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Second-hand smoke and human lung cancer

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Abstract

Since the early 1980s, there has been growing concern about potential health consequences of exposure to second-hand smoke (SHS). Despite SHS being established as a risk factor for lung cancer development, the estimated risk has remained small yet somehow debatable. Human exposure to SHS is complicated because of temporal variabilities in source, composition, and concentration of SHS. The temporality of exposure to SHS is important for human lung carcinogenesis with a latency of many years. To explore the causal effect of SHS in lung carcinogenesis, exposure assessments should estimate chronic exposure to SHS on an individual basis. However, conventional exposure assessment for SHS relies on one-off or short-term measurements of SHS indices. A more reliable approach would be to use biological markers that are specific for SHS exposure and pertinent to lung cancer. This approach requires an understanding of the underlying mechanisms through which SHS could contribute to lung carcinogenesis. This Review is a synopsis of research on SHS and lung cancer, with special focus on hypothetical modes of action of SHS for carcinogenesis, including genotoxic and epigenetic effects.

Keywords

DNA adducts; Lung carcinogenesis; Mutation; Tobacco smoke

Introduction

Second-hand smoke (SHS), otherwise known as ‘environmental tobacco smoke’, is classified as a known human pulmonary carcinogen by various regulatory agencies and government authorities^{1–3}. Since the 1980s, epidemiologic studies have attempted to establish a link between SHS exposure and lung cancer development⁴. The attempts have essentially shown an association between exposure to SHS and risk for development of lung cancer^{5,6}; however, the estimated risk has remained small yet somehow disputable^{4,7}. The uncertainties have arisen from inadequate exposure assessment for SHS⁴. Human exposure to SHS is complicated because of temporal variabilities in source, composition and concentration of emanating SHS⁷. The temporality of exposure to SHS is significant for human lung cancer, which has a long latency of many years⁸. To find the etiologic involvement of an agent in lung carcinogenesis, exposure assessment should estimate chronic exposure rather than single- or short period of time exposure to the agent of interest⁹. Thus, conventional exposure assessment using indices of SHS, as measured in the ambient air or in the body fluids of exposed individuals, at certain times is not optimal for estimating lifelong SHS exposure¹⁰. A more reliable approach would be to utilize biological markers that are specific for SHS exposure and pertinent to lung cancer (Fig. 1). Such biomarkers should integrate exposure to SHS over a relevant time period of significance for lung carcinogenesis^{7,11,12}.

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Exposure to tobacco smoke or its constituents is known to trigger a cascade of events in the multi-stage process of lung carcinogenesis¹³. Of these, genotoxic and epigenetic effects are of paramount importance because they occur frequently and in the early stages of lung carcinogenesis^{14–16}. Specifically, formation of persistent DNA damage at key cancer-related genes, as well as epigenetic silencing of cancer relevant genes are common events in smoking-associated lung cancer (*see* Fig. 2)^{14–16}. Increasing the knowledge of SHS-induced genotoxic and epigenetic effects may help determine the mechanistic involvement of SHS in lung carcinogenesis, thereby facilitating the estimation of lung cancer risk in relation to SHS exposure. In addition, a better understanding of the underlying mechanism(s) through which SHS may contribute to lung carcinogenesis may help identify unique biological markers that can be used for early detection and prognosis of lung cancer as well as for treatment of this lethal disease. This review article is an outline of research on SHS and lung cancer, with special emphasis on hypothetical modes of action of SHS of relevance for carcinogenesis, including genotoxic and epigenetic effects. The review summarizes the current state of knowledge as “although the causal link between SHS exposure and lung cancer development is well established^{1–3}, the exact mechanism(s) of action of SHS of relevance for lung carcinogenesis remains to be determined”.

Search strategy and selection criteria

References were identified through Pub Med search using the following terms: ‘smoke’, ‘tobacco’, ‘lung cancer’, ‘environmental tobacco smoke’, and ‘second-hand smoke’. To limit the number of citations, updated review articles were used rather than individual research articles unless otherwise indicated. Except for one historical paper (ref. 17), all citations are from English-written references. Data restrictions were not applied. The coverage of published work is intended to be illustrative rather than absolutely exhaustive.

Chemical composition of SHS

Tobacco combustion results in the formation of mainstream smoke and sidestream smoke¹⁸. Mainstream smoke is generated during puff drawing from the burning cone and hot zone of a tobacco product, which travels through the tobacco column outward mouthpiece¹⁹. Sidestream smoke is emitted from the smoldering coal of a tobacco product into the air between puffs²⁰. Both mainstream smoke and sidestream smoke are comprised of (I) vapor phase containing volatile agents, *e.g.*, benzene, vinyl chloride, acrolein, etc. and (II) particulate phase (tar) containing semi-volatile and non-volatile agents, such as alkaloids, *e.g.*, nicotine and its derivatives, aromatic amines, polycyclic aromatic hydrocarbons (PAH), etc.^{21,22}. For the most part, the chemical compositions of sidestream smoke and mainstream smoke are qualitatively similar¹³. However, because sidestream smoke is produced at lower burning temperature, the quantities of its chemical constituents in both vapor and particulate phase differ from those of mainstream smoke, *e.g.*, sidestream smoke is richer than mainstream smoke in certain carcinogens, *e.g.*, aromatic amines^{10,19,22,23}.

SHS is a mixture of mainstream smoke and sidestream smoke^{10,23}. Whereas sidestream smoke comprises ~85% of total SHS, mainstream smoke constitutes <15% of the overall SHS, *i.e.*, the smoke first inhaled by an active smoker and then exhaled, while being briefly retained in the lung and scrubbed of some of its constituents, most notably, nicotine, carbon monoxide, and much of the particulate matter²⁴. Minor contributors to SHS include small amounts of smoke that escape during puff drawing from the burning cone of tobacco product and some vapor phase agents that diffuse through the wrapping materials *e.g.*, cigarette paper, into the air¹⁰. Once released into the environment, SHS may further aggregate with pollutants already present in the air and change character^{10,23}. Thus, the physicochemistry of SHS might considerably be different from that of mainstream smoke^{10,19,22,23}. Nonetheless, most toxic or carcinogenic agents present in mainstream smoke can also be found in SHS, of course, in

different concentrations due to aging and dilution with ambient air ^{10,19,23}. Smokers, who actively inhale massive doses of mainstream smoke-carcinogens, have higher intake of carcinogens relative to SHS-exposed individuals. However, the observation that sidestream smoke-condensate is more potent than mainstream smoke-condensate in inducing mouse skin tumors has given rise to the idea that SHS imposed on non-smokers might be even more carcinogenic than mainstream smoke inhaled by active smokers ^{25,26}.

Human lung cancer and SHS

Since the early 1980s, there has been growing concern about potential health consequences of exposure to SHS ^{3,4}. Of the multitude of adverse health effects associated with smoking, lung cancer has been a focus of attention for studies of SHS exposure and ill-health effects ⁴. Such attention has been given due to the graveness of this disease, which is often diagnosed at late stages, with poor responses to surgical, chemotherapeutic, and/or radiotherapeutic regimens that lead to high mortality ⁸. To date, epidemiologic studies on lung cancer and SHS have been observational ⁴. As such, they have attempted to correlate the incidence of lung cancer in chronically exposed individuals to SHS, *i.e.*, smokers' spouses or children, with their exposure level to this complex mixture ^{4,12}. However, the critical information on SHS exposure has been elicited from sources that are vulnerable to bias because of a variety of reasons ²⁷. For example, questionnaire- or interview-derived information is known to be subject to recall bias; the implications of such bias are different in case of prospective (*i.e.*, cohort) and retrospective (*i.e.*, case-control) studies ²¹. More preferable has been the use of external or internal dosimetry data, *i.e.*, measurement of SHS constituents in the ambient air or determination of SHS components/metabolites in various body matrices, respectively ^{9–11,13,21}. The latter, however, can mostly provide information on recent exposure spanning a short period of time, *e.g.*, a few hours/days ^{9–11}. Such rough estimation of SHS exposure may cause misclassification of subjects' longtime exposure levels. Consequently, the corresponding risk assessment for lung cancer development can be debatable and open to criticism ^{10,21}.

Animal studies of SHS inhalation

Given the above-mentioned difficulties in studying SHS carcinogenicity in humans, long-term smoke inhalation experiments in animals would seem an attractive alternative for investigating SHS-induced carcinogenesis. In fact, as early as 1930, mice were experimentally exposed to cigarette smoke, albeit with no proof of tumorigenicity ¹⁷. Subsequent studies in rodents, *e.g.*, mice, rats and hamsters, dogs, and nonhuman primates confirmed that producing lung tumorigenesis in animal models of inhalation smoke is inevitably difficult ^{28–30}.

A significant issue in rodent experiments has been the accuracy of delivering smoking dose because nose-only exposure, although more precise and accurate ³⁰, may induce stress and cause discomfort, whereas whole-body exposure can result in transdermal and gastrointestinal absorption of smoke materials by the animals (from grooming) ^{28,29}. The former may hinder experimental progress or cause retardation of the disease process, whereas the latter may confound exposure route and site-specificity of tumorigenesis ^{28,30}. Inherent in rodent models is also their incomparability to humans regarding the inhalation of smoke ³⁰. Murines are obligatory nose breathers with complex nasal turbinates, which efficiently filter inhalatory smoke ²⁹. In addition, a change in breathing pattern, *i.e.*, shallow respiration, becomes evident in animals forcibly exposed to smoke ³⁰. Consequently, there is scant smoke deposition in the lower airways of rodents experimentally exposed to smoke ^{28–30}. To compensate, the animals are treated with much higher doses of smoke than those humans in real life are usually exposed to ^{28,29}. For example, most smoke inhalation studies in rodents administer doses of smoke with an average total particulate matter, which corresponds to a human smoking of 2–4 packs of cigarettes per day ³⁰. Often, animals exposed to smoke exhibit appetite depression and reduced food intake; the resulting caloric restriction being known to impede tumor initiation and

promotion^{28–31}. For manifestation of tumorigenicity, therefore, a recovery period of a few months in clean air is required following the smoke exposure of the animals in some protocols/models^{28,29}. Nonetheless, smoke inhalation studies without recovery period, but of longer exposure duration, have also yielded lung tumors in mice and rats^{28,30,31}.

Histopathologically, smoke-associated lung tumors in humans and experimental animals bear limited resemblance to one another⁸. In humans, most lung tumors are located in the bronchial tree, classified as non-small-cell lung cancer, including (I) squamous cell carcinoma, (II) adenocarcinoma, and (III) large cell carcinoma, or small cell lung cancer, while bronchoalveolar adenocarcinoma, which occurs in the peripheral lung, is less frequent⁸. Of these, tumors originating at distal airways aggressively invade adjacent tissues and frequently metastasize to other organs, *e.g.*, brain and liver⁸. Smoke-induced tumors in mice largely occur in the peripheral lung as areas of hyperplasia, growing into adenoma, and eventually progressing to adenocarcinoma with negligible invasion of or metastasis to other organs^{28,29}. Hamsters experimentally exposed to smoke develop almost exclusively laryngeal tumors but not tumors in the deep airways³⁰. Rats exposed to inhalatory smoke show an inconsistent induction of nasal cavity tumors and non-neoplastic and neoplastic lung lesions^{28,29}. A small number of smoke inhalation studies has been performed in larger animal species, mainly due to the logistic limitations involved therein^{3,28,30}. For example, studies in dogs trained to inhale smoke or subjected to a highly invasive route of exposure, *i.e.*, tracheotomy, as well as studies in nonhuman primates have only been performed for short duration of exposure and in small group sizes^{3,28,30}. Altogether, after decades of laborious, costly, and long-term animal studies that required sacrifice of thousands of test animals, we need to realize that smoke inhalation of experimental animals for tumorigenicity is far from replicating human smoke-related pulmonary carcinogenesis.

Mechanisms of SHS carcinogenicity

Several constituents of SHS are known carcinogens in experimental animals and/or humans, including aromatic amines, *e.g.*, 4-aminobiphenyl, 2-naphthylamine, PAH, *e.g.*, benzo[a]pyrene, tobacco specific nitrosamines, *e.g.*, 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone (NNK), benzene, cadmium, nickel, etc.^{9,22,33}. The lists of the International Agency for Research on Cancer for known and suspected tobacco smoke carcinogens are presented in Web-Table 1, and Web-Tables 2–3, respectively³³. A genotoxic mode of action for some of these carcinogens has been elucidated, which relies upon their ability to either directly or after biotransformation generate electrophilic species capable of forming covalently bound DNA lesions, *i.e.*, DNA adducts^{9,13,22}. Formation of DNA adducts is an event of potential significance in initiating carcinogenesis inasmuch as irreparable (persistent) DNA adducts may be misinstructional during DNA replication, thus, giving rise to mutations³⁴. Targeted mutations in key genes encoding proteins for, *e.g.*, cell-cycle and growth control might lead to tumorigenesis (Fig. 2)^{34,35}. Furthermore, an epigenetic mode of action for some SHS-derived carcinogens can be proposed that involves DNA and histone modifications, which result in gene silencing without affecting the coding sequence of the turned-off gene (Fig. 2)^{36,37}. Epigenetic silencing of cancer-related genes has been shown to occur frequently and in the early stages of carcinogenesis^{38,39}. It is plausible that SHS-induced genotoxic and epigenetic effects, once identified mechanistically, can be utilized as integrated biomarkers of exposure and early effects for pulmonary carcinogenesis (*see*, Fig. 2).

Mechanistic investigations of SHS-derived genotoxins

Many genotoxic carcinogens are known to leave unique signatures on cancer-related genes⁴⁰. The signature of carcinogens is manifested by the induction of specific types of mutations, *e.g.*, base substitutions or frameshifts, at distinctive locations along oncogenes and/or tumor suppressor genes⁴⁰. These characteristic mutations are often preceded by the formation of

carcinogen-induced DNA damage at the mutated sites⁴⁰. This phenomenon is best exemplified by solar ultraviolet irradiation linked to *TP53* mutations, a highly frequent event in sunlight-associated skin cancers⁴¹. The mutational spectrum of the *TP53* gene in sunlight-attributable skin cancers is characterized by single C→T or tandem CC→TT transitions at specific dipyrimidine sequences, which correspond to the major hotspots of ultraviolet-induced DNA damage found in this tumor suppressor gene⁴¹.

Today, investigations of human cancer etiology often employ genomic sequencing technologies to correlate the pattern of mutations specific for each type of human cancer with the experimentally established signature of carcinogens^{40,42}. Obviously, experimental exposure of humans to carcinogens is unethical and out of the question. Thus, the signature of carcinogens can only be ascertained in relevant model systems⁴⁰. Currently, DNA-lesion footprinting in conjunction with mutagenicity analysis is used to determine carcinogens signature in various *in vitro* and/or *in vivo* model systems⁴⁰. The sensitive and specific DNA-lesion footprinting methodologies, *e.g.*, ligation-mediated- and terminal transferase-dependent polymerase chain reactions, together with versatile *in vitro* and *in vivo* mutagenesis assays have enabled correlation studies of DNA damage and mutation at the level of nucleotide resolution⁴⁰. Accordingly, the signature of a variety of carcinogens, including those present in SHS, has been established in exogenous reporter genes as well as in endogenous cancer-related genes^{40,43–45}.

To investigate the etiologic involvement of SHS in human lung carcinogenesis, future investigations should employ DNA-lesion footprinting combined with mutagenicity analysis in cancer relevant genes, such as the *RAS* oncogenes⁴⁴ and *TP53* tumor suppressor gene³⁵. For example, the *TP53* gene mutations, which are frequent event in smoke-related lung cancer, mainly occur at specific CpG-containing hotspots (*see*, Fig. 3), resulting in a distinctive mutational spectrum, *i.e.*, G→T transversions predominantly found on the non-transcribed strand of this gene (*see*, Fig. 4)^{35,46}. Of relevance, this pattern of mutation is also the established signature of several constituents of SHS^{35,47}. Of significance, it has also been demonstrated that smoke-derived PAH can preferentially form DNA adducts at the above-mentioned *TP53* lung cancer mutational hotspots (*see*, Fig. 5)^{14,48}. It needs to be acknowledged that DNA-lesion footprinting of smoke-derived carcinogens has been rather successful for PAH-related DNA adducts, yet this concerns mainly *TP53* mutations, which represent only one set of alterations observed in smoking-associated lung cancer. Similar footprinting of DNA lesions derived from other tobacco carcinogens (and in other genes) has been less successful and it is not clear at present whether the *TP53* story is a paradigm or an exception. Attempts have been made for DNA footprinting of tobacco-specific nitrosamines, *e.g.*, NNK^{49,50}, and other tobacco carcinogens, *e.g.*, acetaldehyde⁵¹, in the *TP53* and *RAS* genes, although the correlation between DNA damage and mutagenesis in the latter cases has not been straight forward.

Prospectively, correlation studies of DNA damage and mutagenesis in the *TP53* gene can utilize footprinting of smoke-related DNA-adducts together with highly accurate and high-throughput DNA sequencing methodologies, *e.g.*, Solexa, ABI SOLID, using the genomic DNA of SHS-exposed individuals. The latter will enable sequencing of millions of fragments of the *TP53* gene in samples of individuals chronically exposed to SHS. Among these large numbers of sequence reads, mutations should be found that are similar to those present in lung tumors of smokers, and co-localize with hotspots of SHS-induced DNA adduction in this tumor suppressor gene. It needs to be mentioned that because the lower respiratory tract is a general target of most inhalatory carcinogens, including those present in SHS, the above-mentioned analyses should be performed using biological samples taken from the deep airways. Currently, however, invasive methods are mostly available for routine collection of samples from the lower respiratory tract, *e.g.*, bronchoalveolar lavage. This is a major hindrance for population-

based studies in which volunteers are understandably unwilling to undergo invasive sampling methods. Further refinements in developing methodologies, *e.g.*, ‘sputum induction’, which has shown great promise as a non-invasive and easy-to-perform technique for sampling lower airways⁵², will be required to resolve the issue of target-tissue collection for investigations of SHS carcinogenicity in humans.

Other key issues awaiting further exploration include determination of the type, frequency, and distribution of DNA lesions in cancer-related genes consequent to SHS exposure in nonsmokers as compared to those caused by mainstream smoke in smokers. This would help determine whether lung cancer development in smokers *versus* non-smokers exposed to SHS is only a question of smoking dose, *i.e.*, to verify whether the different risk to develop lung cancer is only dependent upon smoking dose because, after all, SHS is still cigarette smoke. There is, however, some evidence, both chemical and biological, that sidestream smoke, the main component of SHS, is different from mainstream smoke inhaled by active smokers^{25, 26}.

Lastly, a few epidemiologic studies have investigated the impact of SHS exposure on lung cancer development by analyzing *TP53* and *K-RAS* mutations in lung tumors of non-smokers exposed to SHS^{53–55}. While these studies suffer from some limitations, *i.e.*, limited statistical power due to the small number of subjects analyzed and uncertainties in ascertainment of subjects’ past exposure to SHS, they represent the first step in the above-mentioned line of mechanistic research. Thus, these epidemiologic studies could be used as starting point for recommendations for future mechanistic investigations.

Mechanistic investigations of SHS-derived epimutagens

Cancer is a chronic disease characterized by significant alterations in normal patterns of gene expression³⁶. Epigenetic mechanisms are profoundly affected in various types of cancer and contribute to the initiation and progression of the disease phenotype^{37–39}. Epigenetic alterations are defined as heritable modifications to the DNA with the potential to change gene expression while the primary DNA sequence remains conserved³⁶. The epigenetic control of gene expression is, therefore, reversible as it does not involve DNA sequence^{36–38}. Epigenetic changes are very frequent events in human lung cancer³⁹. These changes affect several hundreds of genes in individual tumors, and some of them quite frequently, in a patient population⁵⁶.

Two types of epigenetic changes are most notable: (I) hypermethylation of CpG islands and (II) a more global hypomethylation of the DNA in tumors. DNA hypomethylation in cancer tissue was first observed over two decades ago³⁸. In the 1990s, researchers reported hypermethylation of CpG islands of several known and putative tumor suppressor genes and other genes involved in important genome defense pathways, such as checkpoint control and DNA repair^{36, 57}. Today, there are many reports that have documented methylation of CpG islands associated with a large number of different genes, including almost every type of human cancer. In lung cancer, methylated CpG islands include those associated, for example, with *CDKN2A*, *RASSF1A*, *RARβ*, *MGMT*, *GSTP1*, *CDH13*, *APC*, *DAPK*, *TIMP3*, and several homeobox genes^{36,37,39,55,58}. Methylation of CpG islands occurs early during lung cancer pathogenesis⁵⁹, and in some cases can predict the existence of a lung tumor years ahead of clinical diagnosis⁶⁰.

It seems reasonable to hypothesize that cigarette smoke and SHS-associated carcinogens can induce either CpG island hypermethylation or hypomethylation of repetitive elements, or both, in normal lung tissue of smokers or in tissue of individuals chronically exposed to SHS (*see*, Fig. 2). However, there have been very few studies to determine the exact role of carcinogens in initiating gene-specific promoter hypermethylation and/or global DNA hypomethylation in

human tumors. Several studies have compared methylation of specific genes or a series of genes in lung tumors of smokers and non-smokers and have often arrived at different conclusions. Some studies have determined that DNA hypermethylation of specific genes is more frequent in tumors from smokers than in tumors from nonsmokers^{61–63} and others have seen no such difference^{64,65}. In most cases, information on SHS exposure is not available or is not quantifiable for individuals classified as nonsmokers. Interestingly, in one case promoter hypermethylation of the *O⁶-methylguanine-DNA methyltransferase* gene was more common in lung adenocarcinomas from never-smokers than smokers and was associated with tumor progression⁶⁶.

Despite of their potentially important effects on human health, the number of confirmed epimutagens is very small, presumably because there are no efficient screening assays to identify them^{67,68}. Studies examining DNA methylation changes in carcinogen-induced mouse lung tumors have consistently shown that different lung carcinogens induce promoter hypermethylation of different genes, for example *CDKN2 (p16)* and *DAPK* at different frequencies³⁹. However, these studies do not address the issue as to whether the carcinogens induce epigenetic events at an early, tumor-initiating stage, or at a later stage during tumor promotion.

Some carcinogenic metals, such as nickel and cadmium (present in SHS; *see*, Web-Table 1), can promote epigenetic gene silencing most likely through mechanisms that first involve binding of the metal ions to histones with a subsequent modulation of histone modifications, which will eventually lead to changes in gene transcription and DNA methylation^{69,70}. However, there have been very few studies examining whether cigarette smoke carcinogens can directly induce DNA methylation changes. These studies have either been limited to a few single genes^{71,72}, or have examined DNA methylation changes in total genomic DNA only⁷³. Thus, the specific nature and extent of such methylation changes has remained unknown. Benzo[a]pyrene, a carcinogen present in SHS, can inhibit DNA methyltransferases⁷⁴ and exposure of cells to this compound leads to reactivation of LINE-1 retrotransposons⁷⁵ presumably by DNA demethylation. Cigarette smoke extract was shown to directly induce demethylation of the synuclein- γ gene in human cells⁷¹. In addition to inhibiting DNA methylation, Benzo[a]pyrene metabolites are also able to induce DNA hypermethylation as shown for the *retinoic acid receptor beta2* gene⁷².

The potential epigenetic toxicity of the majority of SHS carcinogens remains unexplored. Future investigations using *in vitro* systems, animal models, and biospecimens from chronically exposed individuals will be required to determine if SHS induces lung cancer through an epigenetic pathway, particularly involving aberrant DNA methylation. These investigations should employ state-of-the-art DNA methylation analysis in combination with microarray platforms⁵⁶ to find aberrations in the methylation status of individual cancer-relevant genes as well as of large number of genes genome-wide. Since the “epigenotoxic” effects of SHS are virtually unknown, these studies should clarify how the methylation patterns in chronically SHS-exposed individuals are different from those in non-exposed control populations, and how they may be similar to methylation patterns found in tobacco smokers.

Concluding remarks

Environmental factors play a determining role in human cancer³⁴. Many cancer-causing agents (carcinogens) are present in the air we breathe, in the food we eat, and in the water we drink^{34,45}. Humans’ constant and to some extent unavoidable exposure to environmental carcinogens makes investigation of cancer etiology extremely complicated. The complexity of human cancer etiology is particularly challenging for those types of cancer with long latency, which are associated with exposure to ubiquitous environmental carcinogens³⁴. The small and

somehow disputable risk of lung cancer development in relation to SHS exposure exemplifies the intricacy of establishing human cancer etiology when omnipresent carcinogens are concerned. Because of temporal variabilities in source, composition and concentration of SHS, conventional exposure assessment using indices of SHS, as measured in the ambient air or in the body fluids of exposed individuals, at certain times has failed to estimate long-term SHS exposure¹⁰. Consequently, although the causal link between SHS exposure and lung cancer development is well-established^{1–3}, the estimated risk for developing lung cancer consequent to SHS exposure remains somewhat debatable. Establishing the mechanism(s) of action of SHS of relevance for carcinogenesis can help identify unique biological markers that can be used for assessing lung cancer risk in relation to SHS exposure.

Because SHS contains basically the same carcinogens as mainstream smoke, albeit at different concentrations³³, it is conceivable that SHS may induce genotoxic and epigenetic effects similar to those already established for mainstream smoke. The well-characterized genotoxic and epigenetic effects of mainstream smoke include the formation of persistent DNA adducts at crucial cancer-related genes, and transcriptional silencing of cancer relevant genes, respectively (*see* Fig. 2)^{15,16,34}. These genotoxic and epigenetic effects have already been observed in different *in vitro* and/or *in vivo* systems, including animal models and humans, *e.g.*, cell culture, tissues, biopsies, etc., and have been shown to be related to tumor development. Future investigations should employ comprehensive footprinting of SHS-induced DNA adducts in relation to mutagenicity together with thorough analysis of DNA methylation status in relation to gene expression in cancer-related genes. Not only can these investigations improve our knowledge of the underlying mechanism(s) through which SHS may contribute to lung carcinogenesis, but they may also help identify specific biomarkers that can be used for early detection and prognosis of lung cancer as well as for assessment of its treatment strategies.

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Figure 1. Causal role of SHS in human lung cancer development

Mechanistic research into SHS-induced lung carcinogenesis may help identify unique biological markers that could be used for early detection and prognosis of lung cancer as well as for treatment of this lethal disease.

Figure 2. Schematic model of SHS-induced lung carcinogenesis

This hypothetical model simplistically shows two distinct pathways of genotoxic and epigenetic effects, which might contribute to human lung cancer development, including formation of persistent DNA damage at key cancer-related genes, and aberrant DNA methylation (global hypomethylation or locus-specific CpG island hypermethylation), respectively.

Figure 3. Codon distribution of the *TP53* tumor suppressor gene in tobacco smoke-associated lung cancer ($n = 2340$)

Data were obtained from the *TP53* mutation database of the International Agency for Research on Cancer (<http://www-p53.iarc.fr/p53DataBase.htm>; R12 version). Entries with confounding exposure to asbestos, mustard gas or radon were excluded. Codons containing methylated CpG sequences are indicated by asterisks (*).

Figure 4. Mutation spectrum of the *TP53* tumor suppressor gene in tobacco smoke-associated lung cancer ($n = 2340$)

Data were obtained from the *TP53* mutation database of the International Agency for Research on Cancer (<http://www-p53.iarc.fr/p53DataBase.htm>; R12 version). Entries with confounding exposure to asbestos, mustard gas or radon were excluded.

Figure 5. Schematic model of the mechanism of PAH-related DNA-damage leading to *TP53* mutation

The proposed model demonstrates the targeting of methylated CpG dinucleotides by a PAH compound (benzo[a]pyrene diol epoxide) leading to G→T transversions at mutational hotspots.

Table 1

The International Agency for Research on Cancer (IARC) Group 1 compounds (known human carcinogens) reported in mainstream cigarette smoke

Compound name	Molecular formula	Present in particulate phase (P) or vapor phase (V) or both (P & V)
4-Aminobiphenyl	C ₁₂ H ₁₁ N	P
Arsenic	As	P
Benzene	C ₆ H ₆	V
Beryllium	Be	P
Cadmium	Cd	P
Chromium (VI) compounds	Cr	P & V
Ethylene oxide	C ₂ H ₄ O	V
2-Naphthylamine	C ₁₀ H ₉ N	P
Nickel compounds	Ni	P & V
Radon-222 and its decay products (²¹⁰ Polonium)	Rn (²¹⁰ Po)	P
Vinyl chloride	C ₂ H ₃ Cl	V

This list is based upon evaluation of international, as well as domestic cigarettes. The diversities of cigarette blends and design configurations are inherent in this list. Thus, an individual cigarette style marketed in the US would not be expected to contain each of the herein listed compounds. Data were adapted from ref. (33).

Table 2

The International Agency for Research on Cancer (IARC) Group 2A compounds (probable human carcinogens) reported in mainstream cigarette smoke

Compound name	Molecular formula	Present in particulate phase (P) or vapor phase (V) or both (P & V)
Acrylamide	C ₃ H ₅ NO	V
2-Amino-3-methyl-3 <i>H</i> -imidazo[4,5- <i>f</i>]quinoline (IQ)	C ₁₁ H ₁₀ N ₄	P
Benz[<i>a</i>]anthracene	C ₁₈ H ₁₂	P
Benzo[<i>a</i>]pyrene	C ₂₀ H ₁₂	P
1,3-Butadiene	C ₄ H ₆	V
Dibenz[<i>a,h</i>]anthracene	C ₂₂ H ₁₄	P
Formaldehyde	CH ₂ O	V
Glycidol	C ₃ H ₆ O ₂	V
Nitrogen mustard	C ₅ H ₁₁ Cl ₂ N	P & V
<i>N</i> -Nitrosodimethylamine (DMN)	C ₂ H ₆ N ₂ O	V
<i>N</i> -Nitrosodiethylamine (DEN)	C ₄ H ₁₀ N ₂ O	V
Tetrachloroethylene	C ₂ Cl ₄	V
<i>o</i> -Toluidine (2-methylaniline)	C ₇ H ₉ N	P
Trichloroethylene	C ₂ HCl ₃	V

This list is based upon evaluation of international, as well as domestic cigarettes. The diversities of cigarette blends and design configurations are inherent in this list. Thus, an individual cigarette style marketed in the US would not be expected to contain each of the herein listed compounds. Data were adapted from ref. (33).

The International Agency for Research on Cancer (IARC) Group 2B compounds (possible human carcinogens) reported in mainstream cigarette smoke

Table 3

Classification		Compound name	Molecular formula	Present in particulate phase (P) or vapor phase (V) or both (P & V)
Hydrocarbons	Aliphatic or monocyclic	Isoprene	C ₅ H ₈	V
		4-Vinylcyclohexene	C ₈ H ₁₂	V
	Polycyclic	Benzo[<i>b</i>]fluoranthene	C ₂₀ H ₁₂	P
		Benzo[<i>j</i>]fluoranthene	C ₂₀ H ₁₂	P
		Benzo[<i>k</i>]fluoranthene	C ₂₀ H ₁₂	P
		5-Methyl-chrysene	C ₁₉ H ₁₄	P
		Dibenzo[<i>a,e</i>]pyrene	C ₂₄ H ₁₄	P
		Dibenzo[<i>a,h</i>]pyrene	C ₂₄ H ₁₄	P
		Dibenzo[<i>a,i</i>]pyrene	C ₂₄ H ₁₄	P
		Dibenzo[<i>a,l</i>]pyrene	C ₂₄ H ₁₄	P
	Aromatic	Indeno[1,2,3- <i>cd</i>]pyrene	C ₂₂ H ₁₂	P
		Ethylbenzene	C ₈ H ₁₀	V
		Styrene	C ₈ H ₈	V
		Acetaldehyde	C ₂ H ₄ O	V
	Aliphatic	Furan	C ₄ H ₄ O	V
		Vinyl acetate	C ₄ H ₆ O	V
Propylene oxide		C ₃ H ₆ O	V	
Benzo[<i>b</i>]furan		C ₈ H ₆ O	P	
Catechol (1,2-benzenediol)		C ₆ H ₆ O ₂	P	
3,4-Dihydroxycinnamic acid (caffeic acid)		C ₉ H ₈ O ₄	P	
Aromatic	Safrrole	C ₁₀ H ₁₀ O ₂	P	
	<i>o</i> -Anisidine	C ₇ H ₉ NO	P	
	<i>p</i> -Chloroaniline	C ₆ H ₆ ClN	P	
	2,6-Xyldine (2,6-dimethylaniline)	C ₈ H ₁₁ N	P	
	Dibenz[<i>a,h</i>]acridine	C ₂₁ H ₁₃ N	P	
Nitrogen containing components	Aza-arenes	Dibenz[<i>a,j</i>]acridine	C ₂₁ H ₁₃ N	P

Classification		Compound name	Molecular formula	Present in particulate phase (P) or vapor phase (V) or both (P & V)
N-Heterocyclic amines		7 <i>H</i> -Dibenzo[<i>c,g</i>]carbazole	C ₂₀ H ₁₃ N	P
		2-Aminodipyrido[1,2- <i>α</i> :3',2'- <i>d</i>]imidazole (Glu-P-2)	C ₁₀ H ₈ N ₄	P
		2-Amino-6-methyldipyrido[1,2- <i>α</i> :3',2'- <i>d</i>]imidazole (Glu-P-1)	C ₁₁ H ₁₀ N ₄	P
		2-Amino-1-methyl-6-phenyl-1- <i>H</i> -imidazo [4,5- <i>b</i>]pyridine (PhIP)	C ₁₃ H ₁₂ N ₄	P
		2-Amino-3,4-dimethyl-3 <i>H</i> -imidazo [4,5- <i>f</i>]quinoline (MeIQ)	C ₁₂ H ₁₂ N ₄	P
		2-Amino-9 <i>H</i> -pyrido[2,3- <i>b</i>]indole (AaC)	C ₁₁ H ₉ N ₃	P
		2-Amino-3-methyl-9 <i>H</i> -pyrido[2,3- <i>b</i>]indole (MeAaC)	C ₁₂ H ₁₁ N ₃	P
		3-Amino-1,4-dimethyl-5 <i>H</i> -pyrido [4,3- <i>b</i>]indole (Trp-P-1)	C ₁₃ H ₁₃ N ₃	P
		3-Amino-1-methyl-5 <i>H</i> -pyrido [4,3- <i>b</i>]indole (Trp-P-2)	C ₁₂ H ₁₁ N ₃	P
		<i>N</i> -Nitrosodi- <i>n</i> -butylamine (NDBA)	C ₈ H ₁₈ N ₂ O	V
		<i>N</i> -Nitrosodi- <i>n</i> -propylamine (NDPA)	C ₆ H ₁₄ N ₂ O	V
		<i>N</i> -Nitrosoethylmethylamine (NEMA, MEN)	C ₃ H ₈ N ₂ O	V
		<i>N</i> -Nitrosodiethanolamine (NDELA)	C ₄ H ₁₀ N ₂ O ₃	V
		4-(<i>N</i> -Nitrosomethylamino)-1-(3-pyridinyl)-1-butanone (NNIK)	C ₁₀ H ₁₃ N ₃ O ₂	P
		<i>N</i> '-Nitrosomornicotine (NNN)	C ₉ H ₁₁ N ₃ O	P
		<i>N</i> -Nitrosopiperidine (NPIP, NPP)	C ₅ H ₁₀ N ₂ O	P & V
		<i>N</i> -Nitrosopyrrolidine (NPYR, NPY)	C ₄ H ₈ N ₂ O	V
		Acetamide	C ₂ H ₅ NO	P
		Acrylonitrile	C ₃ H ₃ N	V
		2-Nitropropane	C ₃ H ₇ NO ₂	V
Miscellaneous		Nitrobenzene	C ₆ H ₅ NO ₂	P
		Chloroform	CHCl ₃	V
		<i>p,p'</i> -DDT	C ₁₄ H ₉ Cl ₅	P
		Heptachlor	C ₁₀ H ₅ Cl ₇	P
		Nitromethane	CH ₃ NO ₂	V
		Urethane (carbanic acid, ethyl ester)	C ₃ H ₇ NO ₂	P
		Hydrazine	H ₄ N ₂	V
		Cobalt	Co	P
Inorganic components and metals				

Classification		Compound name	Molecular formula	Present in particulate phase (P) or vapor phase (V) or both (P & V)
		Lead	Pb	P

This list is based upon evaluation of international, as well as domestic cigarettes. The diversities of cigarette blends and design configurations are inherent in this list. Thus, an individual cigarette style marketed in the US would not be expected to contain each of the herein listed compounds. Data were adapted from ref. (33).