Some Recent Topics in Cigarette Smoke Science

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Abstract: This short review summarises some fundamental aspects of cigarette combustion and smoke formation, including how cigarettes burn, how cigarette smoke is formed and the complex and reactive nature of it's composition. Particular emphasis has been placed on important factors which have to be controlled when generating, trapping and analyzing cigarette smoke. Examples are provided which demonstrate the sensitivity of cigarette smoke composition to the way it is produced and measured, a subject of particular importance for redox sensitive species such as free radicals and multiple valency-state metals. Recent regulatory interest in smoke constituent yields is summarized, as well as risk assessment approaches which have sought to identify the smoke constituents which make the greatest contribution to smoking related diseases. Limitations of these approaches are discussed, and a number of other aspects of cigarette smoke that have been suggested to contribute to the incidence of smoking related diseases are highlighted.

Keywords: Cigarette, Smoke Chemistry, Combustion, Toxicants.

INTRODUCTION

The purpose of this review is to provide readers of this special issue with an update on some key topics associated with the subject of cigarette smoke free radical chemistry. A detailed understanding of the complexities of cigarette smoke chemistry is essential for any scientist interested in free radical reactions in cigarette smoke or their possible biological consequences. Comprehensive reviews on cigarette smoke chemistry have been published [1-5], which collectively represent our current understanding of this field. However, on-going research in a number of areas of cigarette combustion science is continuing to deepen our understanding of the formation, dynamics and consequences of smoke chemistry.

This article briefly summarises current understanding of how a cigarette burns and how smoke is formed. Key principles of smoke chemistry, with its diversity of constituents, reactivity, and analytical challenges are also described. One of the prime drivers of research into the chemistry of cigarette smoke is its toxicity. Recent progress in identification and prioritisation of the smoke constituents potentially associated with the on-set of smoking related diseases is discussed, together with current regulatory proposals to disclose and limit toxicant yields from cigarettes. Some limitations of current prioritisation approaches are highlighted, and other recent proposals of a number of cigarette smoke attributes which may contribute to its toxicity, including the presence of free radicals, are critiqued.

THE BURNING CIGARETTE

Cigarette burning processes and the resulting smoke chemistry are strongly influenced by both cigarette design variables [6-10] and the smoking conditions employed (whether human or smoking machine mediated). Cigarette smoke is a highly concentrated and dynamic aerosol system consisting of several thousand chemical substances, which are distributed and partitioned between the aerosol particles and the gases surrounding them. The complexity mainly results from multiple thermolytic processes that occur within the confines of the cigarette and from the complex chemical composition of tobacco. The thermolytic processes involve pyrolysis, combustion, and distillation. Once formed, the tobacco smoke aerosol within the rod is subject to a number of processes including diffusion (room temperature as well as thermally assisted via virgin and semi-charred tobacco paper), condensation, filtration and elution [2].

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According to Baker [2], when a cigarette is lit the tobacco temperature rises rapidly and a hot carbonaceous coal forms at the lit end of the cigarette. Peak puff temperatures of the coal can exceed 900°C during a 2-second puff [11, 12]. At the end of a puff the coal temperature declines in-line with the reduction in air flow. The temperature of the coal continues to fall for up to about 15 seconds after the end of the puff; it reaches a value of around 600 - 700 °C, which is approximately stable until the start of the next puff. The temperature of the tobacco falls significantly within a few millimetres downstream of the coal, and during smoking most of the tobacco rod is at approximately ambient temperature. The sequence of puffing and smouldering consumes the tobacco at different rates but the net effect is a progressive movement of the coal towards the mouth end of the cigarette. The chemical composition of the burning tobacco is modified during the puffing and smouldering sequence: volatile components are released from the combusting solid, the degrading tobacco carbonises, incoming air oxidises the carbonised material, and an inorganic residue is left in the form of cigarette ash [13].

The high temperature inside the coal during a puff causes an increase in the viscosity of the air flowing through the coal and a concomitant increase in the draw resistance of air through the cigarette. This effect forces air to be drawn primarily into the periphery of the coal at the paper burn line rather than through the axial centre of the coal. This air flow pattern results in the formation of a region immediately behind the coal that is depleted in oxygen but still hot enough to promote thermal decomposition of the tobacco. Large amounts of volatile and semi-volatile smoke constituents evolve from this zone resulting, in part, from the thermal decomposition of tobacco, and in part from distillation of volatile constituents native to tobacco; this area of the cigarette is therefore termed the pyrolysis/distillation zone. Cigarette smoke begins as a supersaturated vapour behind the burning tobacco coal; the rapid decline in temperature that the vapour experiences as it travels away from the coal down the tobacco rod, together with the presence of nucleating sites within the gas causes condensation of vapour into aerosol particles of varying sizes.

The smoke that is drawn through the cigarette rod during a puff is subjected to is filtered by the remaining tobacco rod and the cigarette filter. As the smoke travels down the tobacco rod the smaller particles also grow due to coagulation and condensation; larger droplets have sufficient inertia to avoid collision with other particles but they are partially removed by impaction onto the tobacco and filter fibre surfaces. Smoke yields are generally lower in the first puff than in later puffs, due to the time required to form the coal and more extensive filtration of smoke by the tobacco rod, although exceptions do exist [14, 15]. In the final puffs of a ciga-

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Table 1. The Main Classes of Chemical Compounds in Cigarette Smoke [5]

Hydrocarbons	Nitriles
Alcohols and Phytosterols	Acyclic Amines
Aldehydes and Ketones	Amides
Carboxylic Acids	Imides
Esters	*N*-Nitrosamines
Lactones	Nitroalkanes, Nitroarenes, Nitrophenols
Anhydrides	Nitrogen Heterocyclic Components
Carbohydrates & their derivatives	Permanent Gases (N_2, CO_2, CO, etc.)
Phenols and Quinones	Metallic elements, Isotopes, Ions, Salts
Ethers	Nucleotides, Free Radicals

rette, the smoke yields are generally higher due to the shorter filtration path that the smoke traverses and redistillation of smoke filtered in earlier puffs. Fresh smoke emerging from the mouth-end of the cigarette is termed "mainstream smoke" and consists of 10^{10} particles per cm^3 and the majority of the aerosol particles have a particle size between 0.1 to 1.0 μm [2, 16, 17].

A second stream of smoke emerges from the cigarette at the area of the paper burn line during the smoulder period, and is termed "sidestream smoke". At the paper burn line the porosity of paper increases significantly as its main component cellulose decomposes, allowing vapour from within the cigarette to escape. The vapour emerging from the paper burn line cools rapidly in the more dilute atmosphere outside of the cigarette, which leads to the formation of a plume of relatively small aerosol particles which become visible below 150 °C. The smoke plume is drawn back towards the coal through convection, before forming the visible plume of smoke which is driven upward away from the cigarette by buoyancy. The properties of both mainstream and sidestream aerosol particles and the overall chemical composition of the smoke continue to change after formation and exiting the cigarette.

CHEMICAL COMPOSITION OF CIGARETTE SMOKE

A critical challenge in studying any effect of cigarette smoking has always been to identify and quantify chemical constituents present in smoke [1, 5]. The total number of distinct chemical species present in cigarette smoke is unknown. Some researchers have speculated that as many as 100,000 compounds may be present in smoke [18]. Recent high resolution GCxGC-TOF analyses separated approximately 30,000 peaks from a mainstream smoke sample [19], although not all of these peaks were distinct chemical species.

The efforts and advances made over the past 60 years in identifying individual smoke constituents have recently been summarised by Rodgman and Perfetti [5]. They have compiled a list of over 5300 identified compounds in cigarette smoke, representing almost all known organic chemical classes (Table **1**), including saturated and unsaturated hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids, esters, phenols, nitriles, terpenoids, and alkaloids.

The number of identified species is certain to increase with advances in analytical chemistry techniques, as well as our demand for a deeper understanding of smoke chemistry. However, experience to date has shown that an understanding of cigarette smoke composition cannot be achieved without due consideration of the following factors:

- Methods for smoke generation
- Choice of smoking regimes
- Approaches used for trapping smoke
- The reactivity of smoke

Smoke Generation

Laboratories in the tobacco industry and specialised contract laboratories have developed and refined analytical procedures for the repeatable and reproducible generation of cigarette smoke (ISO 3308 - Routine Analytical Cigarette-Smoking Machine - Definitions and Standard Conditions; ISO 3402 - Tobacco and Tobacco products - Atmosphere for Conditioning and Testing; ISO 4387 - Cigarettes - Determination of Total and Nicotine-Free Dry Particulate Matter Using a Routine Analytical Smoking Machine). Fundamental to the study of cigarette smoke is the use of a smoke production system, which reproduces the basic phenomena occurring in cigarette smoking by humans, i.e. the pulsed nature of smoking with short term, intermittent high temperature puffs and a longer, lower temperature smouldering, these different thermal events have important implications for the yields and relative chemical composition generated, as different smoke constituents tend to have very different formation temperatures and rates. Researchers sometimes employ systems such as a vacuum pump to continuously draw on a lit cigarette to produce smoke samples; such an approach overly simplifies the smoke formation process by not reproducing the thermal events of smoking and by imposing a non-representive flow pattern during the draw. The current generation of commercial smoking engines (e.g., www.borgwaldt.de, www.cerulean.com, and www.tricitymachineworks.com) are highly sophisticated devices capable of smoking cigarettes with a high level of repeatability and reproducibility. They are the result of many years of development work which have resulted in reliable standardised approaches with which to smoke cigarettes [20]. These methods define a number of important environmental parameters (e.g. cigarette moisture levels, atmospheric humidity, temperature and air-flow surrounding the test cigarettes); all of which are known to affect the quantity and relative composition of the smoke produced. When employed correctly the methods for quantifying tar, nicotine and carbon monoxide in mainstream smoke can result in very low repeatability and reproducibility errors [20, 21].

Smoking Regimes

However, all machine-smoking approaches have their limitations [4, 22] particularly the inability to reflect the diversity of human smoking patterns, and exposure to smoke. In an effort to provide smoke yields more representative of human exposure than the ISO/FTC regimes, a range of routine smoking regimes (Table **2**) have been proposed [4]. The newer regimes tend to be described as "intense" smoking regimes, and feature larger puff volumes (hence faster flow rates), more frequent puffing, and partial or complete blocking of the ventilation holes in the cigarette filter. Nevertheless, despite the range of parameters employed researchers have concluded that none of these regimes fully reflect the exposure that all smokers receive from the range of cigarettes used globally [23].

Table 2. Machine Smoking Regimes Proposed by Various Regulatory Authorities [4]

	FTC Method	ISO 4387	Massachusetts Method	Canadian "Intense"
Puff volume (cm^3)	35	35	45	55
Puff frequency (s)	60	60	30	30
Puff duration (s)	2	2	2	2
Ventilation blocking (%)	0	0	50	100

Table 3. Trapping Techniques Used in the Analysis of Cigarette Smoke [21]

Analyte Group	Trapping Media
Tar, Nicotine, Phenolics, TSNAs, Benzo[*a*]pyrene	Cambridge Filter Pad (CFP)
Aromatic amines	a) CFP b) Impinger
Semi-volatiles (pyridine, quinoline, styrene)	a) CFP/methanol-triethylamine impinger b) CFP/methanol impinger c) Acid treated CFP/methanol impinger d) CFP/XAD-4 sorbent tube
HCN	a) CFP/impinger b) Impinger c) CFP/Ascarite trap d) CFP/activated silica gel e) CFP/glass tube with NaOH on support f) Glass syringe
Carbonyls	a) CFP/impinger b) Impinger c) Wash bottles d) CFP+2 DNPH treated filter pads e) Activated silica gel
Selected Volatiles (1,3 butadiene, isoprene, acrylonitrile, benzene, toluene)	a) Glass syringe b) CFP/methanol impinger c) CFP/ethanol impinger d) CFP/Tedlar bag
Ammonia	a) CFP/Impinger b) Impinger c) Separatory funnel d) CFP/solid sorbent tube e) Electrostatic precipitator/impinger
CO	a) Gas bag
NO	a) None – gas analysis puff by puff of smoke gas phase b) None – gas analysis puff by puff of diluted smoke gas phase c) Gas collection bag

Of the machine smoking parameters that have been proposed the so-called Canadian "Intense" method has become the focus of recent attention, and is an emerging global standard [23, 24]. However, much of the existing understanding of smoke formation mechanisms has been developed from experiments conducted under conditions related to ISO 4387. More intense smoking regimes change the thermal history of burning cigarettes, resulting in relative increases or decreases in the chemical compositions [25-27] which are difficult to interpret based on the current understanding of cigarette combustion. Consequently, many of the established principles of tobacco combustion and smoke chemistry may need to be re-evaluated under these revised smoking parameters.

Smoke Trapping Techniques

Routine analyses of cigarette smoke constituents have concentrated on trapping and accumulating smoke from multiple cigarettes, either to provide sufficient material for quantification or as a means of minimising variability. The most extensively measured aspects of cigarette smoke, e.g., the tar (or nicotine-free-dry-particulate matter) and nicotine assays conventionally employ glass fibre pads (Cambridge Filter Pads, or CFPs) to trap the particulate matter. Gases such as carbon monoxide are analysed using gas sampling bags. In recent years, there has been increased focus on minor smoke constituents with potential toxicological relevance, and a diversity of approaches [20, 21, 28] for their trapping and analysis have been adopted (Table **3**).

Trapping methods for particulate phase compounds are consistently based around CFPs as they are > 99% effective as traps for particulate matter from cigarettes [29]. However, there is considerable diversity in the range of extraction solvents and analysis techniques used. The more volatile compounds show a greater diversity of trapping and analysis techniques. Unless standardised methods are developed for the quantification of these smoke constituents, significant inter-laboratory or inter-study variability will continue to exist [20, 21].

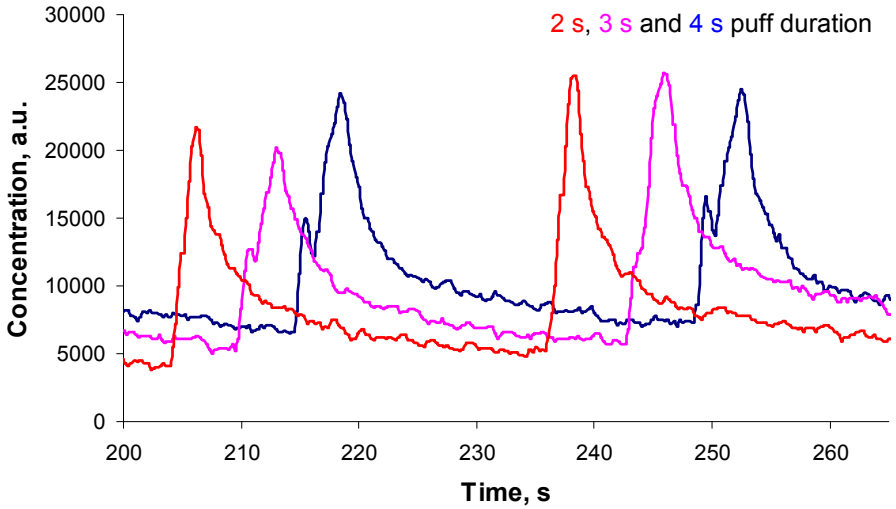

Fig. (1). Consecutive puff-resolved profiles of benzene acquired at three puff durations. 35 mL puff, once every 30 s, 3R4F research reference cigarette [42].

The diverse range of trapping techniques described above rely either on the inherent stability of the species under investigation, or or rely on some aspect of their chemical reactivity to effect an efficient trapping arrangement. While convenient in some cases for trapping stable smoke constituents, the reactivity of other constituents is a factor that has to be borne in mind when studying smoke, as the introduction of artefacts is potentially a major issue [4, 29].

Reactivity of Smoke

It has been known for some time that separating smoke into gaseous and particulate phases by using Cambridge filter pads can create artefacts during smoke analysis [4, 29], as the procedure prevents interactions between smoke constituents residing in the two phases. With most common smoke analysis methods a considerable amount of time elapses between smoke generation and quantification. Ageing of smoke, a phenomenon that refers to any change in smoke composition during the period between smoke leaving the cigarette filter, and its subsequent analysis is also a potential source of artefacts. This is particularly true for highly reactive species such as free radicals and nitroso compounds [30-33].

One aspect of the chemical reactivity of cigarette smoke which has not been studied extensively to date is the redox behaviour of smoke and how it changes post formation, as first reported by Schmeltz *et al*. [34]. The gaseous phase of smoke has generally been viewed as more oxidising, and the particulate phase as more reducing in nature. The behaviour of free radicals as well as the presence of various transition metals (e.g. Cu, Fe, As, Cd, Cr, Pb, Ni and Se) has long been associated with the redox potential of smoke [35, 36]. The redox behaviour for trace metals in smoke may also influence the potential toxicity through their speciation. For example there is sufficient evidence for the carcinogenicity of chromium in the +6 oxidation state while chromium in the +3 oxidation state is considered not classifiable as to its carcinogenicity to humans [37]. Information on the oxidation state and/or speciation of metals in cigarette smoke is still rare in the literature, which makes accurate assessment of their toxicology difficult.

In the case of arsenic, the most toxic species are generally considered to be the inorganic species, e.g., arsenite ($As^{III}O_3^{3-}$) and arsenate ($As^{V}O_4^{3-}$); the trivalent species being more toxic than the pentavalent ones. In contrast, organic arsenicals in food and plant materials (e.g. arsenobetaine, monomethyl arsenic acids, arsenosugars, etc.) have little or no reported toxicity [38]. A preliminary experiment on arsenic speciation in cigarette smoke using synchrotron X-ray absorption spectroscopy has been carried out [39]. The key advantage of this technique is that virtually no sample preparation is needed, which is particularly desirable considering the redox sensitive nature of smoke and the targeted elements.

The information obtained on the oxidation states of As in cured tobacco leaf, cigarette ash, and smoke particulate matter reveals a dynamic redox equilibrium between As^{III} and As^{V} during smoke formation and transfer processes as well as in the smoke particulate matter upon ageing. However, the levels of arsenic species present in these samples were too low for the synchrotron X-ray absorption technique to ascertain their speciation. This picture mirrors reasonably well with the measured redox potential of smoke, which has been suggested to be mainly driven by free radical reactions [34, 40].

Real-time Smoke Analysis

Complementary to routine smoke analyses, real-time cigarette smoke measurements have highlighted the dynamic and reactive nature of some smoke constituents [15, 41]. These on-line approaches do not rely on smoke trapping and/or extended sample preparations. They therefore allow for analysis of smoke constituents close to the point of formation and provide an opportunity for researchers to follow their subsequent chemistry on timescales relevant to human smoking. These techniques are helping to provide greater mechanistic insight into the physics and chemistry behind smoke formation.

In a recent example, a novel real-time detection system was designed to investigate the formation of a number of volatile smoke constituents (nitric oxide, acetaldehyde, acetone, benzene, toluene, 1,3 butadiene, isoprene and carbon dioxide) in the mainstream smoke emerging from the filters of 3R4F research reference cigarettes [42]. Typical plots from two consecutive puffs of three different puff durations are shown in Fig. (**1**), using benzene as an example. Under the identical puff volume of 35 mL, a small "shoulder" peak prior to the main puff peak began to show for the 3-s and 4-s puff durations. The area occupied by the "shoulder" peak appears to increase (as a percentage of the total puff peak area) with increasing puff length or decreasing flow through the cigarette. Because the mass spectrometer's sampling point was immediately next to the mouth end of the cigarette filter, these two separate peaks reflect two separate intricate smoke generation processes occurring within the cigarette. Broadly speaking, the first "shoulder" peak can be attributed to the smoulder-generated species that are formed before the puff, trapped within the cigarette column and then released into

Table 4. A Hoffmann Analyte List with Associated Disease [8]

Disease	Contributor	Enhancing Agent
Tobacco dependence	Major: Nicotine	Minor tobacco alkaloids, flavour components, acetaldehyde
Lung cancer	Major: PAH, TSNAs	Catechol (co-carcinogen)
	Minor: ^{210}Polonium, formaldehyde, acetaldehyde	Weakly acidic promoters, volatile aldehydes, NO_x, (precursors of *N*-nitrosamines)
Cardiovascular diseases	Major: "Tar", CO	Nicotine, 1,3-butadiene
	Minor: HCN, NO_x, CS_2, Cd, Zn	
Chronic Obstructive Pulmonary Disease	Major: "Tar", NO_x, HCN, volatile aldehydes	Inducers of superoxide and H_2O_2

the mainstream at the start of subsequent puff. The second, major peak can be attributed to smoke formed by tobacco burnt in the puff. In the case of benzene, up to 30% in the mainstream smoke yield measured during a puff could be generated during the preceding smouldering period.

The two sources were separated for some volatile species but not for others. This indicates that interactions occur to different extents between different smoke constituents and the tobacco rod or filter. One of the more important implications of this study is that mainstream smoke is a mixture of "freshly" produced smoke and "pre-existing" smoke; the two types of smoke not only differ in the extent of ageing but are also formed by the different combustion and pyrolysis conditions occurring in the puffing and smouldering phases of a burning cigarette. This is a particularly important consideration when studying highly reactive smoke constituents like free radicals.

The discussion above highlights the diversity of species present in smoke, their inter-dependence and their varying reactivities. The consequence of this is that unambiguous identification and accurate quantification of individual smoke components can be a significant analytical challenge [4, 29]. It is essential that analytical methods are employed which are appropriate to the species being measured, and which reflect the way that they are generated in a cigarette. Standardised, internationally recognised methods are essential to ensure that comparable data are obtained in different laboratories. For a number of stable species, such as carbon monoxide, nicotine and tobacco specific nitrosamines (TSNAs) this has been addressed, but for the majority of species in smoke, and for reactive species such as free radicals this task has barely begun. Advances in modern analytical and experimental techniques will continue to play an important role in improving our knowledge of the complex and dynamic nature of cigarette smoke.

TOXICOLOGICAL PRIORITISATION OF SMOKE CONSTITUENTS

There have been a vast number of scientific studies examining the toxicity of individual chemicals, and some compounds that are found in smoke have been identified as either carcinogenic, or toxic in other respects. However, despite decades of work the connection between specific constituents of smoke and smoking related diseases remains unclear. Understanding which of the thousands of smoke constituents are associated with smoking related diseases, and their relative contributions, is an obvious first step towards reducing the harm associated with smoking. However, the large and ever increasing number of constituents identified in cigarette smoke presents a significant challenge for scientists working in this area.

However, over the last thirty years various priority lists of smoke toxicants have been compiled [3, 8, 43-48]. The most frequently cited toxicants listed in the literature are the various "Hoffmann analytes" proposed by Hoffmann *et al.* [8]. Table **4** illustrates one version of this list, which classifies smoke constituents according to three of the most prevalent smoking related diseases.

Some regulators have used these lists to identify sets of smoke constituents for which brand-by-brand disclosure is required, either on an annual basis (Health Canada (HC), British Columbia (BC), Brazil (ANVISA), Taiwan) or as a one-off disclosure (Massachusetts, Australia, UK) [28, 49]. The constituents chosen, listed in Table **5**, show a great deal of similarity between different regulatory authorities, and also feature compounds with common functional groups (e.g. carbonyls, phenols, metals and TSNAs).

A number of studies [25-27, 49, 50] and market surveys on commercial cigarettes have all been reported on this group of smoke toxicants (Table **5**) [28, 51] under FTC/ISO, Massachusetts or Health Canada "Intense" smoking regimes.

Table 5. Smoke Constituents Selected by Different Regulatory Authorities

Constituent	BC(a)	HC(b)	ANVISA(c)	Taiwan (d)	Massachusetts (e)	Australia (f)	UK (g)
Tar	√	√	√	√	√	√	√
Nicotine	√	√	√	√	√	√	√
CO	√	√	√	√	√	√	√
Ammonia	√	√	√	-	√	√	√
HCN	√	√	√	√	√	√	√
NO	√	√	-	-	√	√	√
NO_x	-	√	√	-	-	√	-
N'-nitrosoanabasine (NAB)	√	√	√	-	√	√	√
N'-nitrosoanatabine (NAT)	√	√	√	-	√	√	√
N'-nitrosonornicotine (NNN)	√	√	√	-	√	√	√
4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)	√	√	√	-	√	√	√

Table 5. contd…

Constituent	BC(a)	HC(b)	ANVISA(c)	Taiwan (d)	Massachusetts (e)	Australia (f)	UK (g)
Benzo[*a*]pyrene (B[*a*]P)	√	√	√	√	√	√	√
Formaldehyde	√	√	√	√	√	√	√
Acetaldehyde	√	√	√	-	√	√	√
Propionaldehyde	√	√	√	-	√	√	√
Butyraldehyde	√	√	√	-	√	√	√
Acrolein	√	√	√	-	√	√	√
Crotonaldehyde	√	√	√	-	√	√	√
Acetone	√	√	√	-	√	√	√
Methyl ethyl ketone	√	-	√	-	√	√	√
Arsenic	-	-	√*	-	√	-	√
Cadmium	√	√	√*	-	√	√	√
Chromium	-	-	√*	-	√	-	√
Lead	√	√	√*	-	√	√	√
Mercury	√	√	√*	-	√	√	√
Nickel	-	-	√*	-	√	-	√
Selenium	-	-	√*	-	√	-	√
Acrylonitrile	√	√	√	-	√	√	√
1,3-Butadiene	√	√	√	-	√	√	√
Isoprene	√	√	√	-	√	√	√
Benzene	√	√	√	√	√	√	√
Toluene	√	√	√	-	√	√	√
Styrene	√	√	√	-	√	√	√
Pyridine	√	√	√	-	√	√	√
Quinoline	√	√	√	-	√	√	√
1-Aminonaphthalene	√	√	√	-	√	√	√
2-Aminonaphthalene	√	√	√	-	√	√	√
3-Aminobiphenyl	√	√	√	-	√	√	√
4-Aminobiphenyl	√	√	√	-	√	√	√
Phenol	√	√	√	-	√	√	√
o-Cresol	√	√	√	-	√	√	√
m-Cresol	√	√	√	-	√	√	√
p-Cresol			√	-			√
Hydroquinone	√	√	√	-	√	√	√
Catechol	√	√	√	-	√	√	√
Resorcinol	√	√	√	-	√	√	√
Eugenol	-	√*	√	-	-	-	-
Menthol	-	-	√*	-	-	-	-

* = Optional

a = available on request from HLTH.TobaccoInfo@gov.bc.ca

b = http://www.hc-sc.gc.ca/hc-ps/pubs/tobac-tabac/tir-rft/emissions-eng.php accessed 10/06/2010

c = Brazil Resolution RDC No. 90 of the Federal Sanitation Agency effective 27 December 2007 (re-published), (www.anvisa.gov.br)

d = http://www.health99.doh.gov.tw/box2/smokefreelife/law.aspx, accessed 14/6/2010

e = http://www.legacy.library.ucsf.edu/tid/yek21c00/pdf?search=%22borgerding%20bodnar%20wingate%20the%201999%20massachusetts%20benchmark%20study%22, accessed 10/6/2010

f = http://www.health.gov.au/internet/main/publishing.nsf/Content/health-pubhlth-strateg-drugs-tobacco-emis_data.htm, accessed 10/6/2010

g = Gregg *et al.* (2004)

Under the US Family Smoking Prevention and Tobacco Control Act (P.L. 111-31) of 2009 the FDA was given authority to regulate tobacco products. Included under this authority is the establishment of a list of harmful and potentially harmful constituents in tobacco products, including smoke constituents. The FDA established a Tobacco Products Scientific Advisory Committee (TPSAC) with the remit of reviewing and evaluating safety, dependence, and health issues relating to tobacco products and providing appropriate advice, information and recommendations to the Commissioner of Food and Drugs. In August 2010 TPSAC met to discuss a proposed initial list of harmful or potentially harmful constituents based on the following criteria:

- Identified as a known or probable human carcinogen by either IARC or EPA.
- Identified as possible human carcinogen by IARC or EPA; identified by NIOSH as a potential occupational carcinogen
- Identified by EPA or ATSDR as having adverse respiratory or cardiac effects
- Identified by the California EPA as a reproductive or developmental toxicant

Table 5a. Additional Smoke Constituents Proposed by FDA TPSAC [87]

Amines and Amides		**PAHs/Aromatics**	
Acetamide	2,6 dimethylaniline	Benz[a]anthracene	Dibenz[a,h]anthracene
o-Anisidine	Ethyl carbamate	Benz[j]aceanthrylene	Dibenzo[a,e]pyrene
Acrylamide	o-Toluidine	Benzo[b]fluoranthene	Dibenzo[a,h]pyrene
N-Heterocyclic Amines		Benzo[k]fluoranthene	Dibenzo[a,i]pyrene
A-a-C (2-Amino-9H-pyrido[2,3-b]indole)	Glu-P-1 (2-Amino-6-methyldipyrido[1,2-a:3',2'd]imidazole)	Benzo[c]phenanthrene	Dibenzo[a,l]pyrene
Glu-P-2 (2-Amino dipyrido[1,2-a:3',2'd] imidazole)	IQ (2-Amino-3-methyl imidazo[4,5-f] quinolene)	Chrysene	5-Methylchrysene
MeA-a-C (2-Amino-3-methyl)-9H-pyrido[2,3-b]indole)	Trp-P-1 (3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole)	Cyclopenta[c,d]pyrene	Indeno[1,2,3-cd] pyrene
Trp-P-1 (1-Methyl-3-amino-5H-pyrido[4,3-b]indole)	PhIP (2-Amino-1-methyl-6-phenyl imidazo[4,5-b]pyridine)	Ethylbenzene	Naphthalene
Nitro Compounds		**Aza-Arenes**	
Nitrobenzene	Nitromethane	Dibenz[a,j]acridine	Dibenzo[c,g]carbazole
2-Nitropropane		Dibenz[a,h]acridine	
Furans		**Nitrosamines**	
Benzo[b]furan	Chlorinated dioxins /furans	*N*-Nitrosodiethanolamine (NDELA)	*N*-Nitrosopiperidine (NPIP)
Furan		*N*-Nitrosodimethylamine (NEMA)	*N*-Nitrosopyrrolidine (NPYR)
Inorganics		*N*-Nitrosomethylethylamine	
Beryllium	Cobalt	**Miscellaneous Organics**	
Polonium-210	Hydrazine	Vinyl acetate	Vinyl chloride
Nitrate		Ethylene oxide	Propylene oxide
		Caffeic acid	

- Identified as having potential abuse liability based on peer-reviewed literature, evidence of at least two of the following:
    - Central Nervous System activity, Animal drug discrimination, Conditioned place preference, Animal self-administration, Human self-administration, Drug "liking", Withdrawal.

The initial list discussed by TPSAC in August 2010 contained all of the constituents listed in Table **4** under "Massacheusetts" (other than NAT, 3-Aminobiphenyl and Hydroquinone), and in addition the constituents identified in Table **5a**:

The choice of smoke constituents on many of these regulatory lists have been influenced to greater or lesser degree by risk assessment approaches which have attempted to identify the constituents of smoke which have the greatest impact on smoking related diseases. Common to many of these prioritisation approaches is the use of 'harm potential' values which are normally calculated from available machine-generated cigarette smoke toxicant yields and their known chemical and toxicological properties. Clearly, accurate smoke analysis data is key to the success of these calculations.

Vorhees and colleagues [45, 52] applied regulatory risk assessment methodology to calculate cancer (incremental lifetime cancer risks, or ILCR) and non-cancer risks for cigarette smoke constituents from 25 different cigarette brands. They identified 19 compounds (e.g., polycyclic aromatic hydrocarbons (PAHs), nitrosamines and hydrazine) with chemical-specific cancer risks greater than 10^{-6} (the probability that one in a million individuals will develop cancer) for a smoker of 30 pack-year (20 cigarettes per day for 30 years). This probability value formed the basis for a toxicological risk prioritisation index.

Using a similar approach, Fowles and Dybing [47] ranked 158 smoke components. A cancer risk index was calculated by multiplying the yield of each compound (obtained from a number of different sources) by its cancer potency factor (from US EPA and California EPA databases) and a nominal breathing volume of 20 m^3/day^{-1}. Non-cancer risks were also calculated by dividing the smoke yields by their published Reference Exposure Levels (RELs). The top 5 contributors to cancer risk were calculated as 1,3-butadiene, acrylonitrile, arsenic, acetaldehyde, and benzene. For respiratory effects, the top five toxicants identified through this methodology were acrolein, acetaldehyde, formaldehyde, cadmium, and chromium. For cardiovascular effects the major contributors were calculated to be hydrogen cyanide, arsenic, *o*-, *m*- and *p*-cresol, and carbon monoxide. Different cigarette brands show a variety of smoke constituent yields, and this may influence the ranking orders to some degree. In this respect, Rodgman and Green [3] used a similar methodology to rank 149 smoke components but focused on mainstream smoke yields from one cigarette brand, Kentucky reference cigarette 1R4F. This resulted in a slightly different ranking order from that of Fowles and Dybing's. However, 1,3 butadiene was still calculated to be the greatest contributor to cancer risk.

In 2006, Hecht [48] published a prioritised toxicant list for lung cancer based on a weight of evidence approach. The major toxicants were identified as PAHs and NNK, with minor toxicants being 1,3 butadiene, isoprene, ethylene oxide, ethyl carbamate, aldehydes, benzene, and the trace heavy metals.

More recently the World Health Organization Study Group on Tobacco Product Regulation (TobReg) has published a proposal to regulate 9 smoke toxicants in mainstream smoke based on toxicant/nicotine ratios measured under Canadian "Intense" smoking conditions [53, 54]. These are NNK, NNN, acetaldehyde, acrolein,

Table 6. Nine Smoke Toxicants with Ceiling Levels as Proposed by Burns *et al.* [53]

Toxicant	Level in µg/mg Nicotine (International Brands)	Level in µg/mg Nicotine (Canadian Brands Except US and Gauloises Brands)	Criteria for Limit
NNK	0.072	0.047	Median value
NNN	0.114	0.027	
Acetaldehyde	860	670	125% of the median value
Acrolein	83	97	
Benzene	48	50	
Benzo[*a*]pyrene	0.011	0.011	
1,3 Butadiene	67	53	
CO	18400	15400	
Formaldehyde	47	97	

formaldehyde, benzene, benzo[*a*]pyrene and carbon monoxide (Table **6**). A further 9 toxicants were considered to be high priority for disclosure and monitoring: acrylonitrile, 4-aminobiphenyl, 2-aminonaphthalene, cadmium, catechol, crotonaldehyde, hydrogen cyanide, hydroquinone, and nitrogen oxides. "Ceiling levels" were proposed for the first group of 9 toxicants; regulatory authorities in individual countries were recommended to establish the range of values for the products on sale on their markets and then to define ceiling levels specific to their market. Ceiling levels were recommended to be based on the median value for the nitrosamine/nicotine ratios, and 125% of the median market values for the other toxicant/nicotine ratios. The authors provided two sets of example ceiling levels based on a subset of Health Canada data from 2004 and a survey of 49 Philip Morris brands (Table **6**).

Quantitative Risk Assessment

In addition to prioritising smoke toxicants, the risk assessment approach has also been applied to evaluate whether or not the risks of human cancer predicted by these approaches can account for observed population levels of smoking related cancers. Fowles and Dybing [47] Cancer Risk Indices, calculated from ISO smoking yields, accounted for approximately 20% of observed cancer death rates; this estimate was subsequently updated [55] using more intense smoking regimes and estimates, to a figure of approximately 35% of observed lifetime cancers. Pankow *et al.* [56], using a similar approach, concluded that the calculated lung cancer risk was less than 10% of the observed population-average lung cancer risk. Pankow *et al.* suggested that the poor match arose from either use of inaccurate Cancer Slope Factors (or cancer potency factors) for carcinogens in smoke, not accounting for all of the carcinogens in smoke, or incomplete consideration of the possible roles (e.g. cancer promoters, irritants) of other smoke constituents.

Watanabe *et al.* [57] employed a refined methodology to calculate the ILCR values for benzo[*a*]pyrene, NNN, and NNK, in order to estimate the contribution of these species as predicted using the methodology, to the lung cancer risks derived from the American Cancer Society Cancer Prevention Studies I and II [57]. Two improvements were made to the calculations: the use of mainstream smoke yields measured under human smoking conditions and the use of Monte Carlo simulation techniques to derive a distribution probability for ILCR (rather than the deterministic values used by all in previous calculations). Using this approach, the median ILCR value for NNK was found to be ~ 18-fold and 120-fold higher than the median values for NNN and benzo[*a*]pyrene, respectively, making NNK the most potent smoke constituent for cancer. The authors used a Cancer Slope Factor (CSF) for NNK derived from experiments in which animals were exposed to NNK in drinking water, as no CSF inhalation value for humans was available. However, overall, the median ILCR values for NNK explained only 0.2 – 1.0% of the lung cancer risk for male smokers of "regular" or "light" cigarettes and 0.7 – 21% for female smokers.

The discussion above has shown that despite the considerable efforts devoted to establish causal relationships between individual smoke toxicants and population levels of smoking related diseases there are still significant gaps in our understanding. The approaches used by different researchers share some common limitations [58]:

- Potentially incomplete inclusion of all carcinogens in smoke
- Some discrepancies in the cancer status of smoke constituents as defined by different regulatory bodies (e.g., IARC and US EPA)
- Lack of accurate cancer potency factors for many smoke constituents
- Smoking machine based yield data is available for less than 5% of mainstream smoke constituents,
- The models used do not accommodate interactions between different constituents in the complex mixture of smoke beyond simple additive factors
- Available data comes from a variety of laboratories using differing analytical methodologies and products
- Actual human exposure data, and allowance for the amount absorbed by the smoker, is rarely available

OTHER POSSIBLE CONTRIBUTORS TO SMOKE TOXICITY

One explanation that has been advanced to explain the poor prediction of observed disease rates by these models is that other aspects of cigarette smoke contribute significantly to its overall toxicity [47, 56]. These factors are briefly reviewed below.

Air-borne particulate matter of varying compositions has received some attention in recent years. Respirable particulates, such as diesel soot or inert dust particles, have been suggested to play a role in respiratory and cardiovascular health problems in the general population [59]. Similarly, inhaled fine particles either from ambient air pollution and/or from cigarette smoking [60-62], and their interaction with chemical toxicants once inhaled into the lung [63], have been suggested to play a synergistic role in smoking related diseases.

A number of researchers [64-68] have investigated the possibility of charcoal and cellulose acetate particles being delivered from cigarette filters to smokers' lungs during smoking. More recent studies established that although low levels of particles could be released from the cigarette filters, e.g. approximately 2 ng of charcoal particles released per cigarette [69] and less than 10 particles per cellulose acetate filter cigarette [70], the particle size of the released particles was such that they would deposit in the upper

Table 7. Some Examples of Mainstream Smoke Free Radical Contributors

Mainstream Smoke Free Radical Agents	Designation	IARC* Classification	US EPA* Classification
Nitrogen oxide	Primary free radical	4	D
Catechol	Precursor	2B	D
Hydrogen peroxide	Precursor	3	D
Hydroquinone	Precursor	3	D
Nitrogen dioxide	Precursor	4	D
Sulfur dioxide	Precursor	3	D
Copper	Catalyst	4	D
Iron	Catalyst	3	D

* The US EPA Class D indicates the agent is not classified, i.e., either the agency has not yet reached a conclusion on the compound or that insufficient data are currently available for classification.

airways [71]. These researchers have concluded that exposure from particles directly migrating from conventional cellulose acetate cigarette filters, with or without added charcoal, are unlikely to pose a health hazard.

Other recent studies have suggested that different classes of bacteria and pathogenic organisms found on cut tobacco taken from cigarettes may also have a role to play in smoke toxicity [72]. The fact that traces of tobacco particles are found on unsmoked cigarette filters was suggested as an alternative source of exposure to these organisms [73]. The presence of bacteria during tobacco curing and processing has long been associated with the formation of tobacco-specific nitrosamines [74]. However, direct inhalation of these pathogenic microbes, which may lead to lung inflammation, has not received much attention. In addition, no study has so far been carried out on whether or not these biological species can survive the combustion conditions in a burning cigarette and be transferred into the mainstream cigarette smoke.

The presence of radioactive species in smoke received considerable attention in the 1960s as a possible hazard to smokers. Attention focused primarily on polonium-210 (^{210}Po), an intense α-emitter found on tobacco and in cigarette smoke. Interest in this area declined late in the 1960s when the low levels of ^{210}Po, and its relatively rapid lung clearance, led researchers to conclude that ^{210}Po was unlikely to be a major factor in smoking related diseases [75]. In 1974 Martell [76] proposed an alternative mechanism, whereby lung exposure to the less soluble β-emitter ^{210}Pb, from cigarette smoke, and it's radioactive decay while in the lung, could lead to the generation of ^{210}Po *in-situ* and hence more prolonged irradiation of the lung tissue of smokers. This area continues to receive on-going attention, with some studies reporting levels of ^{210}Po and ^{210}Pb in tobaccos or smoke from various countries or regions [77-80].

The presence of free radical species in cigarette smoke has been known since the 1950s [5, 31-33]. Yet these remain some of the least characterised species in cigarette smoke, largely because of their highly reactive nature. For example, there is no definitive list available which details all of the free radicals identified in cigarette smoke, the most complete current list is that of Rodgman and Perfetti [5], which lists fewer than 35 entries. Free radical species in cigarette smoke have been suggested on many occasions to play a role in smoking related diseases [31, 47, 81]. However, in all the toxicological evaluations of cigarette smoke constituents carried out so far, lack of information on free radical yields (except for NO) has prevented them from being assessed in the prioritisation exercises. Table **7** lists eight smoke constituents, either as known free radicals (e.g., NO) or proposed to be precursors for smoke free radicals. Although others could have been listed, those in Table **7** are selected to illustrate several points. Hydroquinone and catechol are directly involved in the formation of the majority of the particulate-phase free radicals in the mainstream smoke [31, 32, 82]. However, it is estimated that only a small fraction of the hydroquinone or catechol is involved in the radical formation (based on a comparison of the levels of the radical species to the amount of hydroquinone or catechol in smoke). H_2O_2, NO_2, and sulfur dioxide are included in the precursor category because they can react to produce many free radicals [33, 83]. The two metals, copper and iron, may play a catalytic role *in vivo* for the formation of certain types of free radicals via the so-called Fenton mechanism and the same may be true for other transitional metals found in smoke [84]. However, as discussed above, very little is known about the oxidation states or chemical form of metals in the smoke and the exact role played in free radical generation [39, 85].

In the past few years fresh results have been obtained which add significantly to the science of cigarette smoke free radicals [32, 33, 82]. Some fundamental principles in this area have been challenged by recent work. In addition, advances have been made in understanding the role played by free radical species in biological systems [81, 86]. The articles in this special mini-review series summarise the progress and also the knowledge gaps in these areas.

SUMMARY

This review has discussed some of the fundamental aspects of cigarette combustion, including how cigarettes burn, how smoke is formed and the complexity of cigarette smoke composition. Particular emphasis has been placed on current best practise in the generation and measurement of smoke. When generating cigarette smoke it is critical to use techniques which reproduce the basic combustion phenomena of a burning cigarette, as the physico-chemical characteristics behind smoke formation are highly sensitive to the puffing parameters employed. It is also essential to ensure that the sampling and measurement techniques employed preserve the true chemical nature of the species under investigation. This is particularly important for highly reactive species such as free radicals. Ultimately, standardisation of smoke analysis procedures between different laboratories is necessary to provide a consistent basis for scientific and regulatory consideration.

Consideration also needs to be given to the choice of smoking regime; the ISO smoking regime which for many years was the standard approach to smoke generation and comparison, is now being complimented by the Canadian "Intense" regime. Although no machine based smoking regime can estimate the exposure of individual human smokers to toxicants, this newer regime has gained considerable support in recent years. Much of the fundamental science of cigarette combustion has been established using ISO smoking, and the full impact of using intense smoking conditions has yet to be fully established. This is particularly true with changes in constituent yields when moving from ISO to Canadian "Intense", which cannot always be explained based on current understanding of smoke formation.

The review also highlighted a number of on-going challenges in identifying the precise chemical composition of cigarette smoke, and attempts to establish associations between smoke constituents and the occurrence of smoking-related diseases. The main issues confounding both of these goals are the complexity, reactivity and dynamic nature of tobacco smoke. Various risk assessment approaches have led to the development of prioritised lists of smoke toxicants; however, these approaches fail to predict the observed incidence of smoking related diseases on a population basis. The limitations of these approaches appear to arise from incomplete identification of all toxicants in smoke, a lack of data on human exposure to smoke toxicants, and the current simplicity of the models used. Some researchers have suggested that aspects of smoke not included in the prioritisation exercises may account for the shortfall between model predictions and actual incidences of smoking related diseases. One such group of highly reactive compounds, cigarette smoke free radicals, have long been proposed as playing a role in the development of smoking related diseases. The rest of this review series examines the current state of knowledge on the chemistry, analytical measurements and biological impact of these species.

REFERENCES

[1] Wynder, E.L., Hoffmann, D. *Tobacco and Tobacco Smoke Studies in Experimental Carcinogenesis.* New York: Academic Press, **1967**.

[2] Baker, R.R, Smoke chemistry. In: *Tobacco Production, Chemistry and Technology*; Davis, D.L., Nielsen, M.T., Ed. Oxford: Blackwell Science Ltd., **1999**, pp. 398-439.

[3] Rodgman, A.; Green, C.R. Toxic chemicals in cigarette mainstream smoke – hazard and hoopla. *Beitr. zur Tabakforsch. Int.* **2003**, *20*, 481-545.

[4] Borgerding, M.F.; Klus, H. Analysis of complex mixtures – cigarette smoke. *Exp. Toxicol. Pathol.*, **2005**, *57*, 43-73.

[5] Rodgman, A.; Perfetti, T.A. *The Chemical Components of Tobacco and Tobacco Smoke*. Boca Raton, FL: CRC Press, **2008**.

[6] Baker, R.R. Smoke generation inside a burning cigarette: modifying combustion to develop cigarettes that may be less hazardous to health. *Prog. Energy Combust. Sci.* **2006**, *32*, 373-385.

[7] Norman, A., Cigarette design and materials. In: *Tobacco Production, Chemistry and Technology*. Davis, D.L., Nielsen, M.T., Ed. Oxford: Blackwell Science Ltd. **1999**, pp. 353-387.

[8] Hoffmann, D.; Djordjevic, M.V.; Hoffmann, I. The changing cigarette. *Prev. Med.* **1997**, *26*, 427-434.

[9] Bokelman, G.H.; Ryan Jr., W.S. Analyses of bright and burley tobacco lamina and stems. *Beitr. Tabakforsch. Int.* **1985**, *13*, 29-36.

[10] Leffingwell, J.C., Leaf chemistry. A. Basic chemical constituents of tobacco leaf and differences among tobacco types. In: *Tobacco Production, Chemistry and Technology*. Davis, D.L., Nielsen, M.T., Ed. Oxford: Blackwell Science Ltd. **1999**, pp. 265-284.

[11] Liu, C.; Woodcock, D. Observing the peripheral burning of cigarettes by an infrared technique. *Beitr. zur Tabakforsch. Int.* **2002**, *20*, 257-264.

[12] Lyman, C.S.; Perfetti, T.A.; Riggs, D.M.; Morgan, W.T. Thermal emissivity and cigarette coal temperature during smolder. *Beitr. zur Tabakforsch. Int.* **2003**, *20*, 381-388.

[13] Muramatsu, M. Studies on the transport phenomena in naturally smouldering cigarettes. *Sci. Papers Central Res. Inst. Jpn. Tob. Salt. Mon. Corp.*, **1981**, *123*, 9-77.

[14] Baren, R.E.; Parrish, M.E.; Shafer, K.H.; Harward, Q.; Shi, D.D.; Nelson, J.B.; McManus, M.S.; Zahniser, M.S. Quad quantum cascade laser spectrometer with dual gas cells for the simultaneous analysis of mainstream and sidestream cigarette smoke. *Spectrochim. Acta A.* **2004**, *60*, 3437-3447.

[15] Adam, T.; Mitschke, S.; Baker, R.R. Investigation of tobacco pyrolysis gases and puff-by-puff resolved cigarette smoke by single photon ionization (SPI) – time-of-flight mass spectrometry (TOFMS). *Beitr. zur Tabakforsch. Int.* **2009**, *23/24*, 203-226.

[16] Ingebrethsen, B.J. Aerosol studies of cigarette smoke. *Recent Adv. Tob. Sci.*, **1986**, *12*, 54-142.

[17] Adam, T.; McAughey, J.; McGrath, C.; Mocker, C.; Zimmermann, R. Simultaneous on-line size and chemical analysis of gas phase and particulate phase of cigarette mainstream smoke. *Anal. Bioanal. Chem.* **2009**, *394*, 1193-1203.

[18] Wakeham, H., Recent Trends in Tobacco and Tobacco Smoke Research. Schmeltz, I., Ed. Symposium on the Composition of Tobacco and Tobacco Smoke, *Am. Chem. Soc. Mtg*., Washington, DC, New York: Plenum Press, **1972**, pp.1-20.

[19] Dallüge, J.; van Rijn, M.; Beens, J.; Vreuls, R.J.J.; Brinkman, U.A.T. Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometric detection applied to the determination of pesticides in food extracts. *J. Chromatogr. A.* **2002**, *965*, 207-217.

[20] Purkis, S.W.; Drake, L.; Meger, M.; Mariner, D.C. A review of the UK methodology used for monitoring cigarette smoke yields, aspects of analytical data variability and their impact on current and future regulatory compliance. *Regul. Toxicol. Pharmacol*., **2010**, *56*, 365-373.

[21] Intorp, M.; Purkis, S.; Whittaker, M.; Wright, W. Determination of "Hoffmann Analytes" in cigarette mainstream smoke, the CORESTA 2006 joint experiment. *Beitr. zur Tabakforsch. Int.*, **2009**, *23*, 161-202.

[22] Dixon, M.; Borgerding, M.F. Recent advances in the application and understanding of alternative smoking regimes. *Recent Adv. Tob. Sci.,* **2006**, *32*, 3-84.

[23] Hammond, D.; Wiebel, F.; Kozlowski, L.T.; Borland, R.; Cummings, K.M.; O'Connor, R.J.; McNeill, A.; Connolly, G.N.; Arnott, D.; Fong, G.T. Review: revising the machine smoking regime for cigarette emissions: implications for tobacco control policy. *Tob. Control.* **2007**, *16*, 8-14.

[24] Hammond D.; O'Connor, R.J. Constituents in tobacco and smoke emissions from Canadian cigarettes. *Tob. Control.* **2008**, *17*(Suppl.1), i24-i31.

[25] Counts, M.E.; Morton, M.J.; Laffoon, S.W.; Cox, R.H.; Lipwicz, P.J. Smoke composition and predicting relationships for international commercial cigarettes smoked with three machine-smoking conditions. *Regul. Toxicol. Pharmacol.* **2005**, *41*, 185-227.

[26] Counts, M.E.; Hsu, F.C.; Laffoon, S.W.; Dwyer, R.W.; Cox, R.H. Mainstream smoke constituent yields and predicting relationships from a worldwide market sample of cigarette brands: ISO smoking conditions. *Regul. Toxicol. Pharmacol.* **2004**, *39*, 111-134.

[27] Counts, M.E.; Hsu, F.C.; Tewes, F.J. Development of a commercial cigarette "market map" comparison methodology for evaluating new or non-conventional cigarettes. *Regul. Toxicol. Pharmacol.* **2006**, *46*, 225-242.

[28] Gregg, E.; Hill, C.; Hollywood, M.; Kearney, M.; McAdam, K.; McLaughlin, D.; Purkis, S; Williams, M. The UK smoke constituents testing study - summary of results and comparison with other studies. *Beitr. zur Tabakforsch. Int.* **2004**, *21*,117-138.

[29] Dube, M.F.; Green, C.R. Methods of collection of smoke for analytical purposes. *Rec. Adv. Tob. Sci.* **1982**, *8*, 42-102.

[30] Mahanama, K.R.R.; Daisey, J.M. Volatile N-nitrosamines in environmental tobacco smoke: Sampling, analysis, emission factors, and indoor air exposures. *Environ. Sci. Technol.* **1996**, *30*, 1477-1484.

[31] Church D.F.; Pryor, W.A. Free-radical chemistry of cigarette smoke and its toxicological implications. *Environ Health Perspect.* **1985**, *64*, 111-126.

[32] Wooten, J.B.; Chouchane, S.; McGrath, T.E., Tobacco Smoke Constituents Affecting Oxidative Stress, in: Cigarette Smoke and Oxidative Stress. Halliwell, B.H., Poulsen, H.E., Ed. Berlin: Springer, **2006**, pp. 5-46.

[33] Wooten, J.B. Gas-phase radicals in whole cigarette smoke: a re-evaluation of the steady-state model. **2010**, Mini-Review in Organic Chem (this issue)

[34] Schmeltz, I.; Tosk, J.; Jacobs, G.; Hoffmann, D. Redox potential and quinine content of cigarette smoke. *Anal. Chem.* **1977**, *49*, 1924-1929.

[35] Cosgrove, J.P.; Borish, E.T.; Church, D.F.; Pryor, W.A. The metal-mediated formation of hydroxyl radical by aqueous extracts of cigarette tar. *Biochem. Biophys. Res. Commun.* **1985**, *132*, 390-396.

[36] Stavrides, J.C. Lung carcinogenesis: pivotal role of metals in tobacco smoke. *Free Radic. Biol. Med*. **2006**, *41*, 1017-1030.

[37] World Health Organization - International Agency for Research on Cancer (IARC): Evaluation of the carcinogenic risk of chemicals to man. Chromium, nickel and welding; IARC. Lyon (France): IARC Monograph 49, **1990**

[38] European Food Safety Authority (EFSA). Scientific opinion on arsenic in food. *ESFA J.* **2009**, *7*, 1351.

[39] Liu, C.; Hu, J.; McAdam, K.G. A feasibility study on oxidation state of arsenic in cut tobacco, mainstream cigarette smoke and cigarette ash by X-ray absorption spectroscopy. *Spectrochim. Acta B.* **2009**, *64*, 1294-1301.

[40] Song, Y.; Buettner, G.R. Thermodymamic and kinetic considerations for the reaction of semiquinone radicals to form superoxide and hydrogen peroxide. *Free Radical. Med*., **2010** (in press)

[41] Mantz, A.W. Editorial comment. *Spectrochim. Acta. A*, **2007**, *67*, 1-3.

[42] Liu, C.; Feng, S.L.; van Heemst, J; McAdam, K.G. New insights into the formation of volatile compounds in mainstream cigarette smoke. *Anal. Bioanal. Chem.* **2009**, *396*, 1817-1830.

[43] Herzfeld, L. A prophylactic plan for reducing risks to smokers. *Notabene Med*. **1982**, *12*, 808-816.

[44] Rickert, W.S.; Kaiserman, M.J. Development of an index for mainstream tobacco smoke based on chemical composition. Presented at the 52nd Tobacco Scientists' Research Conference, **1998**.

[45] Vorhees, D.J.; Dodson, R.E. Estimation to cigarette smokers from smoke constituents in proposed "Testing and Reporting of Constituents of Cigarette Smoke" Regulations", Menzie-Cura & Associates, Inc., **1999**.

[46] Smith, C.J.; Hansch, C. The relative toxicity of compounds in mainstream cigarette smoke. *Food Chem. Toxicol.* **2000**, *38*, 637-646.

[47] Fowles, J.; Dybing, E. Application of toxicological risk assessment principles to the chemical constituents of cigarette smoke. *Tob. Control*. **2003**, *12*, 424-430.

[48] Hecht, S.S. Cigarette smoking: cancer risks, carcinogens, and mechanisms. *Langenbeck's Arch. Surg*. **2006**, *391*, 603-613.

[49] King, B.; Borland, R.; Fowles, J. Mainstream smoke emissions of Australian and Canadian cigarettes. *Nicotine Tob. Res.* **2007**, *9*, 835-844.

[50] Hyodo, T.; Maruta, Y.; Itaya, H.; Mikita, A.; Kodera, T.; Meger, M. Evaluation of functional relationships for predicting mainstream smoke constituent

machine yields for conventional cigarettes from the Japanese market. *Regul. Toxicol. Pharmacol.* **2007**, *48*, 194-224.

[51] Borgerding, M.F.; Bodnar, J.A.; Wingate, D.E. (Compiler); Taylor, C.R.; Reid, J.R.; Sudholz, M.A.; Podraza, K.F.; Hsu, F.S.; Borgerding M.F.; Whidby, J.F. (Principal Investigators). The 1999 Massachusetts Benchmark Study, Final Report July 24, **2000**. [Available from the Massachusetts Department of Public Health under the Massachusetts (US) public records law].

[52] Vorhees, D.J.; Heiger-Bernays, W.; McClean, M.D. Human health risk associated with cigarette smoke: the link between smoke constituents and additives. Menzie-Cura & Associates, Inc. **1997**

[53] Burns, D.M.; Dybing, E.; Gray N.; Hecht, S.; Anderson, C.; Sanner, T.; O'Connor, R.; Djordjevic, M.; Dresler, C.; Hainaut, P; Jarvis, M.; Opperhuizen, A.; Straif, K. Mandated lowering of toxicants in cigarette smoke: a description of the World Health Organization TobReg proposal. *Tob. Control.* **2008**, *17*, 132-141.

[54] WHO Technical Report Series 945, The Scientific Basis of Tobacco Product Regulation: a Report of a Study Group. Geneva: World Health Organisation, **2007**.

[55] Laugesen, M.; Fowles, J. Scope for regulation of cigarette smoke toxicity: The case for including charcoal filters. *N.Z. Med. J*. **2005**, *118*, 1213.

[56] Pankow, J.F.; Watanabe, K.H.; Toccalino, P.L.; Luo, W.T.; Austin, D.F. Calculated cancer risks for conventional and "potentially reduced exposure product" cigarettes. *Cancer Epidemiol. Biomarkers Prev.* **2007**, *16*, 584-592.

[57] Watanabe, K.H.; Djordjevic, M.V.; Stellman, S.D.; Toccalino, P.L.; Austin, D.F.; Pankow, J.F. Incremental lifetime cancer risks computed for benzo[a]pyrene and two tobacco-specific N-nitrosamines in mainstream cigarette smoke compared with lung cancer risks derived from epidemiologic data. *Regul. Toxicol. Pharmacol.* **2009**, 55, 123-133.

[58] Massey, E.; Cunningham, F.; Meredith, C. Tobacco smoke constituents: application of risk assessment models. *Toxicol. Lett.* **2008**, *180*, S71.

[59] WHO Working Group Report, Health Aspects of Air Pollution with Particulate Matter, Ozone and Nitrogen Dioxide. Bonn (Germany): 13-15 January, **2003**.

[60] Mumford, J.L.; He, X.Z.; Chapman, R.S.; Cao, S.R.; Harris, D.B.; Li, X.M.; Xian, Y.L.; Jiang, W.Z.; Xu, C.W.; Chuang, J.C. Lung cancer and indoor air pollution in Xuan Wei. *China. Sci.* **1987**, *235*, 217-220.

[61] Borm, P.J.A.; Schins, R.P.F.; Albrecht, C. Inhaled particles and lung cancer – part B: paradigms and risk assessment. *Int. J. Cancer.* **2004**, *110*, 3-14.

[62] Pope (III), C.A.; Burnett, R.T.; Krewski, D.; Jerett, M.; Shi, Y; Calle, E.E.; Thun, M.J. Cardiovascular mortality and exposure to airborne fine particulate matter and cigarette smoke. *Circulation*, **2009**, *120*, 941-948.

[63] Valavanidis, A.; Vlachogianni, T.; Fiotakis, K. Tobacco smoke: involvement of reactive oxygen species and stable free radicals in mechanisms of oxidative damage, carcinogenesis and synergistic effects with other respirable particles. *Int. J. Environ. Res. Public Health*. **2009**, *6*, 445-462.

[64] Hasegawa I. Carbon fallout determination with Bausch and Lomb image analyzer. PMUSA Inter-Office Correspondence, www.pmdocs.com, Bates numbers 1000362451–2. September, **1975**.

[65] McCormack A. D.; Taylor M. J. Investigation of fibre and particle release from cigarette filters. Paper Presented to CORESTA Smoke and Technology Group, Brighton, England, 11–15, October, **1998**.

[66] Pauly, J.L.; Stegmeier, S.J.; Mayer, A.G. Release of carbon granules from cigarettes with charcoal filters. *Tob. Control.* **1997**, *6*, 33-40.

[67] Pauly, J.L.; Allaart, H.A.; Rodriguez, M.I.; Streck, R.J. Fibers released from cigarette filters: an additional health risk to the smoker? *Cancer Res.* **1995**, *55*, 253-258.

[68] Pauly, J.L.; Mepani, A.B.; Lesses, J.D.; Cummings, K.M.; Streck, R.J. Cigarettes with defective filters marketed for 40 years: what Philip Morris never told smokers. *Tob Control*. **2002**, *11* (Suppl.1), I51-161.

[69] Agyei-Aye, K.; Appleton, A.; Rogers, R.A.; Taylor, C.R. Assessment of the elution of charcoal, cellulose acetate, and other particles from cigarettes with charcoal and activated charcoal/resin filters. *Inhal. Toxicol.* **2004**, *16*, 615-635.

[70] Hengstberger, M.; Stark, M. Fibre and particle release from cigarette filter. *Beitr. zur Tabakforsch. Int.* **2009**, *23*, 338-358.

[71] Collazo, H.; Crow, W.A.; Gardner, L.; Phillips, B.L.; Dyer, J.M.W.; Marple, V.A.; Utell, M.J. Aerodynamic diameter measurement of cellulose acetate fibers from cigarette filters : What is the potential for human exposure? *Inhal. Toxicol.* **2002**, *14*, 247-262.

[72] Sapkota, A.R.; Berger, S.; Vogel, T.M. Human pathogens abundant in the bacterial metagenome of cigarettes. *Environ. Health Persp.* **2010**, *118*, 351-356.

[73] Pauley, J.L.; Smith, L.A.; Rickert, M.H.; Hustson, A.; Paszkiewicz, G.M. Review: is lung inflammation associated with microbes and microbial toxins in cigarette tobacco smoke? *Immunol. Res.* **2010**, *46*, 127-136.

[74] Parsons, L.L.; Smith, M.S.; Hamilton, J.L.; Mackown, C.T. Nitrate reduction during curing and processing of Burley tobacco. *Tob. Sci.* **1986**, *30*, 100-103.

[75] Harley, N.H.; Cohen, B.; Tso, T.C. Polonium-210: A questionable risk factor in smoking related carcinogenesis. Banbury Report (1980)-3: A safe cigarette? In: Gori, G.B., Bock, F.G. (Eds.). Banbury Report 3-A Safe Cigarette! Cold Spring HBrbor, New York, Cold Spring Harbor Laboratory, **1980**, pp. 93-104.

[76] E.A. Martell. Radioactivity of tobacco trichromes and insoluble cigarette smoke particles. *Nature* **1974**, *249*, 215-217.

[77] Peres, A.C.; Hiromoto, G. Evaluation of 210Pb and 210Po in cigarette tobacco produced in Brazil. *J. Environ. Radioact.* **2002**, *62*, 115-119.

[78] Khater, A.E.M.; Al-Sewaidan, H.A.I. Polonium-210 in cigarette tobacco. *Int. J. Low Radiation* **2006**, 224-233.

[79] Desideri, D.; Meli, M.A.; Feduzi, L.; Rosselli, C. 210Po and 210Pb inhalation by cigarette smoking in Italy. *Health Phys.* **2007**, 92, 58-63.

[80] Papastefanou, C. Radioactivity of tobacco leaves and radiaton dose induced from smoking. *Int. J. Environ. Res. Public Health* **2009**, *6*, 558-567.

[81] Halliwell, B.H.; Poulsen, H.E. Ed. Oxidative Stress. in: Cigarette Smoke and Oxidative Stress, Berlin: Springer, **2006**.

[82] Khachatryan, L.; Adounkpe, J.; Asatryan, R.; Dellinger, B. Radicals from the gas-phase pyrolysis of catechol: 1. o-Semiquinone and ipso-catechol radicals. *J. Phys. Chem. A*. **2010**, 114, 2306-2312.

[83] Menshov, V.A.; Trofimov, A.V. Hydrogen peroxide derived from cigarette smoke: "Pardon impossible, to be sent to Siberia?. *Mini-Rev. Org. Chem.* (this review)

[84] See, S.W.; Wang, Y.H.; Balasubramanian, R. Contrasting reactive oxgen species and transition metal concentrations. *Environ. Res.* **2007**, *103*, 317-324.

[85] Weinberg, E.D. Tobacco smoke iron: an initiator/promoter of multiple diseases. *Biometals* **2009**, *22*, 207-210.

[86] Halliwell, B.H. The wanderings of a free radical. *Free Radic. Bio. Med.* **2009**, *46*, 531-542.

[87] Rodgman, A. Problems with the Tobacco Products Scientific Advisory Committee (TPSAC) list of harmful or potential harmful tobacco and/or tobacco smoke components. *Beitr. Zur Tabakforsch. Int.* **2011**, Accepted for publication.

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