

# **Cell and molecular mechanisms of secondhand cigarette smoke-induced inhibition of healing**

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Cigarette smoke is known to inhibit wound healing. We study the effects of second-hand cigarette smoke on the cell and molecular mechanisms of healing in skin, cornea and blood vessels. Skin and cornea wounds are directly exposed to the second-hand smoke, here designated as SSW, whereas the effects on blood vessels occur as a result of exposure to the toxicants in the smoke that are filtered through the lungs and go into circulation. In the skin, we have observed that SSW inhibits fibroblast migration into the wound and disrupts the endoplasmic reticulum, leading to a severe stress response in these cells. In cornea epithelial wounds, we have shown that SSW stimulates inflammation that leads to neutrophil invasion of the stroma which, in turn, prevents wound closure. Inhibition of inflammation by application of dexamethasone partially reverses the smoke effects. The same is observed if neutrophils are depleted by treatment with nitrogen mustard or if proteases secreted by these leukocytes are inhibited by protease inhibitors. We also determined that adhesion and migration of the corneal epithelial cells is inhibited by SSW and can be partially reversed by Thymosine  $\beta$ 4 (T $\beta$ 4). Furthermore, these SSW effects on cornea healing can be completely reversed by application of dexamethasone plus T $\beta$ 4. These findings indicate that people exposed to second-hand smoke and cornea injuries can be treated with anti-inflammatory agents and T $\beta$ 4 both agents approved by the FDA for use in humans.

We have also studied the mechanisms by which second-hand cigarette smoke affects atherosclerotic plaque development and liver metabolism. Mice exposed to second-hand smoke show increased triglycerides and reduced HDL (high-density lipoprotein) in the plasma at the same time that lipid accumulates in walls of large vessels and partially or completely fills small blood vessels of the heart and lungs. Moreover, smoke stimulates the production of pro-inflammatory cytokines and results in a permanent state of generalized inflammation. In addition, second-hand smoke stimulates accumulation of lipids in the liver, leading to non-alcoholic fatty liver disease (NAFLD). Mechanistically, SSW causes NAFLD by inhibiting activity of AMPK, the enzyme that controls the activity of SREBP1, a transcription factor that turns on genes that stimulate lipid synthesis. We are currently looking at the possibility of finding drugs that can reverse the effects of second-hand smoke on liver metabolism and potentially reverse NAFLD.

# **The oviduct and stem cells: what they can tell us about harm reduction products**

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The harmful effects of cigarettes on health were recognized in the 1950s. Cigarette companies subsequently began to develop ideas for making cigarettes that would be less harmful to health. This has spawned a number of harm reduction products which have sometimes been advertized as being safer and better for the users' health than traditional brands of cigarettes. In spite of the availability of harm reduction products for many years, relatively little is known about their actual effect of health.

Strategies for reducing harm have included making "light cigarettes" that are lower in tar and nicotine, curing the tobacco leaf so as to reduce the amount of carcinogens in tobacco and smoke, adding chemicals to tobacco so that it produces fewer carcinogens as it burns, and finally genetically engineering tobacco, for example to have lower or no nicotine. Past work done on humans who smoked light cigarettes has shown that compensatory smoking of light brands can lead to a higher incidence of tumors and thus be more dangerous than traditional brands (Hatsukami et al 2004 *Ann Rev Public Health* 25:377-95). This complication was the first warning sign that harm reduction products may not be as safe as originally claimed.

Our lab has been investigating the toxicity of both mainstream (MS) and sidestream (SS) smoke from traditional and harm reduction cigarettes using a variety of bioassays that measure various physiological process in both oviducts and in embryonic stem cells. Oviducts were chosen as a model for the female reproductive system because they can be explanted from hamsters and studied *in vitro* using four assays developed in our lab. These assays measure ciliary beat frequency, oocyte cumulus complex pick up rate, adhesion of the oocyte cumulus complex to the oviduct, and smooth muscle cell contraction rate (Talbot and Riveles 2005 *Reprod Biol & Endocrinology* 3:25). When a variety of traditional and harm reduction brands were tested in this assay, we found that all inhibited oocyte pick-up rate and that MS smoke from one of the harm reduction brands was more potent than MS smoke from any of the traditional brands that were tested (Riveles et al 2007 *Hum Reprod* 22: 346-355). We further found that SS smoke was more inhibitory than MS smoke in the oocyte pick-up rate assay. Smooth muscle contraction was likewise inhibited by both MS and SS smoke in the oviductal bioassays, and smoke from all brands, including the harm reduction brands, was effective at inhibiting contraction rate. These findings, when taken together, show that both traditional and harm reduction smoke alter oviductal physiology in a manner that impairs pick-up of ovulated oocytes and movement of embryos through the oviduct to the uterus. Our observations on muscle contraction and embryo movement were further confirmed *in vivo* in female hamsters that smoked during pregnancy. Impeding these processes in women could lead to ectopic pregnancy, which is known to increase in smokers and which is the leading cause of death among pregnant women (Bouyer et al 2003 *Am J Epidemiol* 157:185-194).

In addition to examining the female reproductive tract, we are developing a model to study the effects of cigarette smoke on embryos at the earliest stages of development, which are likely to be the most sensitive to environmental toxicants. We have used mouse and human embryonic stem cells to model pre-implantation embryos and found that MS and SS smoke from both traditional and harm reduction cigarettes impairs attachment of stem cells to Matrigel. Moreover, in the mouse system, smoke decreased survival and proliferation and caused apoptosis in both stem cells and pre-implantation mouse embryos (Lin et al 2009, *Hum Reprod* 24:386-72). Of particular importance, we observed that SS smoke from the harm reduction brands was generally more toxic than SS smoke from the traditional brand. These data demonstrate the need for a better understanding of harm reduction products and in particular a better understanding of SS smoke produced by harm reduction cigarettes.

# Hyperpolarization: the emerging techniques for real time imaging and spectroscopy *in vivo*

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The dominant diagnostic role of MRI is assured as the full range of physical NMR-properties, apart from chemical shift, is played out using 80M protons of water. Chemical shift, spin coupling, rf-editing, water suppression, localization and heteronuclear NMR contribute to diagnostic MR spectroscopy of over 100 human disease states (Alzheimer's, epilepsy, multiple sclerosis, inborn errors, infections) and therapeutic monitoring of cancer in thousands of patients. Typically the spins providing MRS diagnosis are four orders of magnitude lower abundance than water (lactate=1mM; choline = 2mM; ATP =3mM; glutamine=4mM). Hyperpolarization (High Definition Magnetic Resonance) techniques now available can close the SNR-gap for diagnostic MRI/MRS by delivering the four orders of magnitude NMR signal enhancement to  $^{13}\text{C}$ ,  $^{15}\text{N}$ ,  $^{31}\text{P}$  or indirectly to  $^1\text{H}$ .

My laboratory has pioneered the development of HD-MR reagents using Parahydrogen Induced Polarization (PHIP) technique in past four years. The molecules hyperpolarized by this relatively inexpensive and easy-to-install technology has shown promises for real time molecular imaging of several types of cancers and atherosclerotic plaque *in vivo* in mouse models. My talk will focus on the development of these reagents and their biomedical applications.

It is remarkable that MR has contributed so much to our biochemical understanding using only part-per-million polarizations. HD-MR may open up an entirely new regime wherein the local status of a tumor or atherosclerotic plaque *in vivo* may be interrogated on time scale of seconds to minutes with unprecedented chemical specificity. This is a new tool of vastly improved sensitivity and has the potential to detect the early stages of cancer and cardiovascular diseases and monitor treatment efficacy. Because of sensitivity gain in HD-MR, these trials can be accomplished in very short times in order of minutes as opposed to over hours. This can potentially lead to high-throughput screening of diseases in a clinical setup.

# Searching for a new biomarker of HDL functionality for cardiovascular disease risk assessment

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Tobacco smoke is the 4<sup>th</sup> cause of death in United States and the only fully preventable one. Among tobacco smoke-related diseases, cardiovascular disease (CVD) is the primary killer. One of the mechanisms by which tobacco smoke facilitates arteriosclerosis and increases the risk of cardiovascular disease is by damaging lipoproteins. Tobacco smoke-derived chemicals directly promote lipoprotein modifications. Furthermore, tobacco smoke exposure increases oxidative damage to lipoproteins by causing cells within atherosclerotic plaques to undergo an immune response that produces enzymes such as myeloperoxidase (MPO), which generate highly oxidative compounds.

High density lipoproteins (HDL) are cardiovascular protective lipoproteins and a substrate of tobacco smoke-induced modifying reactions. The anti-atherogenic function of HDL is attributed to its role in reverse cholesterol transport (RCT), the process by which excess cholesterol is transferred from peripheral cells to the liver for excretion. In the intima layer of the arterial wall, where atherosclerotic plaques build up, cholesterol is mobilized from cholesterol overloaded macrophages by the action of lipid-free/lipid-poor apolipoprotein A-I (apoA-I) via the membrane ATP binding cassette transporter A-1 (ABCA1). However, more than 95% of apoA-I is lipid-associated on mature HDL. Therefore, to initiate RCT apoA-I needs to transition from the HDL-associated state to the lipid-free/lipid-poor (ABCA1 efflux capable) state, which we refer to as apoA-I 'exchangeability'.

Using a fluorescence resonance energy transfer (FRET) based assay, we demonstrated *in vitro* that apoA-I 'exchangeability' is impaired by crosslinking or oxidative reactions, which are similar to those occurring in pathological states involving increased oxidation and inflammation, such as tobacco smoke and diabetes. In particular, MPO-mediated oxidation of HDL reduced 20 folds the rate by which lipid-free/lipid-poor apoA-I exchange off HDL. These results suggest that in the developmental stage of atherosclerosis, dysfunction of HDL in the form of reduced apoA-I 'exchangeability' could be a leading cause of disease progression, by limiting the amount of lipid-free/lipid-poor apoA-I available in the intima for supporting RCT.

In the clinic, the only routine assay of HDL is the measure of HDL-associated cholesterol (HDL-C). HDL-C levels do not always correlate with incidence of CVD mainly because HDL functionality, rather than HDL-C levels *per se*, contribute to lowering CVD risk. Thus, there is a need for clinically viable methods for measuring dysfunctional HDL. Our data suggest that apoA-I 'exchangeability' could be a clinical marker of cholesterol efflux efficiency that correlates with incidence of CVD.

The current assay to measure apoA-I 'exchangeability', however, is not suitable for analyzing clinical samples because other biomolecules present in blood interfere with the measurement and prevent data interpretation. To allow the measurement of apoA-I 'exchangeability' in clinical blood samples we have been developing a fluorescent variant of apoA-I that will be free from the interference of natural fluorophores and capable to report on the apoA-I lipidation state (lipid-free vs. lipid-associated). Full length apoA-I is obtained by ligation of two separately expressed half sequences, apoA-I(1-135) and apoA-I(136-243). A Cys in position 19 of apoA-I(1-135) is labeled with the FRET donor fluorophore before ligating the N- and C-terminal fragments using established expressed protein ligation (EPL) techniques. EPL of the two fragments generates a Cys residue in position 136 of the final full-length apoA-I that is reacted with the FRET acceptor fluorophore.

Preliminary results show that the two apoA-I fragments can be expressed and purified in high yields. The new apoA-I reporter resulting from labeling and ligation of the two apoA-I fragments will be used to determine in human blood samples if tobacco smoke impairs apoA-I exchangeability and if this, in turn, reduces cholesterol efflux efficiency.

Our research improved the understanding of the molecular mechanisms that link tobacco smoke-induced 'oxidative stress' to cardiovascular disease. The new apoA-I fluorescent reporter will validate apoA-I 'exchangeability' as a novel clinical marker for assessing atherosclerosis risk.

# **Peptidomimetic drug design for age-related macular degeneration, a disease linked to smoking**

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Age-related macular degeneration (AMD) is a disease that affects more than 25 million people worldwide, with onset being typically part of normal aging. Smoking is a high-risk factor for AMD and so is cardiovascular disease which is linked to smoking. A genetic predisposition for AMD has been located on the complement system Factor H (FH) gene, involving a tyrosine-to-histidine single nucleotide polymorphism on chromosome 1. The complement system is part of innate immunity and a link between innate and adaptive immunities. Proper regulation of the complement system, by FH and other regulators with similar structural characteristics to FH, is essential for the recognition of self from nonself by the immune system. We have contributed to the development of potent inhibitors of the complement system, targeting the central protein component C3 and its cleavage fragments C3b and C3a. The complement system is activated through three different pathways which converge to protein C3 and subsequently merge to a common pathway. C3 is also a central component for complement system regulation by FH and other regulators. Thus, C3 and its fragments are excellent targets for drug design. The compstatin family peptides and peptidomimetics are excellent examples of complement inhibitors that target AMD, with one being now in clinical trials. We have used nuclear magnetic resonance (NMR) spectroscopy to delineate the structure of several compstatin peptides and peptidomimetics and to generate structure-activity relations. We have introduced structural and physicochemical perturbations in compstatin analogs by systematically replacing key amino acids to delineate their role in structural stability and activity. These studies were responsible to generate sequence templates discriminating amino acids that are indispensable for structure and activity from those that are amenable to further optimization. These sequences and structures formed the basis for computational optimization and rational design studies, which were the springboard to generate the most active peptides and peptidomimetics known to date. We have also developed quasi-dynamic pharmacophore models, based on molecular dynamics simulations, to incorporate peptide conformational flexibility and conformational interconversion in our design. We will present a new generation of potent compstatin analogs which have been developed jointly with the group of professor Chris Floudas of Princeton University, using the structure of C3c-bound compstatin, computational optimization data, rational design, and surface plasmon resonance data. The new analogs have novel sequence, structural, and physicochemical characteristics, which we will present in detail. We will also present our efforts in designing non-peptidic low-molecular mass C3 inhibitors, C3a receptor inhibiting peptides, and determining the structure of a previously known potent C5a receptor antagonist peptidomimetic by NMR. Finally, we will present our electrostatic analysis of FH and how electrostatics affects the interaction of FH with its target protein C3b.

# Targeting PTHrP/PPAR $\gamma$ signaling to prevent nicotine-induced lung injury in the newborn

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Despite known risks and enthusiastic antismoking campaigns, 12% of the U.S. women still smoke during pregnancy, resulting in the birth of approximately 450,000 smoke-exposed infants/year. Due to the lack of molecular understanding of how smoking during pregnancy affects fetal growth, development, and function, it is not surprising that there is no effective intervention to prevent the damaging effects of smoke exposure on the developing fetus. With Tobacco Related Disease Research Program' support and through innovative thinking and the use of the state-of-the-art methods, we have provided seminal insights to normal lung development and molecular mechanisms involved in nicotine-induced lung damage during perinatal period, and these were the focus of my presentation at the Mini Symposium at the University of California, Riverside, on September 25, 2009.

Pulmonary alveolar epithelial-mesenchymal interactions driven by Parathyroid Hormone related Protein (PTHrP) and Peroxisome Proliferator Activated Receptor  $\gamma$  (PPAR $\gamma$ ) that determine the formation of the alveolus culminate in homeostatic control of the alveolar acinus as well. Molecular disruption of this homeostatic pathway results in transdifferentiation of alveolar interstitial fibroblasts (AIFs) to myofibroblasts (MYFs). Using both in vitro and in vivo studies, we have determined the molecular mechanisms involved in nicotine-induced AIF-to-MYF transdifferentiation, and have discovered novel intermediates involved in this process. Using a variety of development and lung injury models, we have demonstrated that nicotine not only induces transdifferentiation of pulmonary AIFs to MYFs, but also has significant molecular, metabolic, and functional effects on alveolar type II cells. This work for the first time has revealed specific molecular pathways involved in lung injury following in utero nicotine exposure, highlighting the break-down of the homeostatic PTHrP/PPAR $\gamma$  epithelial-mesenchymal signaling in in utero nicotine-induced lung injury. This process is driven by PKC activation, which in turn leads to activation of the canonical Wnt signaling during nicotine-induced AIF to MYF transdifferentiation.

These molecular insights to AIF-to-MYF transdifferentiation led us to try rosiglitazone, a PPAR $\gamma$  agonist, as an intervention strategy to prevent nicotine-induced lung injury under both in vitro and in vivo conditions. This work has very clearly demonstrated that PPAR $\gamma$  agonists can not only block the molecular injury following in utero nicotine exposure, but can also prevent the pulmonary functional consequences of this molecular injury, providing a FDA approved drug to prevent in utero nicotine-induced lung damage. This is clearly significant since this intervention can potentially prevent asthma in the offspring following exposure to maternal smoke during the perinatal period. Furthermore, our data show that this intervention not only blocks, but also reverses the in vivo nicotine-induced lung injury.

Lastly, since there is growing evidence that bone marrow derived mesenchymal stem cells (BMDMSCs) are the key players in lung injury/repair, we have generated data showing the effects of *in utero* nicotine exposure on BMDMSC differentiation and the prevention of these effects by PPAR $\gamma$  agonist administration either alone or in combination with Wnt antagonists. These novel insights are likely to be pivotal in further advancing our understanding of the nicotine-induced injury not only in the lung, but also other organs, possibly allowing us to design novel interventions that target specific additional molecular intermediates involved in nicotine-induced organ damage.

# Chemistry and biology of DNA adducts arising from reactive aldehydes in tobacco smoke

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Tobacco smoke contains large quantities of reactive aldehydes including formaldehyde, acetaldehyde, acrolein, methylglyoxal and glyoxal. All these aldehydes can couple with nucleobases to give DNA adducts. In this presentation, we place our emphasis on the DNA adducts induced by methylglyoxal and glyoxal, both of which can couple with guanine to render  $N^2$ -(1-carboxyethyl)-2'-deoxyguanosine ( $N^2$ -CEdG) and  $N^2$ -carboxymethyl-2'-deoxyguanosine ( $N^2$ -CMdG). We developed synthetic routes for the site-specific incorporation of  $N^2$ -CEdG and  $N^2$ -CMdG into oligodeoxyribonucleotides (ODNs) as well as for the preparation of the stable isotope-labeled derivatives of the two nucleosides. The latter nucleosides were employed as internal standards for assessing quantitatively the formation of these two types of DNA lesions in human cells that are exposed with methylglyoxal and glyoxal. The quantification data revealed the dose-dependent formation of the two lesions in human cells.

The lesion-containing ODNs were further incorporated into single- and double-stranded shuttle vectors for in-vivo replication studies using bacteria and mammalian cells as hosts. In this respect, the lesion-carrying vectors, along with the lesion-free control vectors, were transfected into, and allowed for replication in host cells. The progeny vectors were subsequently isolated, PCR amplified, and the PCR products were digested with restriction enzymes. The restriction fragments were then sequenced by mass spectrometry to identify and quantify the replication products, which facilitated the determination of the bypass efficiencies and mutation frequencies of these two lesions in both bacteria and mammalian cells (1-4). Our results revealed that both  $N^2$ -CEdG and  $N^2$ -CMdG could block DNA replication, with the blocking effect being more pronounced in bacteria than mammalian cells (5). In addition, they could induce G→T and/or G→A mutations. Together, the combined chemical and biological approaches allowed for the molecular-level understanding about the biological implications of the major DNA adducts arising from the exposure to tobacco-derived methylglyoxal and glyoxal.

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# **Detrimental effects to the hearing organ after mild chronic exposure to carbon monoxide (a major component of tobacco smoke)**

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Carbon monoxide (CO) is an ever-present gas in the air of our environment, and a significant product of the incomplete combustion of fossil fuels, tobacco products, and industrial activity. Exposure to high CO concentrations (1000 ppm and higher) may be the cause of more than 50% of fatal poisonings in many industrial countries. Several studies on humans (prenatal and postnatal periods) have provided data to show that CO and cigarette smoke affects normal growth and development. The developing central and peripheral nervous system is extremely susceptible to the reduction of oxygen availability produced by CO exposure, and both neurobehavioral and neurochemical alterations have been found in male rat offspring exposed to low levels of CO (75-150 ppm) during gestation (Lopez et al., *Neuroscience* 151:854-67, 2008, Lopez et al., *BMC Neuroscience*, 10:53,1-28, 2009).

*Studies in the hearing organ the cochlea.* Our first studies with our developing rat model showed that chronic exposure to mild concentrations of CO (12 to 50 ppm) in air during the first 3 weeks of postnatal life caused auditory deficits that continued into adulthood (Lopez et al., *J Neurosci. Res.* 74:666-75, 2003; Stockard-Sullivan et al., *J Neurosci. Res.*, 74:644-54, 2003; Webber et al., *J Neurosci. Res.*, 80:620-33, 2005). There was a significant attenuation of the amplitude of the 8th action potential at 50 ppm, which did not recover completely by 73 days of age. Overall the morphology of the cochlea from these animals was normal from the base (where high frequency sounds are detected) to the apical portion (where low frequency sounds are detected). Immunoreactivity for neurofilament proteins was diminished in the somata and fibers of spiral ganglia neurons (SGN). We have recently examined the expression of several proteins significant to oxidative stress and relevant to cochlear development in rats exposed to 25 ppm over the prenatal period embryonic day 5 (E5) to E20 (Lopez et al., *Neuroscience* 151:854-67, 2008). From postnatal day 5 (P5) to P20 after prenatal chronic mild CO exposure there was an increase in inducible nitric oxide synthase (iNOS) and nitrotyrosine in the cochlear vasculature, suggesting that this structure may be the primary target for oxidative stress with consequent morphological deterioration of spiral ganglia neurons (SGN) and inner hair cells.

These results led us to investigate the molecular mechanisms that the inner ear may recruit to protect the sensory hearing organ, the cochlea from chronic very mild CO exposure. A novel tissue globin, neuroglobin (Ngb) was recently identified in mammals. Ngb is located primarily in the brain and in high concentration in the eye, and plays an essential role in oxygen homeostasis in neuronal tissue. We described for the first time the localization of Ngb in the cochlea of the normal rat. We investigated changes in Ngb expression after chronic very mild prenatal and postnatal CO exposure (25 ppm in air). We also determined whether cytochrome-C may also be affected by mild CO exposure. We found that Ngb was expressed in different types of cells of the cochlea including spiral ganglia neurons (SGN) but not in the inner or outer hair cells. After prenatal CO exposure only, and pre- and postnatal CO exposure, Ngb immunoreactivity and mRNA expression were decreased in the SGN. Cytochrome-C protein immunoreactivity decreased in a similar fashion to Ngb (Lopez et al 2009 in revision *Brain Research*). We have extended our studies using the mice model using similar CO exposure paradigms. In addition to the presence of oxidative stress we have been able to identify the cellular targets affected by mild CO. They are specialized structures relevant for potassium transport "the stria vascularis" and we corroborate our findings in the rat regarding the damage to the spiral ganglia neurons (the primary afferents) that send information from the inner ear to the brain for proper hearing.

*Relevance of mild CO concentrations.* The chronic mild CO exposures being used in our studies, (intermittent and averaging about 12 hours in each 24 hour period) are not toxic (poisonous) but at amounts below the upper limits identified by most regulatory agencies as previously indicated. The exposures are the same or less than that reported for the home environment especially where gas appliances are used, and below the concentrations indicated to prevail in active tobacco smokers who are pregnant or breast feed their offspring. For example, no standards for CO exposure have been agreed upon for indoor air and the EPA indicates that average CO levels in homes without gas stoves vary from 0.5 to 5 ppm, (5 ppm is 0.0005% CO in air) (<http://www.epa.gov/iaq/co.html>). We have yet to determine the most sensitive cellular systems affected by making similar studies at 5 ppm; a concentration below the lowest upper level identified by the EPA for ambient air. Our current studies are done at exposures of 25 ppm. We are extending our studies by exposing mice to 100

ppm, to investigate the cellular and molecular effects of CO at 5, 25, and 100 ppm, and long term studies in which animal ear and cerebella will be studied from 6-18 months after CO exposure.

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## **Thirdhand smoke exposure: An unappreciated public health hazard**

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Cigarette smoke, depending upon the temperature of tobacco combustion, is of two major types: (1) mainstream smoke (MSS) generated at approximately 1,200-1,600°C when a smoker inhales the lit cigarette, and (2) sidestream smoke (SSS) generated at much lower temperature from the smoldering end of the cigarette. Secondhand smoke (SHS), is the third type of tobacco smoke which consists of exhaled smoke (15%) and SSS (85%), to which most non-smokers are exposed when close to a smoker or in the indoor space where active smoking occurs. In the last 50 years, a lot of research has been done to study the chemical toxicants present in the MSS, SSS and SHS that cause several tobacco-related diseases such as cardiovascular disease and stroke, cancer, pulmonary disease, and maladies of the reproductive system, developmental defects in babies born to smoking mothers, and age-related macular degeneration, among others.

Fresh MSS, SSS, and SHS are very different from one another in chemical composition, due mainly to different content of volatile and semi- or non-volatile chemicals, and are known to cause pathological damage to varying extent and magnitude on organs and systems in smokers and non-smokers exposed involuntarily to SHS.

Thirdhand smoke, a newly emergent type of tobacco smoke, is the tobacco smoke adsorbed on surfaces where the semi-volatile and non-volatile chemicals undergo modifications to produce new toxicants, which later become desorbed and appear in the micro-environment, posing public health hazards, long after smoking activity has ceased. In other words, mature and stale secondhand smoke adsorbed on surfaces, and constantly changing in chemical composition is thirdhand smoke.

Based on the tobacco industry documents, data from animal experiments conducted by Philip Morris Company documented that fresh SSS is approximately 4-times more toxic (per gm of tobacco) than MSS; tobacco smoke that is 30-minute-old is approximately 2-4 times more toxic than the fresh SSS (per gm tobacco); and the SHS – a mix of aged and fresh tobacco smoke is approximately 6-12 times more toxic than the cigarette smoke inhaled by smokers. Clearly, the process of “aging” of tobacco smoke makes the stale SHS smoke, and therefore, the thirdhand smoke, much more toxic than SHS. The only explanation for this fact is that the “aging” of SHS adsorbed on surfaces gives rise to new toxicants as yet unknown to the scientific community and the public.

Nicotine, the addictive substance in tobacco smoke, has until now been considered as non-toxic in the strictest sense of the term. However, this belief is fast changing by recent studies by Singer et al. (*Atmos. Environ.*, **38**:2483-2494, 2004) and Destailats et al (*Environ. Sci. Technol.*, **40**:1799-1805, 2006) showing that oxidation of adsorbed nicotine by atmospheric ozone generates new toxicants such as formaldehyde, N-dimethylformamide, and nicotinaldehyde, as well as the cancer causing nitrosamines.

California TRDRP is developing a new initiative on thirdhand smoke exposure and health risk assessment. This initiative will encourage scientists to conduct research on thirdhand smoke for better understanding of its chemistry, molecular toxicology, exposure measurements and route of exposure in hotel rooms, rental apartments, motor vehicles and casinos, and to develop physical and physiological metrics for health risk assessment among vulnerable populations including children, hotel room cleaning personnel, passengers in cars in which smoking has occurred, and workers in casinos where smoking is permitted. Research studies will also be directed at the economic impact and policy implications involving thirdhand smoke.