously occupied by smokers showed higher levels of nicotine on their fingers than nonsmokers living in homes previously occupied by nonsmokers (5.85 ng/wipe vs 0.75 ng/wipe). Nonsmokers living in homes previously occupied by smokers also showed significantly elevated levels of urine cotinine (0.61 vs 0.13 ng/mL). This was the case even though the homes had been cleaned before new people moved in and an average of 2 months had passed since the smokers had moved out.

In their study of hotels,[108] Matt et al. found that nonsmokers who stayed one night in hotels without complete smoking bans showed higher levels of finger nicotine (nonsmoker room, 11.94 ng/wipe; in smoker room, 60.3 ng/wipe) and urine cotinine (smoker room, 0.63 ng/mL) than those staying in hotels with complete smoking bans (2.5 ng/wipe nicotine; 0.14 ng/mL urine cotinine). The nonsmokers who stayed in the 10 most polluted rooms identified in the study also showed significant increases in urinary NNAL (0.86 vs 1.25 ng NNAL/mg creatinine).

The long-term exposure risks to THS were further demonstrated in a study of smokers after successful smoking cessation.[111] Nonsmoking cohabitants continued to show elevated levels of cotinine and NNAL up to six months after the smoker had quit and no additional tobacco had been smoked in the home. Compared to baseline levels before the smokers quit (4.6 ng/mL cotinine; 10.0 pg/mL NNAL), cotinine and NNAL in urine had significant initial declines (1 week and 1 month after cessation: 1.3 and 1.6 ng/mL cotinine; 4.2 and 6.0 pg/mL NNAL) without further significant changes until 6 months after cessation.

Even in places where smoking bans are strictly enforced, such as neontatal intensive care units in hospitals, THS can be found. Northrup et al. collected data on THS levels and infant exposure in a neonatal ICU (NICU), using surface nicotine samples from the fingers of smoking mothers, nicotine on the surfaces of infants' crib/incubator and hospital-provided furniture, and by measuring cotinine and NNAL concentrations in infants' urine. Incubators/cribs and other furniture had detectable surface nicotine, and both cotinine and NNAL were detected in the infants' urine. The authors concluded that THS

DOI: 10.1021/acs.chemrestox.6b00343
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

appears to be ubiquitous, even in highly controlled, smoke-free settings.[110]

3.3.4. Biomarkers of Harm. To study THS exposure and carry out a targeted risk assessment, it is necessary to develop specific biomarkers that can be linked to risk. This is currently a very active research area. As discussed above, Consortium studies are in progress on the identification and development of such biomarkers in mammalian systems, based on specific DNA adducts and metabolites derived from NNA, a unique compound in THS. The detection of a specific NNA-DNA adduct in human tissue or peripheral blood cells could be the basis for a definitive biomarker of THS exposure, as well as harm (genotoxicity), which could be utilized in human studies.

3.3.5. THS and Human Disease. Although numerous adverse health effects of active smoking and SHS are well documented, the dangers THS are poorly understood.[77,127−129] As discussed above, many of the constituents of THS have the potential to have serious adverse health effects, but it is not clear if concentrations in indoor environments are sufficient to result in such effects. Epidemiological studies have yet to be conducted on the relationship between THS and health. Such studies will be challenging because most people who are exposed to THS are also exposed to SHS. The diseases most likely to be caused by THS are those that are already known to be caused by SHS exposure, particularly respiratory tract infections and asthma. Animal toxicology studies reviewed elsewhere in this Perspective suggest that THS exposure may also contribute to cancer, impaired wound healing, lung fibrosis, hepatic steatosis, insulin resistance and diabetes, lipid abnormalities, metabolic syndrome, behavior hyperactivity, and impaired immune responses. Dose−response modeling studies indicate a possible association between nitrosamines in THS and cancer.[118]

3.3.6. Vulnerable Populations. Children, especially infants and very young children, are likely to be among the most vulnerable populations in regard to both exposure to and effects of THS.[1,2,5,7,17,26,130] Nearly 88 million US nonsmokers ≥3 years-old living in homes of smokers have ≥0.05 ng/mL serum cotinine (a metabolite of nicotine) and TSNAs in urine.[1] Young children may be highly exposed to THS in house dust and surfaces through the following routes: orally, dermally, and through inhalation.[131] Oral exposure is enhanced in children by frequent hand to mouth behaviors.[132] Dermal exposure is enhanced by crawling and touching behaviors. Children also have thinner skin than adults. Inhalation exposures are enhanced though their short stature and active play near the floor, where they can resuspend fine house dust which can then be inhaled or settle on skin. Kilogram for kilogram, children breathe more air than adults, so that children have greater exposures even in the same environment than do adults. Children also have a larger surface area to body weight ratio than do adults.[133] Children may be more vulnerable to the toxic effects of THS due to the fact that their organ systems are rapidly developing, and children can differ from adults in their ability to detoxify pollutants.[131] For example, newborn children have little ability to detoxify organophosphate pesticides as compared to adults.[134] Younger children also stay close to their parents and caregivers, which means that they cannot avoid SHS and THS if the caregivers smoke. Take-home or para-occupational exposures have been shown to be a significant source of environmental exposures for children of workers exposed to other pollutants such as lead, which can enter the home on the clothes of parents and significantly expose their children.[135] It is likely that the children of nonsmokers who are exposed to SHS at work (such as a casino card dealer) are exposed to THS this way.[77] The importance of house dust as an exposure route has been well established for other environmental pollutants, such as lead and flame retardants.[136,137] Dermal exposure has also been shown as an important route of exposure to pesticides for young children.[138,139] Therefore, it is likely that para-occupational exposures and dust are significant sources of THS exposure.

3.4. THS Toxicity and Policy Implications. *3.4.1. Policy.* Policies have evolved over more than three decades to limit SHS exposure, which has declined greatly in the United States and some other countries.[3] Banning smoking indoors eliminates SHS and potentially THS as well. However, evidence reviewed here shows that THS can persist in a space after smoking is no longer taking place. We lack evidence, however, on how long THS persists in indoor environments, and whether it ever clears from fabrics and construction materials in buildings where smoking took place for long periods of time, e.g., decades. On the basis of knowledge of the components of THS and their toxicity and on the emerging *in vivo* and *in vitro* evidence reported in this Perspective, THS can be assumed to pose a hazard to human health. However, we lack a population-based body of data on the concentrations of THS in various environments and the range of exposures received by the population and particularly by susceptible infants and young children who may experience the highest exposures. We also lack epidemiological evidence on the health risks posed by THS, disentangled from the risks of SHS exposure.

Nonetheless, data collected previously by the Consortium's investigators indicate that THS is considered a problem in some sectors: real estate and motor vehicle sales and the hotel/motel business[3,140] Nonsmokers do not want to purchase homes where smokers have lived, particularly if they have children. In a publication on strategies for SHS control, Samet and colleagues[140] collected information from a convenience sample of individuals working in these sectors, confirming that THS was problematic, although not recognized specifically by the designation of THS.[45] The odor of tobacco smoke, signaling the presence of prior smoking, was problematic for the real estate and used car sales sectors.

Approaches to managing several indoor pollutants were considered as policy models: lead, asbestos, and radon. Each has known hazard, and for each, the presence of the contaminant can be documented by a measurement made according to a standard and accepted protocol. The control strategies are mandated at the federal level for asbestos and lead, while the Environmental Protection Agency, which lacks regulatory jurisdiction for indoor environments, has published a guideline value for indoor radon concentration. Extended to THS, a parallel approach would require a marker for its presence and a risk-based target concentration for its mitigation. The work of the Consortium is developing the foundation for elaborating such an approach.

3.4.2. Avoiding Exposure and Remediation. In the US, diverse companies perform restoration services for home and commercial buildings affected by tobacco smoke, mold, fire, and flood damage. In general, treatments combine removing severely affected materials (such as carpets) or using liquid cleaners. Ammonia-based cleaners are recommended to remove tobacco odors. Restoration companies also use ozone generators to remove intense tobacco odors. The ozone concentrations generated are several orders of magnitude

DOI: 10.1021/acs.chemrestox.6b00343
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

higher than typical levels found in urban atmospheres. There has been very little scientific research to date on how well these treatments work or on the toxic reaction products that may be created. Evidence from ozonation of nicotine suggests that several asthmagens can be found among the byproducts.[26] More research is needed in order to better assess the potential risks associated with the use of high levels of ozone for THS remediation.

4. CONCLUSIONS AND PRIORITIES FOR FURTHER RESEARCH

There is much that remains to be learned about THS, particularly related to the magnitude of the health risk that THS poses to humans. However, based on research carried out over the past few years, by this consortium and others, some conclusions can be made: (1) Tobacco smoke contamination in the form of THS is pervasive. Tobacco-derived substances, including alkaloids and their pyrolysis products, and tobacco specific nitrosamine (TSNA) carcinogens can be measured in homes, other indoor venues such as casinos, and in particulate matter collected outdoors. (2) Laboratory studies have demonstrated that the chemical reactions that take place during the aging of tobacco smoke residues produce secondary organic pollutants, including de novo formation of TSNAs and release of VOCs. Using concentration data and standard methods for the estimation of disability-adjusted life years (DALYs) lost by nonsmokers due to long-term exposure to THS, particulate matter (indexed by $PM_{2.5}$) was estimated to contribute to a majority of the THS-associated disease burden, and acrolein, furan, acrylonitrile, and 1,3-butadiene were the most harmful VOCs among those for which epidemiological and/or toxicological data were available. (3) In studies using *in vitro* systems, THS extracts inhibited cell proliferation, caused a concentration dependent inhibition of cell growth, fragmentation of cells, vesiculation, impaired motility, and caused damage to DNA and mitochondria. *In vitro* evidence for the genotoxicity of THS includes the formation of DNA strand breaks, oxidative damage to DNA, and characterization of NNA adducts with DNA. (4) Toxicology studies using mouse models demonstrated numerous deleterious effects of THS on organ and cellular systems, including delay in wound healing, lung and liver damage, metabolic effects, including elevated triglycerides, increased LDL, decreased HDL defects in insulin metabolism, and permanent changes in peripheral blood immune cell composition. THS-exposed animals showed behavior that was indicative of hyperactivity. Transdermal absorption of the TSNAs NNA and NNK in mice showed the likelihood of this route occurring in humans, a special concern for children who are more likely than adults to be in contact with contaminated surfaces, such as parents' clothes and skin. (5) Biomarkers measured in nonsmokers have documented THS exposure in nonsmokers living in homes of former smokers, nonsmokers staying in hotel rooms with no smoking bans, and subjects visiting casinos after smoking was banned, and infants in a neonatal ICU are exposed to THS toxins. (6) THS is considered a problem in some sectors of the general public, in real estate and motor vehicle sales, and the hotel and motel business.

An overarching goal is to provide a reasonable assessment of the health risks caused by THS exposure. More extensive field studies of THS exposure are needed that incorporate specific markers. A major challenge is to distinguish THS exposure from SHS exposure. For most people exposed to THS, especially adults, it would be hard to rule out SHS exposure outside of their THS-contaminated homes where smoking has ceased to occur, unless a THS-specific biomarker could be found. For infants and very young children, accurate reports from parents might largely rule out SHS exposure. Another approach is laboratory studies in which human subjects are exposed to THS-contaminated articles, such as clothing, with biomarker measurements to measure exposure to assess exposure. A Consortium study is underway in which subjects are being exposed to THS-contaminated clothes, with urinary cotinine and NNAL being used to assess exposure. Although progress has been made, further studies of the chemistry of THS and the extent of environmental contamination are needed to determine which substances are likely to be a health risk. Animal and *in vitro* toxicology studies, especially those with next generation risk assessment approaches, coupled with more extensive human exposure data might be used to develop more extensive models for estimating human health risk.

AUTHOR INFORMATION

Corresponding Author

*Division of Clinical Pharmacology and Experimental Therapeutics, University of California, San Francisco, Box 1220, San Francisco, California 94143-1220, USA. Tel: 415-282-9495. Fax: 415-206-5080. E-mail: peyton.jacob@ucsf.edu.

ORCID

Peyton Jacob III: 0000-0003-3366-2775

Funding

We thank the California Tobacco-Related Disease Research Program (TRDRP) for support of most of the studies described in this Perspective: TRDRP Grant 20PT-0184, Thirdhand Tobacco Smoke Exposure and Health Risk Assessment (Phase-1); TRDRP Grant 23PT-0013, Impacts of Thirdhand Smoke on Public Health (Phase-2). Support from the National Institute on Drug Abuse, P30 DA012393, and from the National Center for Research Resources, S10 RR026437, for laboratory resources at the University of California, San Francisco; support from the U.S. Department of Energy, DE-AC02-05CH11231, and from the TRDRP Grant 19CA-0164 for research at Lawrence Berkeley National Laboratory; from the TRDRP Grant 25IP-0023, and from the U.S. Department of Housing and Urban Development, CAHHU0028-15, for research at San Diego State University, is gratefully acknowledged.

Notes

The authors declare the following competing financial interest(s): Dr Benowitz had served as a paid expert witness in litigation against tobacco companies.

Biographies

Peyton Jacob, III is a Research Chemist in the Division of Clinical Pharmacology at the University of California, San Francisco. His expertise is in the areas of organic and analytical chemistry, in particular chromatography–mass spectrometry methods. He has carried out research on the pharmacology and toxicology of tobacco for over 30 years, including the development of environmental tracers and biomarkers of exposure to carcinogens and other toxic substances, the development of methods for quantitative analysis organic small molecules, and synthesis of stable isotope-labeled tobacco alkaloids used for metabolic studies in humans. His laboratory provides a resource to numerous investigators throughout the US and abroad, for tobacco biomarker analyses in support of collaborative projects.

DOI: 10.1021/acs.chemrestox.6b00343
Chem. Res. Toxicol. XXXX, XXX, XXX–XXX

Neal Benowitz, M.D. is Professor of Medicine and Bioengineering & Therapeutic Sciences and Chief of the Division of Clinical Pharmacology at the University of California San Francisco. He serves as the PI of the California Consortium on Thirdhand Smoke. Dr. Benowitz's research includes studies of biomarkers of exposure and health effects of tobacco products and secondhand smoke.

Hugo Destaillats, Ph.D., is a Staff Scientist and Deputy Leader of the Indoor Environment Group at the Lawrence Berkeley National Laboratory. He holds a Ph.D. in Chemistry from the University of Buenos Aires, Argentina. His research focus is environmental chemistry of the built environment. In particular, Dr. Destaillats has investigated the sources, fate, and transport of indoor pollutants, including those associated with secondhand and thirdhand smoke and electronic cigarettes. He has also developed and characterized novel air cleaning and remediation technologies.

Lara A. Gundel, Ph.D., is a Staff Scientist in the Indoor Environment Group at Lawrence Berkeley National Laboratory. She also serves as the Co−PI and Science Coordinator for the California Consortium on Thirdhand Smoke. Her research focuses on heterogeneous physical chemistry that affects the dynamic behavior of toxicants indoors, particularly secondhand smoke and thirdhand smoke. She spearheaded the groundbreaking measurements of surface-bound nicotine that laid the foundation for LBNL researchers to quantify and model the sorption and desorption behavior of labile organic compounds in SHS as it ages to THS. With Dr. Destaillats she led the pioneering study that showed that adsorbed nicotine reacts with nitrous acid, a common combustion byproduct indoors, to form carcinogenic tobacco specific nitrosamines. Her publications are approximately evenly distributed among the fields of atmospheric science, complex mixtures of toxicants in indoor and outdoor settings, and new instrumentation.

Bo Hang, Ph.D., is a Staff Scientist at the Lawrence Berkeley National Laboratory. He has been working in the field of DNA damage and repair and their roles in environmental mutagenesis and carcinogenesis for more than 25 years. His recent interest is in understanding the biological effects of exposure to thirdhand smoke (THS) and related mechanisms using NexGen risk assessment approaches, including delineating THS genotoxic effects using *in vitro* assays, identifying novel biomarkers of THS exposure such as DNA adducts, and investigating THS impact on health in mouse models and human cohort studies. Dr. Hang is a member of the CA Consortium on Thirdhand Smoke Exposure and Health Effects.

Manuela Martins-Green, Ph.D., is Professor of Cell Biology in the Department of Cell Biology and Neuroscience at the University of California, Riverside. Professor Martins-Green came to the US from Portugal on a Fulbright Fellowship and received a Ph.D. in Zoology with emphasis in Developmental Biology from the University of California, Davis in December 1987. She held a postdoctoral fellowship at the Lawrence Berkeley National Laboratory, a National Research Service Award from NIH, and was Adjunct Assistant Professor at Rockefeller University before joining the UC Riverside faculty in 1993. She is an internationally recognized researcher in the field of response to injury and wound healing. In the past decade, she has been working on understanding how the body responds to injury caused by toxins from tobacco. Recently, her research has concentrated on the effects of toxins from thirdhand smoke (THS) and has shown that mice exposed to THS under conditions that mimic exposure of humans have numerous health problems.

Georg E. Matt, Ph.D., is Professor and Chair of the Department of Psychology, College of Sciences at San Diego State University. His research concerns identifying loopholes in the protection of nonsmokers from exposure to tobacco smoke toxicants. This includes investigating thirdhand smoke pollution and exposure in actual field settings, including, homes, cars, hospitality industry, multiunit housing, and pediatric care. A goal is to develop and evaluate specific measures and general policies to protect nonsmokers from secondhand and thirdhand smoke.

Penelope, J.E. Quintana, Ph.D., received her Ph.D. in Environmental Health Sciences from the University of California, Berkeley and her M.P.H. in Occupational and Environmental Health from San Diego State University (SDSU). She is currently a Professor in the division of Environmental Health at the Graduate School of Public Health at SDSU. She has a research focus on exposures to children and vulnerable populations at the US-Mexico border. She has assessed children's exposure to toxicants in house dust and on surfaces, especially residual tobacco toxicants remaining after smoking has taken place, known as third-hand smoke. She has studied exposure to traffic pollutants as an environmental justice issue at the US−Mexico border, including exposures to pedestrians waiting in lines to cross the US−Mexico border. She is a Scientific Guidance Panel member for the California Environmental Contaminant Biomonitoring Program.

Jonathan M. Samet, M.D., M.S., is Distinguished Professor and Flora L. Thornton Chair of the Department of Preventive Medicine of the University of Southern California. His research has addressed the health risks of active and passive smoking, air pollution, and ionizing radiation. He has also been engaged in activities related to risk assessment and policy.

Suzaynn Schick, Ph.D. is an environmental scientist who studies the health effects of air pollutants. She received her Ph.D. in Biomedical Sciences from the University of California, San Francisco in 2001. She published some of the first data showing that the respiratory toxicity of secondhand smoke is greater than that of the smoke that smokers inhale and that the chemical compounds in secondhand smoke can react to create new, potentially more carcinogenic compounds. She has shown that the majority of the particulate material, nicotine, tobacco-specific nitrosamines, and polycyclic aromatic hydrocarbons in secondhand smoke deposit on indoor surfaces before they can be removed by ventilation. Her lab is a Core for the California Thirdhand Smoke Consortium and produces standardized thirdhand smoke samples for research in laboratories around the world. She studies the cardiovascular and respiratory effects of exposure to secondhand cigarette smoke, thirdhand cigarette smoke, and wood smoke in human subjects. Her clinical research has shown that very short exposures to secondhand smoke cause vascular dysfunction and nasal congestion.

Prue Talbot, Ph.D., is a Professor of Cell Biology and the Director of the UCR Stem Cell Center and Core and the Inland Empire Stem Cell Consortium. Her current position is in the Department of Cell Biology & Neuroscience at the University of California, Riverside, CA. Her laboratory studies the effect of tobacco products/residues, including thirdhand smoke and electronic cigarettes, on human health. Dr. Talbot uses human embryonic stem cells to determine how in utero exposure to nicotine and other chemicals in tobacco products/residues alters prenatal development. Her laboratory also uses a variety of *in vitro* assays to screen chemicals in tobacco products/residues for cytotoxicity and genotoxicity. Her overall goal is to contribute to a better understanding of how thirdhand smoke and electronic cigarettes affect adult and neonatal health.

Noel J. Aquilina, Ph.D., holds a Senior Lecturer position within the Department of Geosciences at the University of Malta, Malta. His Ph.D. at the University of Birmingham, UK focused on personal exposure and microenvironment measurements of VOC and PAH and the development of personal exposure models of the same air toxics.

DOI: 10.1021/acs.chemrestox.6b00343
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

His research interests include improving sampling protocols of airborne tobacco-related carcinogens and contributing to the understanding of their atmospheric behavior in indoor and outdoor microenvironments.

Melbourne Hovell, Ph.D., MPH is Professor in the Graduate School of Public Health, San Diego State University and Director of the Center for Behavioral Epidemiology and Community Health. He has completed 100s of studies and reports, including the first Second Hand Smoke exposure trial demonstrating that children with asthma could be protected from their parents' smoke. This study launched a program of second hand smoke studies and resulted in the identification of unusual patterns of child cotinine assays. This led to the discovery of Thirdhand Smoke with leadership from Georg Matt, Neal Benowitz, and Peyton Jacobs. His current research is focused on protecting children from both second- and thirdhand smoke.

Jian-Hua Mao, Ph.D. is a Geneticist Career Staff Scientist in Lawrence Berkeley National Laboratory. He received his Ph.D. at Department of Radiation Oncology, University of Glasgow, UK, and completed his postdoctoral training at Department of Medical Oncology, the University of Glasgow. Jian-Hua Mao is known for innovative studies on identifying the combinations of genes and their functional polymorphisms that affect the susceptibility to tumor development after environmental exposure, discovering genetic alterations in tumors using recently developed high throughput technologies, such as CGH microarray, SNP microarray, gene expression microarray, and next generation sequencing, and studying the functional and mechanistic role of new discovered genes in tumor development using genetic engineering mice. Dr. Mao has authored more than 120 peer-reviewed publications.

Todd Whitehead, Ph.D., holds an Assistant Researcher position in the School of Public Health at the University of California, Berkeley. His Ph.D. at UC Berkeley focused on assessing children's exposure to indoor contaminants, including constituents of thirdhand smoke. His research interests involve improving exposure science to identify causes of childhood cancer and reducing its incidence.

■ ACKNOWLEDGMENTS

Many colleagues, too numerous to list here, made important contributions to the research described in this Perspective. Their contributions are documented in cited publications. We thank Dr. Anwer Mujeeb of the State of California Tobacco-Related Disease Research Program, Program Officer for the California Consortium on Thirdhand Smoke, who has offered invaluable guidance and support to the Consortium over many years, and Tyson Douglass for editorial assistance.

■ ABBREVIATIONS

B[a]P, benzo[*a*]pyrene; DALYs, disability-adjusted life years; HDL, high-density lipoprotein; HONO, LC-MS/MS, liquid chromatography−tandem mass spectrometry; nitrous acid; *iso*-NNAC, 4-(methylnitrosamino)-4-(3-pyridyl)butyric acid; *iso*-NNAL, 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol; LDL, low-density lipoprotein; NNA, 4-(methylnitrosamino)-4-(3-pyridyl)butanal; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N*′-nitrosonornicotine; NRU, neutral red uptake; PAHs, polycyclic aromatic hydrocarbons; PM, particulate matter; PM2.5, fine particulate matter; ROS, reactive oxygen species; SHS, secondhand smoke; SIMH, stress-induced mitochondrial hyperfusion; THS, thirdhand smoke; TSNA, tobacco-specific nitrosamine; VOCs, volatile organic compounds

■ REFERENCES

(1) Winickoff, J. P., Friebely, J., Tanski, S. E., Sherrod, C., Matt, G. E., Hovell, M. F., and McMillen, R. C. (2009) Beliefs about the health effects of "thirdhand" smoke and home smoking bans. *Pediatrics 123*, e74−9.

(2) Matt, G. E., Quintana, P. J., Destaillats, H., Gundel, L. A., Sleiman, M., Singer, B. C., Jacob, P., Benowitz, N., Winickoff, J. P., Rehan, V., Talbot, P., Schick, S., Samet, J., Wang, Y., Hang, B., Martins-Green, M., Pankow, J. F., and Hovell, M. F. (2011) Thirdhand tobacco smoke: emerging evidence and arguments for a multidisciplinary research agenda. *Environ. Health Perspect 119*, 1218−26.

(3) U.S. Department of Health and Human Services (2014) *The Health Consequences of Smoking: 50 Years of Progress. A Report of the Surgeon General*, Office of the Surgeon General, Washington, DC, http://www.surgeongeneral.gov/library/reports/50-years-of-progress/ .

(4) Schick, S. F., and Glantz, S. (2007) Concentrations of the carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in side-stream cigarette smoke increase after release into indoor air: results from unpublished tobacco industry research. *Cancer Epidemiol., Biomarkers Prev. 16*, 1547−53.

(5) Sleiman, M., Gundel, L. A., Pankow, J. F., Jacob, P., 3rd, Singer, B. C., and Destaillats, H. (2010) 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. 107*, 6576−81.

(6) Petrick, L. M., Svidovsky, A., and Dubowski, Y. (2011) Thirdhand Smoke: Heterogeneous Oxidation of Nicotine and Secondary Aerosol Formation in the Indoor Environment. *Environ. Sci. Technol. 45*, 328−333.

(7) Matt, G. E., Quintana, P. J. E., Hovell, M. F., Bernert, J. T., Song, S., Novianti, N., Juarez, T., Floro, J., Gehrman, C., Garcia, M., and Larson, S. (2004) Households contaminated by environmental tobacco smoke: sources of infant exposures. *Tobacco Control 13*, 29−37.

(8) Sundell, J., Levin, H., Nazaroff, W. W., Cain, W. S., Fisk, W. J., Grimsrud, D. T., Gyntelberg, F., Li, Y., Persily, A. K., Pickering, A. C., Samet, J. M., Spengler, J. D., Taylor, S. T., and Weschler, C. J. (2011) Ventilation rates and health: multidisciplinary review of the scientific literature. *Indoor Air 21*, 191−204.

(9) Roffo, A. H. (1939) Krebserzeugendes Benzpyren, gewonnen aus Tabakteer. *J. Cancer Res. Clin. Oncol. 49*, 588−597.

(10) Ochsner, A., and DeBakey, M. (1999) Primary Pulmonary Malignancy: Treatment by Total Pneumonectomy: Analysis of 79 Collected Cases and Presentation of 7 Personal Cases. *Ochsner J. 1*, 109−125.

(11) Muller, F. H. (1940) Tabakmissbrauch und Lungencarcinom. *J. Cancer Res. Clin. Oncol. 49*, 57−85.

(12) US-DHHS (1964) *Smoking and Health: Report of the Advisory Committee to the Surgeon General of the Public Health Service*, Office of the Surgeon General, Washington, DC.

(13) US-DHHS (1986) *The Health Consequences of Involuntary Smoking: A Report to the Surgeon General, United States Public Health Service*, Office of the Surgeon General, Washington, DC.

(14) Hein, H. O., Suadicani, P., Skov, P., and Gyntelberg, F. (1991) Indoor dust exposure: an unnoticed aspect of involuntary smoking. *Arch. Environ. Health 46*, 98−101.

(15) Matt, G. E., Quintana, P. J., Zakarian, J. M., Fortmann, A. L., Chatfield, D. A., Hoh, E., Uribe, A. M., and Hovell, M. F. (2011) When smokers move out and non-smokers move in: residential thirdhand smoke pollution and exposure. *Tob Control 20*, e1.

(16) Fortmann, A. L., Romero, R. A., Sklar, M., Pham, V., Zakarian, J., Quintana, P. J., Chatfield, D., and Matt, G. E. (2010) Residual tobacco smoke in used cars: futile efforts and persistent pollutants. *Nicotine Tob. Res. 12*, 1029−36.

DOI: 10.1021/acs.chemrestox.6b00343
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

(17) Matt, G. E., Quintana, P. J., Hovell, M. F., Chatfield, D., Ma, D. S., Romero, R., and Uribe, A. (2008) Residual tobacco smoke pollution in used cars for sale: air, dust, and surfaces. *Nicotine Tob. Res. 10*, 1467−75.

(18) ASHRAE (2013) *Position Document on Environmental Tobacco Smoke*, American Society of Heating, Refrigerating and Air-Conditioning Engineers, Atlanta, GA.

(19) Weschler, C. J. (2000) Ozone in indoor environments: concentration and chemistry. *Indoor Air 10*, 269−88.

(20) Weschler, C. J. (2006) Ozone's impact on public health: contributions from indoor exposures to ozone and products of ozone-initiated chemistry. *Environ. Health Perspect 114*, 1489−96.

(21) Weschler, C. J., and Shields, H. C. (1997) Measurements of the Hydroxyl Radical in a Manipulated but Realistic Indoor Environment. *Environ. Sci. Technol. 31*, 3719−3722.

(22) Gómez Alvarez, E., Amedro, D., Afif, C., Gligorovski, S., Schoemaecker, C., Fittschen, C., Doussin, J.-F., and Wortham, H. (2013) Unexpectedly high indoor hydroxyl radical concentrations associated with nitrous acid. *Proc. Natl. Acad. Sci. U. S. A. 110*, 13294−13299.

(23) Gómez Alvarez, E., Sörgel, M., Gligorovski, S., Bassil, S., Bartolomei, V., Coulomb, B., Zetzsch, C., and Wortham, H. (2014) Light-induced nitrous acid (HONO) production from NO2 heterogeneous reactions on household chemicals. *Atmos. Environ. 95*, 391−399.

(24) Singer, B. C., Hodgson, A. T., and Nazaroff, W. W. (2003) Gas-phase organics in environmental tobacco smoke: 2. Exposure-relevant emission factors and indirect exposures from habitual smoking. *Atmos. Environ. 37* (39−40), 5551−5561.

(25) Singer, B. C., Revzan, K. L., Hotchi, T., Hodgson, A. T., and Brown, N. J. (2004) Sorption of organic gases in a furnished room. *Atmos. Environ. 38*, 2483−2494.

(26) Sleiman, M., Destaillats, H., Smith, J. D., Liu, C.-L., Ahmed, M., Wilson, K. R., and Gundel, L. A. (2010) Secondary organic aerosol formation from ozone-initiated reactions with nicotine and second-hand tobacco smoke. *Atmos. Environ. 44*, 4191−4198.

(27) Sleiman, M., Destaillats, H., and Gundel, L. A. (2013) Solid-phase supported profluorescent nitroxide probe for the determination of aerosol-borne reactive oxygen species. *Talanta 116*, 1033−9.

(28) Destaillats, H., Singer, B. C., Lee, S. K., and Gundel, L. A. (2006) Effect of Ozone on Nicotine Desorption from Model Surfaces: Evidence for Heterogeneous Chemistry. *Environ. Sci. Technol. 40*, 1799−1805.

(29) Hecht, S. S., Chen, C.-H. B., Ornaf, R. M., Jacobs, E., Adams, J. D., and Hoffmann, D. (1978) Chemical studies on tobacco smoke. 52. Reaction of nicotine and sodium nitrite: formation of nitrosamines and fragmentation of the pyrrolidine ring. *J. Org. Chem. 43*, 72−76.

(30) Pitts, J. N., Grosjean, D., Van Cauwenberghe, K., Schmid, J. P., and Fitz, D. R. (1978) Photooxidation of aliphatic amines under simulated atmospheric conditions: formation of nitrosamines, nitr-amines, amides, and photochemical oxidant. *Environ. Sci. Technol. 12*, 946−953.

(31) Sleiman, M., Logue, J. M., Luo, W., Pankow, J. F., Gundel, L. A., and Destaillats, H. (2014) Inhalable constituents of thirdhand tobacco smoke: chemical characterization and health impact considerations. *Environ. Sci. Technol. 48*, 13093−101.

(32) Tang, H., Richards, G., Benner, C. L., Tuominen, J. P., Lee, M. L., Lewis, E. A., Hansen, L. D., and Eatough, D. J. (1990) Solanesol: a tracer for environmental tobacco smoke particles. *Environ. Sci. Technol. 24*, 848−852.

(33) Daisey, J. M. (1999) Tracers for assessing exposure to environmental tobacco smoke: what are they tracing? *Environ. Health Perspect 107* (Suppl 2), 319−27.

(34) Douce, D. S., Clench, M. R., and Frost, B. (2001) Variations in the estimation of the contribution of environmental tobacco smoke (ETS) to respirable indoor air particulates obtained by the use of different analytical methods. *J. Environ. Monit. 3*, 295−301.

(35) Jacob, P., 3r, Goniewicz, M. L., Havel, C. M., Schick, S. F., and Benowitz, N. L. (2013) Nicotelline: a proposed biomarker and environmental tracer for particulate matter derived from tobacco smoke. *Chem. Res. Toxicol. 26*, 1615−31.

(36) Ogden, M. W., and Richardson, J. D. (1998) Effect of lighting and storage conditions on the stability of ultraviolet particulate matter, flourescent particulate matter, and solanesol. *Tob. Sci. 42*, 10−15.

(37) Tucker, S. P., and Pretty, J. R. (2005) Identification of oxidation products of solanesol produced during air sampling for tobacco smoke by electrospray mass spectrometry and HPLC. *Analyst 130*, 1414−1424.

(38) Rogge, W. F., Hildemann, L. M., Mazurek, M. A., Cass, G. R., and Simoneit, B. R. T. (1994) Sources of Fine Organic Aerosol. 6. Cigaret Smoke in the Urban Atmosphere. *Environ. Sci. Technol. 28*, 1375−1388.

(39) Radulovic, N. S., and Blagojevic, P. D. (2012) Volatile secondary metabolites of Micromeria dalmatica Benth. (Lamiaceae): biosynthet-ical and chemotaxonomical aspects. *Chem. Biodiversity 9*, 1303−19.

(40) Audino, M., Grice, K., Alexander, R., Boreham, C. J., and Kagi, R. I. (2001) Unusual distribution of monomethylalkanes in Botryococcus braunii−rich samples: origin and significance. *Geochim. Cosmochim. Acta 65*, 1995−2006.

(41) Hammond, S. K., Leaderer, B. P., Roche, A. C., and Schenker, M. (1987) Characterization of Contaminant Emissions from Indoor Sources Collection and analysis of Nicotine as a marker for environmental tobacco smoke. *Atmos. Environ. 21*, 457−462.

(42) Repace, J., Al-Delaimy, W. K., and Bernert, J. T. (2006) Correlating atmospheric and biological markers in studies of secondhand tobacco smoke exposure and dose in children and adults. *J. Occup. Environ. Med. 48*, 181−94.

(43) Pankow, J. F., Mader, B. T., Isabelle, L. M., Luo, W., Pavlick, A., and Liang, C. (1997) Conversion of Nicotine in Tobacco Smoke to Its Volatile and Available Free-Base Form through the Action of Gaseous Ammonia. *Environ. Sci. Technol. 31*, 2428−2433.

(44) Loefroth, G. (1995) Phase Distribution of Nicotine in Real Environments As Determined by Two Sampling Methods. *Environ. Sci. Technol. 29*, 975−978.

(45) Apelberg, B. J., Hepp, L. M., Avila-Tang, E., Gundel, L., Hammond, S. K., Hovell, M. F., Hyland, A., Klepeis, N. E., Madsen, C. C., Navas-Acien, A., Repace, J., Samet, J. M., and Breysse, P. N. (2013) Environmental monitoring of secondhand smoke exposure. *Tob Control 22*, 147−55.

(46) Van Loy, M. D., Riley, W. J., Daisey, J. M., and Nazaroff, W. W. (2001) Dynamic Behavior of Semivolatile Organic Compounds in Indoor Air. 2. Nicotine and Phenanthrene with Carpet and Wallboard. *Environ. Sci. Technol. 35*, 560−567.

(47) Sleiman, M., Maddalena, R. L., Gundel, L. A., and Destaillats, H. (2009) Rapid and sensitive gas chromatography ion-trap mass spectrometry method for the determination of tobacco-specific N-nitrosamines in secondhand smoke. *J. Chromatography A 1216*, 7899−7905.

(48) Whitehead, T. P., Havel, C., Metayer, C., Benowitz, N. L., and Jacob, P., 3rd (2015) Tobacco alkaloids and tobacco-specific nitrosamines in dust from homes of smokeless tobacco users, active smokers, and nontobacco users. *Chem. Res. Toxicol. 28*, 1007−14.

(49) Jacob, P., III, Havel, C., Yu, L., Adhami, N., Flores, C., Martins-Green, M., and Benowitz, N. (2014) *Determination of Two Metabolites of the Tobacco-Specific Nitrosamine NNA (4-(methylnitrosamino)-4-(3-pyridyl)butanal) in Mouse Urine. Application to a Dermal Absorption Study in Mice*, Presented at the 20th Annual Meeting of the Society for Research on Nicotine and Tobacco (SRNT), February 5−8, Seattle WA, USA.

(50) Jacob, P., 3rd, Havel, C., Lee, D. H., Yu, L., Eisner, M. D., and Benowitz, N. L. (2008) Subpicogram per milliliter determination of the tobacco-specific carcinogen metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol in human urine using liquid chromatography-tandem mass spectrometry. *Anal. Chem. 80*, 8115−21.

(51) Benowitz, N. L. (1996) Cotinine as a biomarker of environmental tobacco smoke exposure. *Epidemiol Rev. 18*, 188−204.

(52) Hecht, S. S. (1999) Tobacco smoke carcinogens and lung cancer. *J. Natl. Cancer Inst 91*, 1194−210.

DOI: 10.1021/acs.chemrestox.6b00343
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

(53) Bernert, J. T., Jacob, P., 3rd, Holiday, D. B., Benowitz, N. L., Sosnoff, C. S., Doig, M. V., Feyerabend, C., Aldous, K. M., Sharifi, M., Kellogg, M. D., and Langman, L. J. (2009) Interlaboratory comparability of serum cotinine measurements at smoker and nonsmoker concentration levels: a round-robin study. *Nicotine Tob. Res. 11*, 1458−66.

(54) Jacob, P., III, Yu, L., Duan, M., Ramos, L., Yturralde, O., and Benowitz, N. L. (2011) 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: Anal. Technol. Biomed. Life Sci. 879*, 267−76.

(55) Benowitz, N., Goniewicz, M. L., Eisner, M. D., Lazcano-Ponce, E., Zielinska-Danch, W., Koszowski, B., Sobczak, A., Havel, C., and Jacob, P., 3rd (2010) Urine cotinine underestimates exposure to the tobacco-derived lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in passive compared with active smokers. *Cancer Epidemiol., Biomarkers Prev. 19*, 2795−800.

(56) Hovell, M. F., Zakarian, J. M., Matt, G. E., Liles, S., Jones, J. A., Hofstetter, C. R., Larson, S. N., and Benowitz, N. L. (2009) Counseling to reduce children's secondhand smoke exposure and help parents quit smoking: A controlled trial. *Nicotine Tob. Res. 11*, 1383−94.

(57) Jacob, P., III, Havel, C., Yu, L., Bahl, V., Talbot, P., Schick, S., Hovell, M., Whitehead, T., and Benowitz, N. (2015) In *Assessing Exposure to Thirdhand Smoke (THS) in Humans: The Need for a Comprehensive Approach*, Presented at "Tobacco Control, Research and Education: Joining Forces to Address New Challenges", California Tobacco-Related Disease Research Program, October 27−29, Sacramento CA, USA, 2015.

(58) Farren, N. J., Ramirez, N., Lee, J. D., Finessi, E., Lewis, A. C., and Hamilton, J. F. (2015) Estimated Exposure Risks from Carcinogenic Nitrosamines in Urban Airborne Particulate Matter. *Environ. Sci. Technol. 49*, 9648−56.

(59) Martins-Green, M., Adhami, N., Frankos, M., Valdez, M., Goodwin, B., Lyubovitsky, J., Dhall, S., Garcia, M., Egiebor, I., Martinez, B., Green, H. W., Havel, C., Yu, L., Liles, S., Matt, G., Destaillats, H., Sleiman, M., Gundel, L. A., Benowitz, N., Jacob, P., 3rd, Hovell, M., Winickoff, J. P., and Curras-Collazo, M. (2014) Cigarette smoke toxins deposited on surfaces: implications for human health. *PLoS One 9*, e86391.

(60) Schick, S. F., Farraro, K. F., Perrino, C., Sleiman, M., van de Vossenberg, G., Trinh, M. P., Hammond, S. K., Jenkins, B. M., and Balmes, J. (2014) 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 23*, 152−9.

(61) Murray, D. M., and Burmaster, D. E. (1995) Residential air exchange rates in the United States: Empirical and estimated parametric distributions by season and climatic region. *Risk Anal 15*, 459−465.

(62) Pandian, M. D., Ott, W. R., and Behar, J. V. (1993) Residential air exchange rates for use in indoor air and exposure modeling studies. *J. Expo Anal. Environ. Epidemiol. 3*, 407−416.

(63) Yamamoto, N., Shendell, D. G., Winer, A. M., and Zhang, J. (2010) Residential air exchange rates in three major US metropolitan areas: results from the Relationship Among Indoor, Outdoor, and Personal Air Study 1999−2001. *Indoor Air 20*, 85−90.

(64) Pandian, M. D., Ott, W. R., and Behar, J. V. (1993) Residential air exchange rates for use in indoor air and exposure modeling studies. *J. Expo Anal. Environ. Epidemiol. 3*, 407−16.

(65) Bahl, V., Jacob, P., 3rd, Havel, C., Schick, S. F., and Talbot, P. (2014) Thirdhand cigarette smoke: factors affecting exposure and remediation. *PLoS One 9*, e108258.

(66) Hang, B., Sarker, A. H., Havel, C., Saha, S., Hazra, T. K., Schick, S., Jacob, P., 3rd, Rehan, V. K., Chenna, A., Sharan, D., Sleiman, M., Destaillats, H., and Gundel, L. A. (2013) Thirdhand smoke causes DNA damage in human cells. *Mutagenesis 28*, 381−91.

(67) Hang, B., Lavarone, A., Havel, C., Jacob, P., III, Villalta, P., Matter, B., Sharan, D., Hang, M., Sleiman, M., Destaillats, H., Gundel, L. A., and Chenna, A. (2014) In *NNA, a Thirdhand Smoke Constituent, Induces DNA Damage in Vitro and in Human Cells*, 247th National Meeting of the American Chemical Society (ACS) with Press Release, Dallas, 2014, American Chemical Society, Washington, DC.

(68) Dhall, S., Alamat, R., Castro, A., Sarker, A. H., Mao, J. H., Chan, A., Hang, B., and Martins-Green, M. (2016) Tobacco toxins deposited on surfaces (third hand smoke) impair wound healing. *Clin. Sci. 130*, 1269−84.

(69) Hang, B. (2010) Formation and Repair of Tobacco Carcinogen-Derived Bulky DNA Adducts. *J. Nucleic Acids 2010*, 1−29.

(70) Hecht, S. S. (2012) Lung carcinogenesis by tobacco smoke. *Int. J. Cancer 131*, 2724−32.

(71) Hecht, S. S. (2008) Progress and challenges in selected areas of tobacco carcinogenesis. *Chem. Res. Toxicol. 21*, 160−71.

(72) Xu, B., Chen, M., Yao, M., Ji, X., Mao, Z., Tang, W., Qiao, S., Schick, S. F., Mao, J. H., Hang, B., and Xia, Y. (2015) Metabolomics reveals metabolic changes in male reproductive cells exposed to thirdhand smoke. *Sci. Rep. 5*, 15512.

(73) Talbot, P. (2008) In vitro assessment of reproductive toxicity of tobacco smoke and its constituents. *Birth Defects Res., Part C 84*, 61−72.

(74) Talbot, P., and Lin, S. (2011) The effect of cigarette smoke on fertilization and pre-implantation development: assessment using animal models, clinical data, and stem cells. *Biol. Res. 44*, 189−94.

(75) Talbot, P., and Lin, S. (2011) Mouse and human embryonic stem cells: can they improve human health by preventing disease? *Curr. Top. Med. Chem. 11*, 1638−52.

(76) Zahedi, A., On, V., Lin, S. C., Bays, B. C., Omaiye, E., Bhanu, B., and Talbot, P. (2016) Evaluating Cell Processes, Quality, and Biomarkers in Pluripotent Stem Cells Using Video Bioinformatics. *PLoS One 11*, e0148642.

(77) US-DHHS (2006) *The Health Consequences of Involuntary Exposure to Tobacco Smoke: A Report of the Surgeon General*, U.S. Department of Health and Human Services, Center for Disease Control and Prevention, Coordinating Center for Health Promotion, National Center for Chronic Disease and Health Promotion, Office on Smoking and Health, Atlanta, GA.

(78) Bahl, V., Shim, H. J., Jacob, P., 3rd, Dias, K., Schick, S. F., and Talbot, P. (2016) Thirdhand smoke: Chemical dynamics, cytotoxicity, and genotoxicity in outdoor and indoor environments. *Toxicol. In Vitro 32*, 220−231.

(79) Bahl, V., Weng, N. J., Schick, S. F., Sleiman, M., Whitehead, J., Ibarra, A., and Talbot, P. (2016) Cytotoxicity of Thirdhand Smoke and Identification of Acrolein as a Volatile Thirdhand Smoke Chemical That Inhibits Cell Proliferation. *Toxicol. Sci. 150*, 234−46.

(80) Bahl, V., Johnson, K., Phandthong, R., Zahedi, A., Schick, S. F., and Talbot, P. (2016) Thirdhand Cigarette Smoke Causes Stress-Induced Mitochondrial Hyperfusion and Alters the Transcriptional Profile of Stem Cells. *Toxicol. Sci. 153*, 55−69.

(81) Figueiro, L. R., Dantas, D. C., Linden, R., and Ziulkoski, A. L. (2016) Thirdhand tobacco smoke: procedures to evaluate cytotoxicity in cell cultures. *Toxicol. Mech. Methods 26*, 355−61.

(82) Mossman, B. T. (1983) In vitro approaches for determining mechanisms of toxicity and carcinogenicity by asbestos in the gastrointestinal and respiratory tracts. *Environ. Health Perspect 53*, 155−61.

(83) Riveles, K., Iv, M., Arey, J., and Talbot, P. (2003) Pyridines in cigarette smoke inhibit hamster oviductal functioning in picomolar doses. *Reprod. Toxicol. 17*, 191−202.

(84) Riveles, K., Roza, R., Arey, J., and Talbot, P. (2004) Pyrazine derivatives in cigarette smoke inhibit hamster oviductal functioning. *Reprod. Biol. Endocrinol. 2*, 23.

(85) Riveles, K., Roza, R., and Talbot, P. (2005) Phenols, quinolines, indoles, benzene, and 2-cyclopenten-1-ones are oviductal toxicants in cigarette smoke. *Toxicol. Sci. 86*, 141−51.

DOI: 10.1021/acs.chemrestox.6b00343
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

(86) Yu, R., Wu, M., Lin, S., and Talbot, P. (2006) Cigarette Smoke Toxicants Alter Growth and Survival of Cultured Mammalian Cells. *Toxicol. Sci. 93*, 82−95.
(87) Ueta, I., Saito, Y., Teraoka, K., Miura, T., and Jinno, K. (2010) Determination of volatile organic compounds for a systematic evaluation of third-hand smoking. *Anal. Sci. 26* (5), 569−74.
(88) van der Bliek, A. M. (2009) Fussy mitochondria fuse in response to stress. *EMBO J. 28*, 1533−4.
(89) Chen, H., Vermulst, M., Wang, Y. E., Chomyn, A., Prolla, T. A., McCaffery, J. M., and Chan, D. C. (2010) Mitochondrial fusion is required for MTDNA stability in skeletal muscle and tolerance of MTDNA mutations. *Cell 141*, 280−9.
(90) Picard, M., Wallace, D. C., and Burelle, Y. (2016) The rise of mitochondria in medicine. *Mitochondrion 30*, 105−116.
(91) Grandjean, P., Bellinger, D., Bergman, A., Cordier, S., Davey-Smith, G., Eskenazi, B., Gee, D., Gray, K., Hanson, M., van den Hazel, P., Heindel, J. J., Heinzow, B., Hertz-Picciotto, I., Hu, H., Huang, T. T., Jensen, T. K., Landrigan, P. J., McMillen, I. C., Murata, K., Ritz, B., Schoeters, G., Skakkebaek, N. E., Skerfving, S., and Weihe, P. (2008) The faroes statement: human health effects of developmental exposure to chemicals in our environment. *Basic Clin. Pharmacol. Toxicol. 102*, 73−5.
(92) Teague-Enterprises Inhalation exposure apparatus, available at: http://www.teague-ent.com/equipment/smoking-machines http://www.teague-ent.com/equipment/smoking-machines (Sep 1, 2016).
(93) NIH The FTC Cigarette Test Method for Determining Tar, Nicotine, and Carbon Monoxide Yields of U.S. Cigarettes. http://cancercontrol.cancer.gov/brp/TCRB/monographs/7/.
(94) Sorensen, L., and Gottrup, F. (2009) Smoking and postsurgical wound healing − an update on mechanism and biological factors. *Adv. Wound Care, 1*.
(95) Cutroneo, K. R., White, S. L., Phan, S. H., and Ehrlich, H. P. (2007) Therapies for bleomycin induced lung fibrosis through regulation of TGF-beta1 induced collagen gene expression. *J. Cell. Physiol. 211*, 585−9.
(96) Azambuja, E., Fleck, J. F., Batista, R. G., and Menna Barreto, S. S. (2005) Bleomycin lung toxicity: who are the patients with increased risk? *Pulm. Pharmacol. Ther. 18*, 363−6.
(97) Kupiainen, H., Kinnula, V. L., Lindqvist, A., Postma, D. S., Boezen, H. M., Laitinen, T., and Kilpelainen, M. (2012) Successful Smoking Cessation in COPD: Association with Comorbidities and Mortality. *Pulm. Med. 2012*, 725024.
(98) Yuan, H., Shyy, J. Y. J., and Martins-Green, M. (2009) Second-hand smoke stimulates lipid accumulation in the liver by modulating AMPK and SREBP-1. *J. Hepatol. 51*, 535−47.
(99) Venn, A., and Britton, J. (2007) Exposure to secondhand smoke and biomarkers of cardiovascular disease risk in never-smoking adults. *Circulation 115*, 990−5.
(100) Barnoya, J., and Glantz, S. A. (2005) Cardiovascular effects of secondhand smoke: nearly as large as smoking. *Circulation 111*, 2684−98.
(101) Raupach, T., Schafer, K., Konstantinides, S., and Andreas, S. (2006) Secondhand smoke as an acute threat for the cardiovascular system: a change in paradigm. *Eur. Heart J. 27*, 386−92.
(102) Anthony, H. M. (1989) Reactive changes in the blood of smokers and the development of arterial diseases and COPD, a review: evidence of associations between changes and subsequent disease with implications for the evaluation of harmful effects of cigarettes, and for susceptibility to the chronic effects of inhaled pollutants. *Rev. Environ. Health 8*, 25−86.
(103) Kisfali, P., Polgar, N., Safrany, E., Sumegi, K., Melegh, B. I., Bene, J., Weber, A., Hetyesy, K., and Melegh, B. (2010) Triglyceride level affecting shared susceptibility genes in metabolic syndrome and coronary artery disease. *Curr. Med. Chem. 17* (30), 3533−41.
(104) Adhami, N., Starck, S. R., Flores, C., and Martins Green, M. (2016) A health threat to bystanders living in the homes of smokers: how smoke toxins deposited on surfaces can cause insulin resistance. *PLoS One 11*, e0149510.
(105) Karim, Z. A., Alshbool, F. Z., Vemana, H. P., Adhami, N., Dhall, S., Espinosa, E. V., Martins Green, M., and Khasawneh, F. T. (2015) Third-hand smoke: impact on hemostasis and thrombogenesis. *J. Cardiovasc. Pharmacol. 66*, 177−182.
(106) Hang, B., et al., submitted for publication.
(107) Matt, G. E., Fortmann, A. L., Quintana, P. J., Zakarian, J. M., Romero, R. A., Chatfield, D. A., Hoh, E., and Hovell, M. F. (2013) Towards smoke-free rental cars: an evaluation of voluntary smoking restrictions in California. *Tob Control 22*, 201−7.
(108) Matt, G. E., Quintana, P. J., Fortmann, A. L., Zakarian, J. M., Galaviz, V. E., Chatfield, D. A., Hoh, E., Hovell, M. F., and Winston, C. (2014) Thirdhand smoke and exposure in California hotels: non-smoking rooms fail to protect non-smoking hotel guests from tobacco smoke exposure. *Tob Control 23*, 264−72.
(109) Kassem, N. O., Daffa, R. M., Liles, S., Jackson, S. R., Kassem, N. O., Younis, M. A., Mehta, S., Chen, M., Jacob, P., 3rd, Carmella, S. G., Chatfield, D. A., Benowitz, N. L., Matt, G. E., Hecht, S. S., and Hovell, M. F. (2014) Children's exposure to secondhand and thirdhand smoke carcinogens and toxicants in homes of hookah smokers. *Nicotine Tob. Res. 16*, 961−75.
(110) Northrup, T. F., Khan, A. M., Jacob, P., Benowitz, N. L., Hoh, E., Hovell, M. F., Matt, G. E., and Stotts, A. L. (2015) Thirdhand smoke contamination in hospital settings: assessing exposure risk for vulnerable paediatric patients. *Tobacco Control*, DOI: 10.1136/tobaccocontrol-2015-052506.
(111) Matt, G. E., Quintana, P., Zakarian, J., Hoh, E., Hovell, M. F., Mahabee-Gittens, M., and Chatfield, D. (2016) When Smokers Quit: Exposure to Nicotine and Carcinogen Persists from Thirdhand Smoke Population [In Press]. *Tobacco Control*, DOI: 10.1136/tobaccocontrol-2016-053119.
(112) Quintana, P. J. E., Matt, G. E., Hoh, E., Zakarian, J., Destaillats, H., Dhaliwal, N., Chowdhury, Z., Watanabe, K., Sleiman, M., Gundel, L. A., Russell, M. L., Theweny, T., Arceo, J., Muniz, M., Flores, V., and Klepeis, N. (2015) *Thirdhand Tobacco Smoke Pollutants in a Casino Measured before and after Smoke-Free Status*. International Society of Exposure Science (ISES) Conference, Henderson, NV, October, 2015. http://www.ises2015.org/.
(113) Quintana, P. J., Matt, G. E., Chatfield, D., Zakarian, J. M., Fortmann, A. L., and Hoh, E. (2013) Wipe sampling for nicotine as a marker of thirdhand tobacco smoke contamination on surfaces in homes, cars, and hotels. *Nicotine Tob. Res. 15*, 1555−63.
(114) Van Loy, M. D., Nazaroff, W. W., and Daisey, J. M. (1998) Nicotine as a Marker for Environmental Tobacco Smoke: Implications of Sorption on Indoor Surface Materials. *J. Air Waste Manage. Assoc. 48*, 959−968.
(115) Thomas, J. L., Hecht, S. S., Luo, X., Ming, X., Ahluwalia, J. S., and Carmella, S. G. (2014) Thirdhand Tobacco Smoke: A Tobacco-Specific Lung Carcinogen on Surfaces in Smokers' Homes. *Nicotine Tob. Res. 16*, 26−32.
(116) Zhang, S., Qiao, S., Chen, M., Xia, Y., Hang, B., and Cheng, S. (2015) An investigation of thirdhand smoke pollution in 3 types of places of Nanjing, 2014. *Zhonghua Yu Fang Yi Xue Za Zhi (Chinese Journal of Preventive Medicine) 49*, 31−5.
(117) Hoh, E., Hunt, R. N., Quintana, P. J., Zakarian, J. M., Chatfield, D. A., Wittry, B. C., Rodriguez, E., and Matt, G. E. (2012) Environmental tobacco smoke as a source of polycyclic aromatic hydrocarbons in settled household dust. *Environ. Sci. Technol. 46*, 4174−83.
(118) Ramirez, N., Ozel, M. Z., Lewis, A. C., Marce, R. M., Borrull, F., and Hamilton, J. F. (2014) Exposure to nitrosamines in thirdhand tobacco smoke increases cancer risk in non-smokers. *Environ. Int. 71*, 139−47.
(119) World-Lung-Foundation, Cigarette Consumption (2015) The Tobacco Atlas, available at http://www.tobaccoatlas.org/topic/cigarette-use-globally/.
(120) IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, World Health Organization (2004) Tobacco smoke and involuntary smoking. *IARC Monogr. Eval. Carcinog. Risks Hum., 83*,

DOI: 10.1021/acs.chemrestox.6b00343
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX

1200, available at http://monographs.iarc.fr/ENG/Monographs/vol83/mono83.pdf.
(121) Cecinato, A., Balducci, C., Romagnoli, P., and Perilli, M. (2012) Airborne psychotropic substances in eight Italian big cities: burdens and behaviours. *Environ. Pollut. 171*, 140−7.
(122) Lopez, M. J., Fernandez, E., Gorini, G., Moshammer, H., Polanska, K., Clancy, L., Dautzenberg, B., Delrieu, A., Invernizzi, G., Munoz, G., Precioso, J., Ruprecht, A., Stansty, P., Hanke, W., and Nebot, M. (2012) Exposure to secondhand smoke in terraces and other outdoor areas of hospitality venues in eight European countries. *PLoS One 7*, e42130.
(123) Sureda, X., Martinez-Sanchez, J. M., Lopez, M. J., Fu, M., Aguero, F., Salto, E., Nebot, M., and Fernandez, E. (2012) Secondhand smoke levels in public building main entrances: outdoor and indoor PM2.5 assessment. *Tobacco Control 21*, 543−8.
(124) Hood, N. E., Ferketich, A. K., Klein, E. G., Pirie, P., and Wewers, M. E. (2014) Associations between self-reported in-home smoking behaviours and surface nicotine concentrations in multiunit subsidised housing. *Tobacco Control 23*, 27−32.
(125) St. Helen, G., Bernert, J. T., Hall, D. B., Sosnoff, C. S., Xia, Y., Balmes, J. R., Vena, J. E., Wang, J. S., Holland, N. T., and Naeher, L. P. (2012) Exposure to secondhand smoke outside of a bar and a restaurant and tobacco exposure biomarkers in nonsmokers. *Environ. Health Perspect 120*, 1010−6.
(126) Bostrom, C. E., Gerde, P., Hanberg, A., Jernstrom, B., Johansson, C., Kyrklund, T., Rannug, A., Tornqvist, M., Victorin, K., and Westerholm, R. (2002) Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. *Environ. Health Perspect 110* (Suppl 3), 451−88.
(127) Giovino, G. A., Mirza, S. A., Samet, J. M., Gupta, P. C., Jarvis, M. J., Bhala, N., Peto, R., Zatonski, W., Hsia, J., Morton, J., Palipudi, K. M., and Asma, S. (2012) Tobacco use in 3 billion individuals from 16 countries: an analysis of nationally representative cross-sectional household surveys. *Lancet 380*, 668−79.
(128) US-FDA Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke: Established List, available at http://www.fda.gov/TobaccoProducts/GuidanceComplianceRegulatoryInformation/ucm297786.htm (Sep 1).
(129) Asotra, K. (2009) In *Thirdhand Smoke Exposure: An Unappreciated Public Health Hazard*, Presented at the International Symposium on Cancer Chemoprevention and Translational Research, School of Life Sciences, Jawaharlal Nehru University, New Delhi, India.
(130) Drehmer, J. E., Ossip, D. J., Rigotti, N. A., Nabi-Burza, E., Woo, H., Wasserman, R. C., Chang, Y., and Winickoff, J. P. (2012) Pediatrician interventions and thirdhand smoke beliefs of parents. *Am. J. Prev. Med. 43*, 533−6.
(131) WHO Children's Health and the Environment, available at http://www.who.int/ceh/capacity/Children_are_not_little_adults.pdf.
(132) US-EPA (2008) *Child-Specific Exposure Factors Handbook (Final Report)*, EPA/600/R-06/096F, U.S. Environmental Protection Agency, Washington, DC.
(133) WHO Summary of Principles for Evaluating Health Risks in Children Associated with Exposure to Chemicals, available at http://www.who.int/ceh/publications/health_risks_exposure_chemicals/en/ .
(134) Huen, K., Bradman, A., Harley, K., Yousefi, P., Boyd Barr, D., Eskenazi, B., and Holland, N. (2012) Organophosphate pesticide levels in blood and urine of women and newborns living in an agricultural community. *Environ. Res. 117*, 8−16.
(135) CDC (2012) Take-home lead exposure among children with relatives employed at a battery recycling facility - Puerto Rico, 2011. Centers for Disease Control and Prevention. *MMWR 61* (47), 967−70.
(136) Jones-Otazo, H. A., Clarke, J. P., Diamond, M. L., Archbold, J. A., Ferguson, G., Harner, T., Richardson, G. M., Ryan, J. J., and Wilford, B. (2005) Is house dust the missing exposure pathway for PBDEs? An analysis of the urban fate and human exposure to PBDEs. *Environ. Sci. Technol. 39*, 5121−30.
(137) Lanphear, B. P., Matte, T. D., Rogers, J., Clickner, R. P., Dietz, B., Bornschein, R. L., Succop, P., Mahaffey, K. R., Dixon, S., Galke, W., Rabinowitz, M., Farfel, M., Rohde, C., Schwartz, J., Ashley, P., and Jacobs, D. E. (1998) The contribution of lead-contaminated house dust and residential soil to children's blood lead levels. A pooled analysis of 12 epidemiologic studies. *Environ. Res. 79*, 51−68.
(138) Quandt, S. A., Arcury, T. A., Rao, P., Snively, B. M., Camann, D. E., Doran, A. M., Yau, A. Y., Hoppin, J. A., and Jackson, D. S. (2004) Agricultural and residential pesticides in wipe samples from farmworker family residences in North Carolina and Virginia. *Environ. Health Perspect 112*, 382−7.
(139) Morgan, M. K., Sheldon, L. S., Croghan, C. W., Jones, P. A., Robertson, G. L., Chuang, J. C., Wilson, N. K., and Lyu, C. W. (2005) Exposures of preschool children to chlorpyrifos and its degradation product 3,5,6-trichloro-2-pyridinol in their everyday environments. *J. Exposure Anal. Environ. Epidemiol. 15*, 297−309.
(140) Samet, J. M., Chanson, D., and Wipfli, H. (2015) The Challenges of Limiting Exposure to THS in Vulnerable Populations. *Curr. Environ. Health Rep 2*, 215−25.

DOI: 10.1021/acs.chemrestox.6b00343
Chem. Res. Toxicol. XXXX, XXX, XXX−XXX