THE TOBACCO-SPECIFIC NITROSAMINES FROM THIRDHAND TOBACCO SMOKE AS A NEW CONCERN TO PUBLIC HEALTH

by

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ABSTRACT

Smoking has long been recognized as a hugely serious public health issue. The effects of secondhand smoke have been attached great attention by health agencies worldwide over the past decades. A new risk of tobacco smoke exposure, thirdhand smoke (THS), has been identified in recent years. Tobacco-specific nitrosamines (TSNAs) are generated by chemical reactions between THS and common air pollutants, which are characterized as a carcinogenic group of substances. Public health has started to consider TSNAs as a new source of environmental pollutant. Even though a small number of studies has begun investigating TSNAs from THS in a variety of contexts and testing its possible health effects, the toxicity of THS and how TSNAs impact on health are still under investigation. The objective of this essay is to review existing evidence on TSNAs and health implication for TSNAs, focusing on how THS differs from secondhand smoke, current evidence regarding the formation of TSNAs and its potential health impacts and addressing possible assessments for THS exposure.

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INTRODUCTION

Smoking has long been recognized as a hugely serious public health issue. In 2012, an estimated 18.1% (42.1 million) of U.S. adults were current cigarette smokers. Of these, 78.4% (33.0 million) smoked every day, and 21.6% (9.1 million) smoked some days [1]. In order to reach the objectives of the *Health People 2020* [2], a significant overall smoking prevalence declined from 20.9% to 18.1% during past 30 years in the United States [1]. Beyond that, the effects of secondhand smoke have been attached great attention by health agencies worldwide over the past decades. The United States and other countries have steadily decreased secondhand smoke exposure over time by implementing smoking ban in workplaces and public areas, including restaurants and bars [3, 4]. The 2010 Morbidity and Mortality Weekly Report on nonsmokers' exposure to secondhand smoke presented that an estimated 88 million nonsmokers were exposed to secondhand smoke in the United States during 2007-2008 [3].

In recent years, a new risk of tobacco smoke exposure, thirdhand smoke (THS), has been identified [5]. It was defined as residual nicotine that remains after someone has stopped smoking, reabsorbs into the gas-phase and reacts with oxidants and other compounds in the environment to yield secondary pollutants [6]. A significant finding from a 2010 study addressed that a carcinogenic group of substances, called tobacco-specific nitrosamines (TSNAs), was formed by chemical reactions between residual nicotine with common ambient pollutants, including nitrous acid (HONO) [7]. The study discovered that TSNAs were formed rapidly and that THS could get more toxic with time [7]. Moreover, THS exposure

results from inhalation, ingestion, or dermal contact of THS contaminants in the air, in dust and on surfaces [8]. Based on the above, THS is a potential health hazard and it may be more toxic than secondhand smoke in some ways. TSNAs will be considered as a new source of environmental pollutant. Public Health has started to generate concern with regard to THS [9]. To date, a small but increasing number of experts have begun investigating TSNAs from THS in a variety of contexts and testing its possible health effects [10]. Scientists put forward different views of THS from various professional backgrounds, and even doubt the definition of THS itself [10]. Most evidence supports the widespread presence of THS in indoor environments. However, due to the magnitude of the problem and the shortage of attention and resources, the toxicity of THS and how TSNAs impact on health are still under investigation [10]. The objective of this essay is to review existing evidences on TSNAs and health implications for TSNAs, focusing on how THS differs from secondhand smoke, current evidence regarding the formation of TSNAs and its potential health impacts, and addressing possible assessments for THS exposure.

THE NEW TERM: THIRDHAND SMOKE

Secondhand smoke (SHS) is smoke from other people's tobacco and breathing it in brings non-smokers many of the same health risks as active smoking [11]. It has been demonstrated that the chemicals resulting in cancer are present in higher concentrations on SHS than in the smoke inhaled by smokers themselves [11]. In the past few years, many of available studies have indicated that inhaling SHS can cause adverse health effects. Most people have awareness that visible SHS pose threats to their health [10]. In 2009, a report on public attitudes about smoke residue which was called "thirdhand smoke (THS)" was published by Dr. Jonathan Winickoff and his colleagues at Massachusetts General Hospital in Boston [4]. Winickoff mentioned in an interview that a new term needed to be generated due to less public awareness of invisible smoke left in the air and the residue left on surfaces. In his study, he found parents from focus groups would try to decrease the invisible smoke by turning on a fan and defined SHS as visible tobacco smoke to distinguish it from THS. However, not everyone agrees with the new term [10]. Some experts hold the view that almost everything left in the air after the cigarette is put out are SHS [10]. From the opinion of Dr. Gundel, who studies chemical reactions in smoke reside: "As an environmental chemist and as someone knowledgeable about how air moves around in buildings and such, the differentiation between SHS and THS is that after someone has stopped smoking it is going to take about three air exchanges to get rid of 99% of the SHS that is in the air. Anything that is left in the environment after that is going to be THS [10]." A common

accepted definition is that THS consists of residual nicotine which accumulates on surfaces (furniture, walls, skin, clothing) and in dust after smoke has been put out, re-emitted into the gas-phase and reacts with other atmospheric species (O_3, nitrous acid (HONO), NO_x) to produce secondary pollutants [6,7,8,9,11,12]. Several studies have illustrated that some gas- and particle- phase THS compounds can persist for extended periods from days to even months in all indoor environments (e.g., walls, floors, carpet, curtains, pillows mattresses, clothes, drapes and even skin and hair) in which tobacco smoke has been produced [8, 13, 14]. It has been recognized that THS creates special risks for nonsmokers who spend time indoors in proximity to polluted surfaces [12].

Although THS and SHS exposure are closely related and co-exist in the early period of THS formation, there are three distinguishing features between THS exposure and SHS exposure [8, 12]. First of all, while SHS exposure comes from the involuntary inhalation of smoke from other people's tobacco, THS exposure results from the involuntary inhalation, ingestion or dermal contact of pollutants present in air, in dust and on surfaces [12]. Second, SHS inhaled is repeated in high levels over short intervals. In contrast, THS exposure takes long periods in cumulative low levels [8]. Third, THS may persist in the indoor environments even for several days or months after tobacco have been smoked after SHS which is removal through ventilation [12].

The 2006 the U.S. Surgeon General concluded that there was no safe level of exposure to tobacco smoke after intervening years of research, both SHS and THS exposure represent unappreciated health hazards [15]. Total tobacco smoke exposure is the cumulative involuntary exposure to tobacco smoke contaminants during and after the time in which

cigarettes are smoked [12]. An expanding body of evidence indicates that indoor environment will be contaminated by tobacco smoke after the visible smoke dissipates [10]. In other words, although smoke has been snuffed out, the risk of tobacco smoke exposure may remain in the absence of further smoking instead of termination [5].

THE NEW CARCINOGENS: TOBACCO-SPECIFIC NITROSAMINES (TSNAs)

After initial releasing tobacco smoke contaminants, it will undergo physical and chemical transformation [8, 12]. Nicotine, as the precursor of carcinogens present in tobacco smoke, is the most abundant organic compound released during smoking [7]. Compared with most other tobacco smoke constituents that showed more moderate sorptive tendency, nicotine can deposit almost entirely on indoor surfaces, such as walls, floors, carpeting, drapes and furniture, and will remain from a few seconds to several weeks or months [12, 14].

A study from Sleiman et al. has found that tobacco-specific nitrosamines (TSNAs) were formed by chemical reactions between residual nicotine with nitrous acid (HONO) [7]. HONO is typically found indoors with gas-burning appliances and heterogeneous conversion of atmospheric nitrogen oxides [7, 12]. Cellulose was used as a model surface of indoor material by researchers at the Lawrence Berkeley National Laboratory [7]. They exposed cellulose substrates to cigarette smoke and then exposed it to a "high but reasonable" concentration of HONO for three hours [14]. Nicotine adsorbed to cellulose showed high reactivity toward HONO through liquid chromatography–tandem mass spectrometry [7, 14]. They discovered the levels of newly formed TSNA's were 10 times higher after HONO exposure [7]. TSNAs are regarded as the most broadly acting and potent carcinogens present in unburned tobacco and tobacco smoke, which consists of 1-(N-methyl-N-nitrosamino)–1–(2-pyridinyl) –4– butanal (NNA), 4-(methylnitrosamino)-1-(3-pyridinyl)-1-butanone (NNK),

and N-nitroso nornicotine (NNN) [7, 12, 14]. Additional tests were carried out to assess the stability of TSNAs dissolved in water-methanol and were subsequently exposed to HONO under the same conditions as above [7]. The results showed that NNK and NNN were more stable than NNA, which was lost in two hours. These findings suggested that TSNAs conducted partial degradation by HONO after their fast initial formation, and then reach steady-state concentrations [7]. These TSNAs are likely associated with indoor surfaces and dust. Thus, more TSNAs are formed when higher levels of smoking accumulate at a regular pace in indoor environment [5]. Both the structures of these compounds and their mechanisms of formation are shown in Figure 1 [7]. Fig.1A presents the main physical-chemical processes involved in the surface-mediated nitrosation of nicotine. Fig.1B is the proposed mechanism for the formation of TSNAs, in which (a), (b) and (c) represent mechanisms for the formation of NNA, NNK and NNN respectively. NO^+ is assumed to be the main reactive species, which removes one electron from the pyrrolidine nitrogen of nicotine, leading to the formation of an unstable cationic intermediate in the first place. Next, a second NO^+ abstracts a hydrogen atom from one of the three α-carbon atoms (a, b, and c) to form an iminium cation, which is then hydrolyzed by sorbed water molecules. Finally, NNA, NNK and NNN are formed by nitrosation of the secondary amines by HONO [7, 12]. It has been shown that when SHS ages and turns into THS, NNK concentrations are enhanced [17]. However, NNA is not reported to be present in freshly emitted tobacco smoke, probably because of its reactivity and low vapor pressure during tobacco pyrolysis [16]. The Sleiman et al. study has shown that NNA was the major TSNA product in THS [7]. In addition to TSNAs, nitrosation of nicotine generated secondary products in the gas phase and on surfaces,

including TSNAs degradation products such as N-nitroso-pyrrolidine (itself a carcinogenic volatile nitrosamine), a surface-bound product formed through C-nitrosation of NNK, and a stable pyrazole compound, resulting from NNA decomposition [18, 19]. The latter compound, pyrazole, was identified as a tracer for the THS products formed by HONO-nicotine chemistry, because it was reported in a higher concentration than the total TSNAs and it did not present in freshly emitted SHS [19].

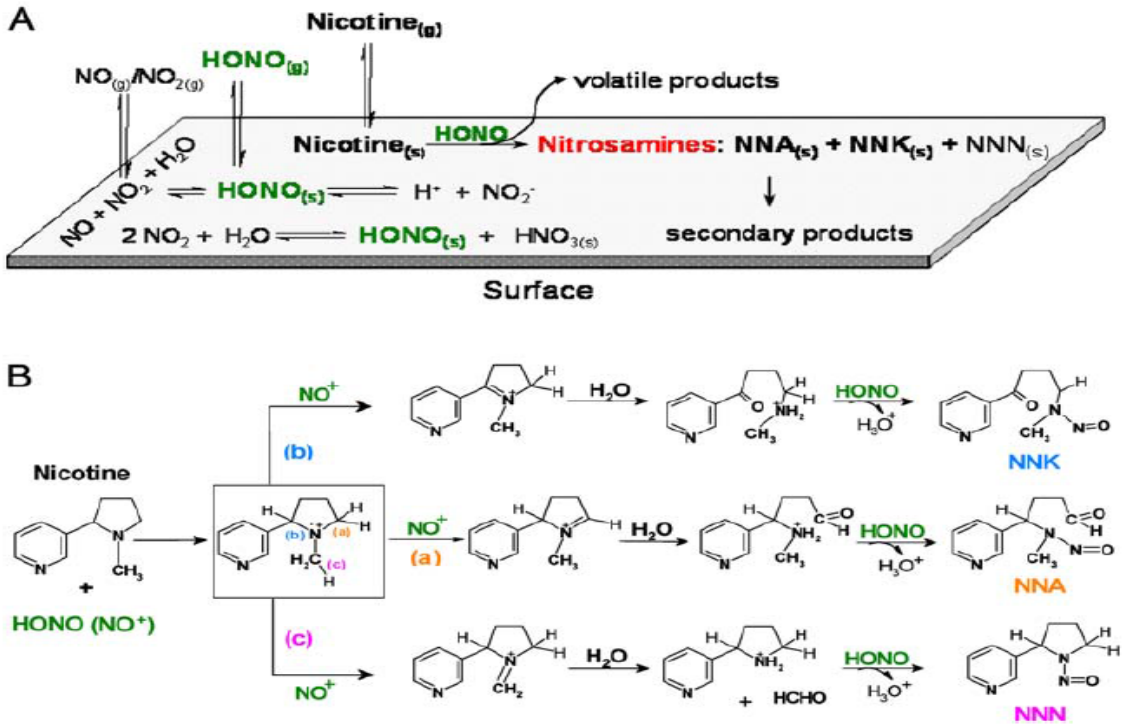

Figure 1. Physical-chemical processes of reactions between the absorbed nicotine and nitrous acid. (A) The three pathways of the formation of $HONO_{(S)}$ and illustration of surface-mediated nitrosation of nicotine. (B) Proposed mechanism for the formation of TSNAs [7].

THE CURRENT HEALTH EFFECTS OF TOBACCO-SPECIFIC NITROSAMINES (TSNAs)

As mentioned earlier, comparing THS with SHS and active smoking, available evidence suggests that exposure to THS involves very different time profiles of exposure, different pollutant concentrations in different media, and different relative contributions of exposure routes [20]. Consequently, it is hard to compare pollutant concentrations between THS and SHS, because health risks of THS may include some of those of SHS and active smoking as well as new ones not yet directly associated with tobacco smoke [12]. For example, air nicotine levels and urine cotinine levels can be markers of SHS when active smoking occurs [20]. However, these two kinds of testing methods are not likely to be the best indicators of THS pollution and other compounds on surface and in dust [12]. Currently, assessing the independent health risks of THS is premature while human exposure to constituents of THS has not been well characterized [12]. In this case, one can consider how some of the known THS components could affect human health. As we know, most studies support carcinogenesis of nicotine. It has adverse effects on the vascular system and may promote inflammation through oxidative stress, as well as it may alter brain and lung development in children [8]. Also, it has been pointed out that nicotine can react with other pollutants to produce new compounds, including TSNAs [8]. On the basis of the formation of TSNAs, it is of concern as a potential hazard of THS. In one study, appreciable levels of surface-bound TSNAs, including NNA and NNK, were detected inside a smoker's vehicle, highlighting their relevance to human exposure and health [7]. The International Agency for Research on

Cancer has identified NNK and NNN as potent carcinogens, especially NNK which is much stronger [21]. NNA carcinogenicity has not been reported, but its mutagenic activity is similar to that of NNN [22].

Lung Carcinogen

It is known that NNK exists in the mainstream and side-stream smoke of all tobacco products [21, 23]. Mainstream smoke levels typically range from 10 to 200 ng per cigarette, while amounts in side-stream smoke are from 50 to 100 ng per cigarette [21, 23, 24]. NNK has been reported as a potent lung carcinogen with a cancer potency of 49 $kgmg^{-1}d^{-1}$ [25]. Hecht et al. tested NNK in laboratory animals, inducing mainly adenocarcinoma of the lung in all species [26]. In some cases of F-344 rats, a majority of low doses of NNK (1 ppm in the drinking water or 0.1 mg/kg by subcutaneous injection) have resulted in incidences of lung tumors [17]. Similar results have been obtained in mice and Syrian golden hamsters [26]. In one study, Sencar mice were treated with NNK by topical application to the skin and lung tumors also have been observed [27]. Thus, NNK may cause lung cancer in humans, but more evidence is required. A recent study was conducted using liquid chromatography-tandem mass spectrometry (LC-MS/MS) to analyze surface dust samples in both the homes of smokers and nonsmokers for the powerful lung carcinogen NNK. The study demonstrated that the differences in occurrence and levels of NNK in the homes of smokers and nonsmokers were significant ($p < .0001$). It was concluded that the powerful tobacco-specific lung carcinogen NNK was present on surfaces in most homes occupied by smokers. The result indicates that the existence of NNK in homes that have been occupied by

smokers would pose threat to adults and children, who may come into contact with surfaces in the home during their activities [17]. To some extent, this may increase the risk of lung cancer after a long time, especially for young children, whose lungs in the process of development are more susceptible to even the lowest level of toxins [8].

Genotoxicity

One of the critical mechanisms responsible for various types of cancer caused by active smoking and SHS exposure is genotoxicity [9]. A small number of studies have been designed to estimate the genotoxic potential of THS, as TSNAs are known to be potential mutagens and carcinogens [9].

A great number of studies have shown that NNK presents its toxic, mutagenic and carcinogenic impact in the case of metabolic activation [26, 28]. Alpha-hydroxylation of NNK can cause the formation of methylating and pyridyloxobutylating agents, and mutations resulting from methyl and pyridyloxobutyl adducts lead to genotoxicity of NNK [26, 29]. One study discovered that NNK, as a photosensitizer, might be activated by UVA to produce NO and other oxidative and alkylative intermediates leading to the formation of 8-oxodG and O^6meG in DNA, which might lead to DNA strand breaks, oxidative and alkylative DNA base damage and mutation. In the study, 8-oxodG and O^6meG accounted for the growing frequency and percentage of GC to CG transversions when DNA reacted with NNK and UVA [30]. This phenomenon has been previously found in tumors from mice treated with pyridoxyloxobutylating agent [30].

Although NNA exists in freshly emitted SHS, it is the predominant TSNA in THS [12].

In the presence of HONO, NNA levels are increased [9]. One report used a 6-thioguanine mutagenicity assay to test mutagenic potential of NNA; it showed that NNA had ability to induce concentration-dependent increases in mutant fractions [31]. To investigate the NNA toxicity, Hang et al. conducted *in vitro* assays to estimate the genotoxicity of NNA in exposed human cell lines. To simulate acute and chronic THS, chromatography paper strips were exposed to tobacco smoke in a closed chamber for various lengths of time. Researchers extracted the compounds on the paper strips and then used them to treat human liver cell lines. The role of cultured cells was to probe for DNA breaks and oxidation, which are strong indicators of genotoxicity. The study discovered cell cultures exposed to NNA showed significantly higher levels of DNA strand breaks and oxidative DNA damage in the alkaline Comet assay of human HepG2 cells exposed to acute or chronic THS for 24h. The Comet assay was used to test genotoxic of NNK in several studies as well. Therefore, NNA was confirmed to be as genotoxic as the carcinogen NNK at nanomolar level exposure. Moreover, the concentrations of NNA in acute and chronic THS exposure are around 2 nM while the acute and chronic concentration of NNK were 4.3 nM and 34 nM respectively. The concentrations of NNA and NNK in THS samples exhibited increased levels of DNA strand breaks [9].

IMPLICATIONS FOR TOBACCO-SPECIFIC NITROSAMINES (TSNAs) EXPOSURE

TSNAs are concerned to be the main hazards of THS. NNK is a lung carcinogen, as well as it has been shown to induce mutations, DNA strand breaks, and oxidative damage under sunlight, in the absence of metabolic activation [7]. NNA carcinogenicity has been reported and that it is able to induce DNA strand breaks and oxidative DNA damage [30]. Due to assess nonsmokers' intake of NNA, it may detect its likely metabolite 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol (iso-NNAL) which is similar to 4-(methylnitrosamino)-1-(3-pyridel)-1-butanol (NNAL) as a biomarker formed by metabolic reduction of NNK [32]. NNAL has been found in urine and blood of people exposed to NNK [21]. A study was attempted to detect iso-NNAL in the urine samples, but it was not successful [13, 33]. The structures of these compounds discussed are shown in Figure 2 [16].

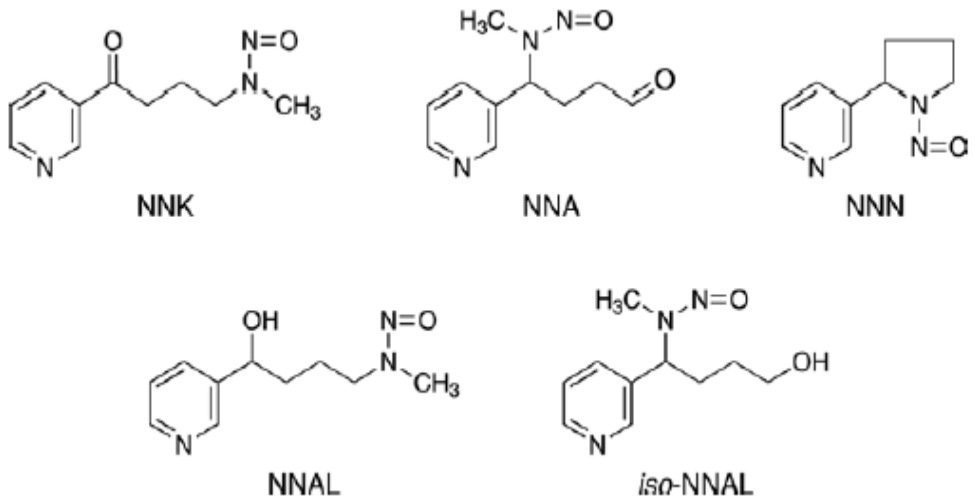

Figure 2. The structures of NNK, NNA, NNN, NNAL and iso-NNAL [16]. NNK, NNA, and NNN consist of TSNAs. NNAL is found in urine and blood of people exposed

to NNK [21]. iso-NNAL has been suggested as a biomarker for NNA exposure but yet to be detected in human urine or blood [13, 33].

The surface-formed TSNAs may enter the body through a variety of potentially important exposure routs [7]. Given low vapor pressure, gas-phase TSNAs seem like to be negligible by direct inhalation [7]. On the contrary, dermal contact with surfaces polluted by TSNAs (walls, doors, carpets, upholstery, pillows, curtains, mattresses, clothes, skin, and hair) and inhalation and ingestion of TSNAs in dust are likely to consist of the main exposure pathways [7, 8, 12, 14]. Table 1 is shown the concentrations between nicotine and TSNAs in households, vehicle surfaces, and human skin [7].

Table 1. The concentrations of nicotine and TSNAs in households, vehicle surfaces, and human skin. NNA and NNK were calculated on the basis of 0.35% and 0.05% of nicotine respectively, expressed in mass units [7].

	Households		Vehicles		Skin	Clothes
	Furniture	Dust	Dashboard	Dust		
[Nicotine] (μgm^{-2})	11-73	0.89-4.43	5.0-8.6	11.6-19.5	0.63-63	1000
[NNA] (μgm^{-2})	37-206	3-15	17-30	41-68	2.2-220	3500
[NNK] (μgm^{-2})	5.3-36.5	0.44-2.2	2.5-4.3	6.1-9.7	0.31-31	500

From Table 1, in the presence of HONO, TSNAs were produced in different levels through different surfaces by reaction with nicotine. The concentrations of nicotine have been measured in dust, dashboard inside vehicles and furniture in smokers' homes (tables and bed frames) [34, 35]. Destaillats et al. discovered cotton could absorb amounts of nicotine up to

100 mgm^2 [36]. Studies suggested that TSNAs from clothes and skin might lead to additional intake through inhalation if part of a breathing zone [9]. TSNAs may be spread from smokers to other persons (e.g., infants and children) and other objects (e.g., food) because they are trapped on the clothes of smokers and non-smokers who were exposed to SHS. Also, TSNAs could be contacted by hands when nonsmokers touch polluted surfaces in smoking environments. Matt et al. found nonsmokers who moved into apartments which were occupied by smokers more than two months earlier were detected to have THS on their hands [12].

Once TSNAs are created, they can persist on surfaces for weeks to months. Given the features of TSNAs with frequent contact with surfaces and dust, infants and children are more prone to be at great risk, especially those who live in homes in which adults smoke, even if smoking occurs at times or in rooms when no children are present [8, 12, 13, 14]. They are particularly more vulnerable than adults for the following reasons. First, they typically spend more time indoors and are involved in greater activity in areas where dust collects and may be resuspended (e.g., carpets on the floor) [12]. House dust has been demonstrated to be the main route of exposure for infants and children from U.S. Environmental Protection Agency [38]. Second, they have age-specific behaviors (e.g., crawling) and like exploring objects with their mouths (e.g., ingesting non-food items) [8,14]. Third, at approximately 0.05–0.25 g day^{-1}, the dust ingestion rate in infants and children is estimated to be more than twice that of adults because of their higher respiration related to lower body weight [13, 26]. Fourth, their brains are more sensitive to even the lowest level of toxins in the process of development [14]. Thus, low doses of TSNAs such as those shown in

Table 1 may cause long-term health impacts on infants and children [13]. Some researchers have asserted that a smoking ban in the home seems likely to be the best way to protect infants and children from the dangers of TSNAs [13]. Using fans and opening a window have been verified to not help eliminate the hazards [14]. It is suggested that smokers who have infants or children are better not to smoke in homes or in cars, wash their hands and change clothes before contacting with infants or children and most importantly, it is beneficial to delay contact with children for up to five minutes after the last cigarette [14].

The highest risk of involuntary exposure to TSNAs occurs in indoor environments with different ownership or occupancy. This kind of environments includes rental apartments, condominiums and houses, hotel rooms, and rental and used cars [12]. Two reasons contribute to this phenomenon. One is no implementation of public smoking bans or poor monitoring of private smoking bans [12]. The other is one or more smokers living in these environments, because smoking adults represent 18.1% of the United States population (10.6% in Utah to 28.3% in Kentucky) [1]. That is to say the probability that one or more smokers are among the occupants is 60% given a smoking prevalence of 0.18. Dr. Kamlesh Asotra, a program director at the Tobacco-Related Disease Research Program, said that maids who came to clean up hotel rooms where smoking was allowed were at risk because it took only thirty minutes for NNA to form and amounts of NNA were present on the surfaces after three hours [10]. In buildings where substantial smoking has occurred, replacing nicotine-laden furnishings, carpets and wallboard can significantly reduce exposure to THS hazards [10].

Even though a growing number of states have implemented smoking ban to protect public health in restaurants, bars and work areas, the home remains a place of intense and

consistent exposure to tobacco smoke for nonsmokers [12]. A study has demonstrated that THS pollutants attracted great concern in multiunit housing where smoking was permitted [38]. TSNAs can persist on the surface and in dust for a long time, which implies it may be able to move along air ducts, through wall and floor cracks, though elevator shafts, and along plumbing and electrical routes to contaminate units on other floors far removed from the smoking area [39]. In that case, disadvantaged and vulnerable populations will be at greater risks in public housing [4]. According to the 2010 U.S. Department of Housing and Urban Development, 32% of households in public housing included elderly persons, 35% disabled persons, and 41% children between 2008 and 2009 [40]. Furthermore, Wilson et al. reported that children who lived in multiunit housing apartments had 140% higher serum cotinine levels than did children who lived in detached housing [41].

To sum up, there is available evidence of a mechanism by which potentially harmful TSNAs may be released into the environment, indicating that TSNAs are ubiquitous and pervasive [12]. TSNAs are considered as potential carcinogens and they can enter body through multiple exposure routes. They are able to exist in air and dust and on surfaces. Nonsmokers who spend time indoors near polluted surfaces are at specials risks of THS. Infants and children are most vulnerable because of their increased exposure and high sensitivity to contaminants than adults [12]. People who live in public housing and places with high rotation frequency suffer from high risks of THS exposure. Various approaches can be considered to limit the impact of these carcinogenic pollutants indoors [13].

CONCLUSIONS

There are a number of studies on the formation of TSNAs through aging and interaction with ambient oxidants such as ozone, oxides of nitrogen and related compounds. These processes need to be studied over days, weeks, and months [8]. However, the characterization of health risk attributable to TSNAs is premature. Although a few studies have shown the carcinogenicity of TSNAs in animal models and *in vitro* experiments, more analysis of the cytotoxicity and genotoxicity of TSNAs are required for a comprehensive assessment THS risks. More research should focus on the chemistry of TSNAs reacting with DNA and testing their carcinogenicity in humans. There is a lack of evidence on TSNAs contamination and biomarkers to confirm individual exposures and correlation of such data to human outcomes. To date, NNAL (a metabolite of NNK) is found in urine and blood of people [21], but iso-NNAL (a metabolite of NNA and biomarkers for TSNAs) has not been detected yet [13, 33]. Future studies are necessary to seek an effective biomarker for NNA (because NNA is the major TSNA formed from the reaction of nicotine and nitrous acid) and develop biomarkers for other TSNAs to enable better diagnosis of THS exposures.

The majority of current studies which assess human exposure to THS mainly focus on the higher concentrations of TSNAs formed in a short period of time and imply that this may lead to adverse health effects on the basis of the impacts of SHS pollutants. In fact, besides experimental studies, some epidemiology studies may be appropriate conducted for analyzing the association of THS exposure with morbidity and mortality, which could include cohort studies. Cohort studies are able to assess long-term human effects. For cohort studies, infants

and children who live in the smoker homes may be the target population because they are more vulnerable to higher THS exposures as a result of their increased contact with dust and surfaces and their close association with smoking adults which has been discussed in the essay. Such studies should attempt to investigate whether infants and children exposed to THS develop adverse impacts and observe the concentration of TSNAs *in vivo* after decades of follow-up visiting and examination. This method may be more straightforward than laboratory experiments on individual exposure because it is hard to simulate exposure in laboratory which is completely the same as real-life situations. Also, the cohort studies may search the symptoms of long-term exposure of THS, contributing to clues on conducting future clinical trials. In addition, besides considering the risks of infants and children, examining occupational exposure risks is significant for the assessment of THS exposure.

It is known that TSNAs remain in dust surfaces and air for a significant period of time. However, little is currently known on how TSNAs accumulate in different media and how different smoking patterns contribute to the accumulation of TSNAs. Developing effective strategies to protect an environment from THS has been considered by experts. Implementation of 100% smoke-free environments in residences, automobiles, and public places are the most effective way to limit the impact of carcinogenic pollutants from tobacco smoke, through elimination of the primary pollution source [13, 14]. However, the success and expectations of such policies in protecting nonsmokers is not at all certain. Replacing nicotine-laden furnishings, carpets and other possible polluted substrates may significantly reduce exposures to THS hazards in indoor environments where substantial smoking has occurred [13]. There is low awareness on how THS be a serious danger for human health [10].

A survey conducted by Winickoff et al. demonstrated that only 65.2% of nonsmokers and 43.2% of smokers believe that THS is harmful to children [4]. Consequently, a THS education campaign about health hazards related to THS exposure appears essential. Increasing public awareness of TSNAs could change attitudes and protect vulnerable populations, especially children. Furthermore, further studies are warranted to investigate the effectiveness of interventions (smoking restrictions, education campaigns, cleaning, clinical trials) which can decrease the risk of THS exposure and reduce the cumulative effects of THS on morbidity and mortality. All in all, more evidence needs to be provided to fill gaps in our current understanding of the chemistry, toxicology, pollution, exposure, clinical significance, and policy implications of THS.

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