

Tobacco Smoke Constituents Affecting Oxidative Stress

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2.1 Introduction

Cigarette smoke is a highly complex aerosol composed of several thousand chemical substances distributed between the gas and the particulate phases. A frequently cited estimate for the number of these constituents is ca. 4,700 (Dube and Green 1982). The enormous complexity of cigarette smoke is the result of multiple thermolytic processes that occur in heated tobacco within the confines of the burning cigarette rod. These processes involve distillation, pyrolysis, and combustion, and are influenced by factors including the design of the cigarette (Norman 1999) and the composition of the tobacco (Bokelman and Ryan 1985; Leffingwell 1999). Numerous organic chemical classes are represented in cigarette smoke including saturated and unsaturated hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids, esters, phenols, nitriles, terpenoids, and alkaloids (Baker 1999; Dube and Green 1982; Hoffmann et al. 2001). Whereas the composition of cigarette smoke is complex, certain smoke constituents have received greater analytical scrutiny than have others, either because of their greater relative abundance in smoke (which makes them easy to analyze), their known pharmacological properties (Seeman et al. 2004), and/or because they are believed to be carcinogenic or potentially harmful to smokers (IARC Monographs 1986; USCPSC 1993).

Large numbers of data on the composition of mainstream smoke have been published, and the subject has been reviewed in detail (Baker 1999). The objective of this chapter is to take a more focused look at the chemical constituents in cigarette smoke that relate to oxidative stress. In particular, we examine smoke constituents that are known to (1) increase oxidant burden, (2) decrease antioxidant protection, or (3) result in the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Section 1 provides a brief description of the thermal conditions inside a burning cigarette and some relevant properties of cigarette smoke. Section 2 is an overview of the existing information related to smoke chemistry and oxidative stress. Section 3 explores how certain tobacco leaf constituents affect the delivery of some of the cigarette smoke constituents known to influence oxidative stress. Phenolic compounds originate from the pyrolysis of polyphenols, carbohydrates, and other precursors in tobacco leaves, whereas trace metal ions present initially in the leaves are known to transfer to cigarette smoke. This discussion draws on recent results from our own laboratory and literature reports. Section 4 discusses the important topic of free radicals, ROS, and reactive RNS,

their potential involvement in the toxicity of cigarette smoke in general, and the in vitro cytotoxicity of individual smoke constituents in particular. Special emphasis is given to the role of free radicals and the redox chemistry of phenolic compounds, including some current results.

2.1.1 Pyrolysis and Combustion inside Cigarettes

Before discussing cigarette smoke and oxidative stress, we present some basic principles of cigarette smoke formation and properties that may help to convey the complexity of tobacco smoke. An elaborate description of the fluctuating thermal gradients and vapor environment inside a cigarette during smoking has been given by Baker (1999). The chemical complexity of cigarette smoke is strongly dependent on the heating conditions inside the lit cigarette. To summarize briefly, when a smoker lights and draws on a cigarette, the temperature of the ignited tobacco rises rapidly, and a hot coal forms at the lit end of the cigarette that is the center of combustion (the combustion zone). Peak temperatures inside the coal can exceed 900 °C. 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 resistance to the draw of air through the cigarette. This effect forces air to be drawn primarily from the periphery of the coal at the paper burn line rather than through the center of the coal. The depletion of oxygen due to combustion inside the coal and the flux of air around the coal results in the formation of a region immediately behind the coal that is depleted of oxygen, but where the temperatures remain high enough to promote the thermal decomposition of the unburned tobacco. For this reason, this area behind the coal is known as the pyrolysis/distillation zone. Copious amounts of volatile and semivolatile smoke constituents evolve from this zone. These constituents result in part from the pyrolysis of tobacco and in part from distillation of volatile constituents native to tobacco because of the heat of the encroaching coal.

The smoke constituents drawn through the cigarette rod during a puff and delivered to the smoker are termed mainstream smoke. In the interim period between puffs when no air is being drawn through the cigarette, the coal undergoes smoldering combustion driven primarily by diffusion of oxygen into the coal. The smoke escapes from the periphery of the coal to the surrounding air. This smoke is termed sidestream smoke. Typical coal temperatures during smoldering combustion are less than 800 °C. The different thermal conditions and air flow through and around the coal during smoldering combustion, in comparison to combustion during a puff, causes the sidestream and mainstream smoke composition to differ significantly, the primary difference being the relative abundance of the smoke constituents (Baker 1999).

2.1.2 Cigarette Smoke Properties

The smoke emitted from a lit cigarette is a dense aerosol composed of microscopic droplets, known as the particulate phase, dispersed in a vapor of air and other gases derived from the burning tobacco. The particulate phase of cigarette smoke overall acts as a reducing agent, which may play a role in its toxicity (Church and Pryor 1985; Lakritz et al. 1972; Schmeltz et al. 1977). There are some 10^9 – 10^{10} particles per cubic centimeter in

fresh mainstream smoke, and the particle size varies from 0.1 to 1.0 μm in diameter. The standard method for separating the particulate fraction of cigarette smoke from the gas-phase constituents is to pass the cigarette smoke through a fiberglass filter called a Cambridge pad (Baker 1999; Dube and Green 1982). This filter has a trapping efficiency of 99% for particles with an aerodynamic diameter larger than 0.1 μm . Thus, the particulate phase of mainstream smoke is defined operationally by the method employed to trap nonvolatile and semivolatile materials.

The total particulate matter (TPM) includes *all* the material collected on the Cambridge pad (Baker 2002). Tar is the term applied to the smoke particulate fraction collected on the Cambridge pad, minus the content of nicotine and water. A small portion of the mainstream smoke constituents are distributed between the gas and particulate phases. These organic substances are described as semivolatile constituents, and they typically have molecular weights in the range of ca. 60–200 (Baker 1999). The gas phase of cigarette smoke is the component that passes unobstructed through the Cambridge filter pad, which includes the gaseous constituents (oxygen, nitrogen, nitric oxide, carbon dioxide, carbon monoxide, etc.) and the volatile and some semivolatile organic constituents.

The TPM collected on a Cambridge filter pad is mostly soluble in either water or organic solvents (at least 95–99%), and thus differs from respirable particulate matter such as carbon black (e.g., diesel exhaust particles and other forms of soot) that is prevalent in the environment. Such materials have been termed poorly soluble particles (PSP) and have recently attracted the interest of researchers because of potential adverse effects related to the generation of ROS. Primarily surface-driven mechanisms have been invoked to explain ROS generation from PSPs and therefore appear to differ from the ROS generation mechanisms of cigarette smoke (Knaapen et al. 2004). ROS generation in cigarette smoke particulate matter is believed to be based on the redox cycling of quinones derived from TPM constituents (Dellinger et al. 2001).

2.2 An Overview of Cigarette Smoke Chemistry and Oxidative Stress

In this section, we discuss a number of chemical classes of smoke constituents that have been documented to affect oxidative stress. Because of the complexity of cigarette smoke, however, it is impossible to be comprehensive, and much remains unknown. We also do not attempt to address the relative importance of the various chemical classes to induce oxidative stress as there are many complex biological interactions and processes involved. Rather, we focus on the chemistry of the smoke constituents, citing the appropriate literature references that make the connection between the smoke constituents and their biological effects. Among the smoke constituents that we include in our overview are organic compounds or metal ions that act as electrophiles, free radicals, reactive anions or metal ions that act as reducing agents (donate an electron), or free radicals or metal ions that act as oxidizing agents (accept an electron).

ROS and RNS are generated when mainstream cigarette smoke interacts with aqueous media or physiological fluids. Some smoke constituents become involved in oxidative stress only after they are chemically modified by metabolic processes *in vivo*. For example, benzo[*a*]pyrene can be metabolized to its corresponding quinone, which can generate ROS via a redox cycling mechanism (Briede et al. 2004; Winston et al. 1993).

This quinone and related substances that are not initially present in cigarette smoke are not otherwise included in our discussion. Another distinction can be made between oxidants that form by the direct action of cigarette smoke constituents and secondary oxidants that form in response to inflammation resulting from smoking-related oxidative stress. These topics are addressed in other chapters.

2.2.1 Particulate-Phase Constituents

2.2.1.1 Free Radicals

Free radicals were discovered in cigarette smoke and other charred organic materials soon after the development of electron paramagnetic resonance spectroscopy (EPR) (Lyons et al. 1958). However, it was not until 1983 that Prof. William A. Pryor of Louisiana State University employed EPR to associate cigarette tar radicals with hydroquinone and catechol, and to suggest their possible involvement with smoking-related diseases (Pryor et al. 1983a, c). Subsequently, the Pryor research group conducted many studies to characterize the smoke radicals, and in vitro assays were performed, suggesting that cigarette smoke could cause oxidative stress or oxidative damage to essential biological molecules. For example, Church and Pryor (1985) proposed that the excess superoxide that forms in lung tissue in response to exposure to cigarette smoke might be one possible mechanism responsible for the inactivation of α_1 -protease inhibitor, a protein associated with the onset of emphysema in deficient individuals. In the same report, the authors noted that cigarette tar incubated with DNA exhibits an EPR signal in the recovered DNA. Later, it was shown that DNA damage could occur by the attack of hydroxyl radicals generated from the bound tar radicals (Pryor 1992; Pryor et al. 1998). Pryor (1992) noted that such molecular damage is not unique to tobacco smoke, but also occurs from smoke from other sources such as diesel fuel and wood.

Extensive studies were initiated by the Pryor group to characterize the cigarette TPM radicals. Organic extracts of cigarette smoke condensate revealed the presence of as many as five different EPR signals (Church and Pryor 1985). Treating the alcoholic extract of TPM with sodium hydroxide in the presence of air gave an EPR spectrum dominated by the characteristic five-line spectrum of the *p*-benzosemiquinone radical, thus revealing an abundant source of radical precursors (Pryor et al. 1983b). Subsequently, the semiquinone radicals were shown to be concentrated in the aqueous extract of cigarette tar (ACT). The EPR spectrum of fresh ACT in air-saturated pH 10 buffer solutions was found to exhibit the intense resonances of the semiquinone radicals of both hydroquinone and catechol. The pattern of resonances in the ACT spectrum was indistinguishable from the combined spectra of pure hydroquinone and catechol allowed to autooxidize in air-saturated solutions at pH 9, showing that the radicals in ACT derive from the hydroquinone and catechol in cigarette smoke.

The Pryor group (Zang et al. 1995) and “Tanigawa et al. (1994)” also demonstrated by EPR spin-trapping experiments that aqueous dimethyl sulfoxide (DMSO) solutions of ACT, buffered at pH 9 and saturated with air, contain superoxide radical anions, one of the ROS involved in oxidative stress. The mechanism proposed for the formation of superoxide in ACT was the autooxidation of the hydroquinone anion (and related anions) in air to give benzosemiquinone radical and superoxide, as shown in Fig. 2.1 (Brunmark and Cadenas 1989; Zang et al. 1995). Spin-trapped adducts of the hydroxyl radical, an-

other important stress-related ROS, were also identified in ACT. The mechanism put forth for the formation of hydroxyl radicals was the catalytic disproportionation of hydrogen peroxide (H_2O_2) by transition metal ions, the well-known Fenton reaction (Cosgrove et al. 1985).

Hydrogen peroxide is a naturally occurring by-product of oxidative stress. It is formed during normal respiration in living organisms by catalytic disproportionation of superoxide radicals by superoxide dismutase (SOD). Another enzyme, catalase, is highly efficient at converting H_2O_2 to “innocuous products, water, and molecular oxygen.” If this cellular defense mechanism is overwhelmed, the excess H_2O_2 can undergo disproportionation via the Fenton reaction to form hydroxyl radicals. The hydroxyl radicals derived from H_2O_2 are highly oxidizing species that are well known to cause oxidative damage to essential biomolecules, including DNA (Halliwell and Gutteridge 1999). H_2O_2 has been found in ACT and in aged unbuffered aqueous solutions of catechol, a smoke constituent abundant in both ACT and TPM. The H_2O_2 concentration in smoke condensate has been shown to increase with age, pH, and temperature (Nakayama et al. 1989; Stone et al. 1995). Exogenous H_2O_2 found in cigarette smoke and H_2O_2 that forms by the physiological response to smoke constituents are presumed to be a source of oxidative stress and/or damage in smokers.

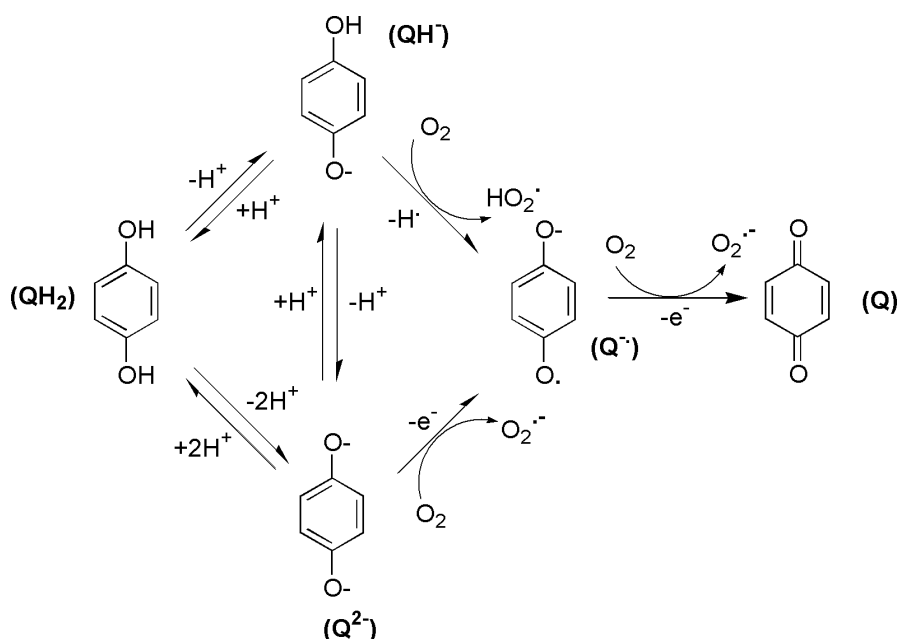


Fig. 2.1 Generation of semiquinone and superoxide radicals by autooxidation of hydroquinone, an abundant dihydroxybenzene found in the particulate phase of cigarette smoke (Zang et al. 1995)

2.2.1.2 Quinones

Quinones are readily formed from cigarette smoke constituents that can undergo auto-oxidation. Benzoquinone, for example, forms by the autooxidation of hydroquinone in

ACT (Sect. 2.1.1) or by oxidation *in vivo* in living organisms. The toxicology of quinones has been studied extensively (Bolton et al. 2000; Monks et al. 1992). In general, the toxicity of quinones is believed to occur by two mechanisms, the redox cycling mechanism, which generates excess ROS as byproducts, and the formation of covalent bonds with essential biological molecules (especially molecules containing thiol groups) (Rodriguez et al. 2004; Seung et al. 1998). Both mechanisms can contribute to the onset of oxidative stress. Quinones derived from cigarette smoke constituents undergo redox cycling in living organisms by entering into the NADPH reductase pathway (Bolton et al. 2000; Hirakawa et al. 2002; Squadrito et al. 2001). The reduction of quinones by NADPH or ascorbate regenerates the parent quinols, thereby creating the redox cycle (Roginsky et al. 1999a). Redox cycling of xenobiotic quinones can significantly increase the cellular burden of ROS and deplete their antioxidant defenses.

Whereas redox cycling of quinones is recognized as a significant source of oxidative stress from cigarette smoke, α,β -unsaturated ketones derived from particulate-phase constituents, such as benzoquinone, can also undergo electrophilic substitution in a manner similar to a number of gas-phase constituents such as acrolein (see Sect. 2.2.3). For example, pure benzoquinone in oxygenated aqueous solutions undergoes Michael addition via a semiquinone intermediate to form intensely colored condensation products; the color of the solution changes to a deep purple within minutes of dissolution, an indication of the presence of conjugated Michael addition products. This can occur for various quinones even at physiological pH, depending on the pK of the parent dihydroxybenzene (Pedersen 2002) and the redox potentials of the corresponding semiquinone radicals (Roginsky et al. 1999b). Quinones react readily with cellular nucleophiles, especially glutathione (GSH) and other thiols (Lau et al. 1988).

2.2.1.3 Trace Heavy Metals

Tobacco plants transport metal ions from the soil through the roots into the leaves (Lougou-Moulin et al. 2004; Tso 1990). Trace amounts of heavy metals accumulate in the leaves, and they are known to transfer in trace quantities from the cured and processed tobacco to mainstream cigarette smoke. These metals include cadmium, lead, mercury, arsenic, iron, copper, chromium, nickel, and selenium (Hoffmann et al. 2001; IARC Monographs 1986; Smith et al. 1997; Stohs and Bagchi 1995). The most abundant redox-inactive metals in cigarette smoke generally are cadmium, lead, mercury, and arsenic. The yield of these metals in cigarette smoke is influenced by cigarette design, but the yield generally correlates with tar yields. The most abundant redox-active metals in cigarette smoke are copper and iron, with copper being more abundant than iron, ca. 0.19 versus ca. 0.042 μg per cigarette, respectively (Stohs et al. 1997).

Many investigations have suggested that metal-induced oxidative stress can be partially responsible for the toxicity of these metals (Ercal et al. 2001). Redox-active metals, such as iron, copper, nickel, and chromium, can undergo redox cycling in oxygenated aqueous solutions, with the concomitant formation of ROS, whereas redox-inactive metals such as lead, cadmium, and mercury can deplete cells of thiol-containing antioxidants and reduce the activity of antioxidant enzymes. Heavy metals can exert other molecular effects such as inhibition of DNA repair and activation of cellular signaling (Bal and Kasprzak 2002; Barchowsky and O'Hara 2003; Kasprzak 2002; Waisberg et al. 2003). Thus, both redox-active and redox-inactive metals can potentially cause an increase in ROS in smokers.

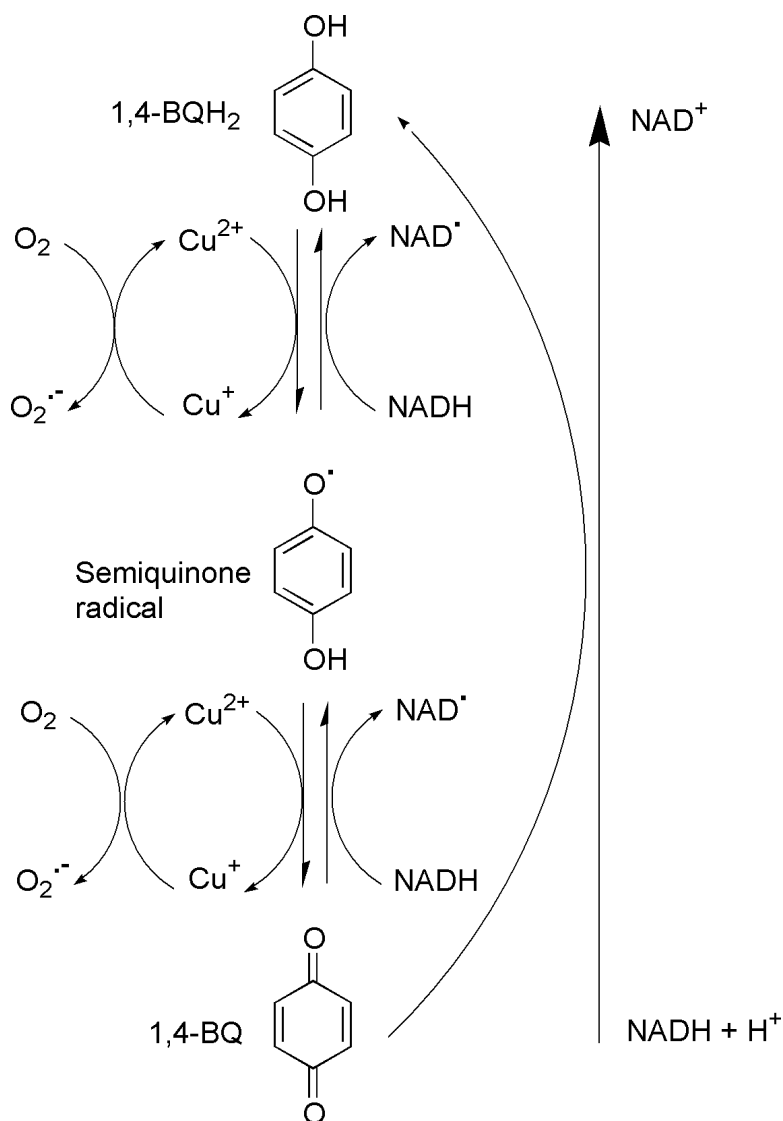


Fig. 2.2 Redox cycling mechanism for the oxidation of quinols to quinones with the formation of reactive oxygen species (Hirakawa et al. 2002)

Transition metals in the tar of cigarette smoke are notable because of their capacity to promote the formation of hydroxyl radicals via the Fenton reaction, both in aqueous extracts of cigarette smoke and in living tissues. In particular, both Fe^{2+} and Cu^{1+} are known to be active in the formation of hydroxyl radicals. These ions can also readily form complexes with many organic molecules, including those that undergo redox cycling (Stohs and Bagchi 1995; Stohs et al. 1997). Cu^{2+} has been shown to oxidize catechol

and hydroquinone to their respective quinones. It can enter into a redox cycle involving hydroquinone in the presence of molecular oxygen, forming semiquinone radicals and generating superoxide radical anions, as shown in Fig. 2.2. In contrast, Fe^{3+} does not significantly enhance the rate of oxidation of hydroquinone (Hirakawa et al. 2002; Li and Trush 1993; Li et al. 1995).

2.2.2 Gas-Phase Constituents

2.2.2.1 Oxidizing Radicals and RNS

Cigarette smoke contains abundant oxidizing agents that are found in the gas-vapor phase (Church and Pryor 1985; Pryor 1992). Even though nitric oxide ($\text{NO}\cdot$) is itself a radical, it is neither particularly reactive nor toxic. $\text{NO}\cdot$ combines slowly with molecular oxygen in air (over a period of seconds) to form the toxic oxidant and nitrating agent, $\text{NO}_2\cdot$. According to a mechanism proposed by Pryor et al. (1983b), $\text{NO}_2\cdot$ reacts rapidly with other smoke constituents such as isoprene and butadiene to form nitroso-carbon-centered radicals. Carbon-centered radicals are generally highly reactive species. The gas-phase carbon-centered radicals in smoke react instantaneously with molecular oxygen to form peroxy radicals that react with smoke gas-phase $\text{NO}\cdot$ to form alkoxy radicals and $\text{NO}_2\cdot$, thereby creating a continuous cycle. There are two interesting consequences of the above reaction scheme: (1) the oxidizing radicals in cigarette smoke are formed by reactions between the gas-phase constituents, and not primarily by pyrolysis or combustion reactions in the burning tobacco, and (2) the radicals collected inside an enclosed container of gas-phase smoke increase until the supply of $\text{NO}\cdot$ is depleted, persisting for several minutes.

Because the radical species that form from reactions of $\text{NO}\cdot$ and other gas-phase smoke constituents are all short-lived, spin-trapping methods must be employed to detect them by EPR spectroscopy, as in the case of the reactive oxygen species. The Pryor group employed the spin trap α -phenyl-*N*-*tert*-butylnitron (PBN) to detect the oxidizing gas-phase radicals in cigarette smoke. The primary spin adducts found in benzene solutions of PBN bubbled with gas-phase smoke are from alkoxy radicals, the least reactive, and therefore the longer-lived of the oxidizing radicals. Other researchers developed alternative methods to detect free radicals. For example, Flicker and Green (1998, 2001) developed a chromatographic-based method that is specific for carbon-centered radicals in whole mainstream smoke (including the TPM and the gas phase). The involvement of gas-phase free radicals in oxidative damage is unclear, because it is generally believed that the reactive gas-phase radicals are quenched immediately on contact with surfaces of the respiratory tract (Rahman and MacNee 1996a; 1996b).

$\text{NO}\cdot$ itself at physiological concentrations (ca. 0.1–10 nM) is relatively unreactive with nonradical molecules (Halliwell and Gutteridge 1999). However, it can react with tyrosyl radical, which is present at the active sites of some enzymes, particularly ribonucleotide reductase (Kwon et al. 1991; Lepoivre et al. 1994). $\text{NO}\cdot$ may be converted to a number of more reactive derivatives, known collectively as RNS, such as $\text{NO}_2\cdot$, N_2O_3 , and N_2O_4 and ONOO^- (peroxynitrite). DNA damage and nitration of tyrosine in cells exposed to the gas phase of cigarette smoke has been attributed to the action of RNS (Eiserich et al. 1994; Spencer et al. 1995). $\text{NO}\cdot$ is reported to enhance the toxicity of phenolic compounds by oxidation to their respective quinones (Urios et al. 2003).

2.2.2.2 Peroxynitrite

Peroxynitrite is an RNS that forms from the reaction of $\text{NO}\cdot$ and superoxide. Peroxynitrite is not itself a free radical, being derived from two free radicals, but it is a powerful oxidant that has been shown to induce damage to essential biomolecules in physiological media (Denicola and Radi 2005; Halliwell and Gutteridge 1999). Simultaneous generation of $\text{NO}\cdot$ and superoxide favors the production of peroxynitrite anion (Beckman et al. 1990). This peroxynitrite-forming reaction has since been shown to be diffusion controlled ($k_{\text{obs}} = 6.7 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$), indicating that competition between $\text{NO}\cdot$ and SOD for superoxide is feasible (Huie and Padmaja 1993), and most of the toxicity of superoxide has been attributed to the formation of peroxynitrite (Koppenol 1998).

It is generally believed that $\text{NO}\cdot$ in cigarette smoke reacts with superoxide derived from the reducing constituents in the particulate phase of cigarette smoke, i.e., dihydroxybenzenes such as hydroquinone and catechol, to form peroxynitrite (Müller et al. 1997). Based on kinetic and other considerations, Squadrito and Pryor (1998) proposed that peroxynitrite readily forms *in vivo*, combining rapidly with abundant intracellular carbon dioxide to form metastable nitrating, nitrosating, and oxidizing intermediates. Apart from carbon dioxide, peroxynitrite is believed to react rapidly only with molecules localized in the cellular vicinity of its formation. Peroxynitrite can react with and inactivate essential proteins including hemoglobin, myeloperoxidase, GSH peroxidase, and others. Because peroxynitrite is short-lived in living tissues and difficult to measure directly, the detection of 3-nitrotyrosine (the nitration product of tyrosine by peroxynitrite) is usually taken as evidence of its existence *in vivo* (Eiserich et al. 1994; Reiter et al. 2000).

Peroxynitrite has been identified as an oxidative stress-inducing compound of aqueous cigarette smoke fractions (Müller and Gebel 1994, 1998; Müller et al. 1997). After depletion of intracellular GSH content by electrophilic aldehydes, peroxynitrite interferes with specific target molecules, resulting in the activation of stress-related signal transduction and gene expression in cigarette smoke-treated cells *in vitro* (Müller and Gebel 1994). Furthermore, gene expression profiling in respiratory tract tissues obtained from cigarette smoke-exposed rats revealed a pronounced activation of stress response via upregulation of oxidative stress-related genes, many of which counteract cigarette smoke-induced peroxynitrite stress (Bosio et al. 2002), although other nitration reactions can occur.

2.2.2.3 Glutathione Depleting Substances

Glutathione is abundant in cytoplasm, nuclei, and mitochondria and is the major water-soluble antioxidant in these cell compartments at millimolar concentrations (Ault and Lawrence 2003). High levels of GSH are found in the extracellular lung lining fluid (about $100 \mu\text{mol/l}$), but not in blood plasma, where concentrations are very low ($<1 \mu\text{mol/l}$). Among the intracellular nonprotein thiols such as cysteine, homocysteine, α -lipoic acid, and coenzyme A, GSH accounts for more than 90% of the total thiols. GSH and other thiols react more easily with α,β -unsaturated aldehydes at the β -carbon than at the carbonyl carbon (Meacher and Menzel 1999). Both α,β -unsaturated and saturated aldehydes are direct-acting chemicals, i.e., they require no metabolic activation. The

yields of acrolein and crotonaldehyde, two α,β -unsaturated aldehydes in cigarette mainstream smoke, range from 5 to 60 and <1 to 25 μg per cigarette, respectively (Counts et al. 2004).

Modifications of intracellular GSH by electrophiles in the gas phase of cigarette smoke were first reported decades ago (Gaisch and Nyffeler 1976; Leuchtenberger et al. 1974, 1976). As shown in Fig. 2.3, electrophilic cigarette smoke constituents react with thiol-containing proteins. Eiserich (1995) reported that the concentration of protein sulfhydryl groups in blood plasma is about 500 μM . After exposure to cigarette smoke, the concentration of protein sulfhydryl groups was reduced by ca. 60%. Reddy et al. (2002) investigated this effect in more detail. Solutions of GSH in phosphate buffer exposed to gas-phase cigarette smoke resulted in a significant depletion of GSH, attributed primarily to reaction with acrolein, and a concomitant appearance of oxidized GSH (GSSG). NO , which is abundant in the cigarette smoke gas phase, can react with GSH to form S-nitroso-GSH (GSNO), but Reddy et al. (2002) found that only ca. 1% of the overall reduction in GSH could be attributed to GSNO formation. A more recent investigation by Cahours et al. (2004), using an alternative assay, showed similar amounts of GSSG and GS-aldehyde formation, but the relative percentage of GSNO accounted for more than 30% of the overall GSH depletion.

Hagedorn et al. (2003) developed a GSH depletion assay for gas-phase, particulate-phase, and whole mainstream cigarette smoke. GSH consumption was reported to be two and three times higher for particulate phase and whole smoke, respectively, in comparison to gas-phase smoke. The assay showed that the depletion of GSH in solutions of GSH treated with gas-phase cigarette smoke correlates well with the cytotoxicity of the gas phase, as determined by the neutral red uptake (NRU) and 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS)-tetrazolium assays.

The conjugation of cigarette smoke electrophiles with GSH can proceed spontaneously or by catalysis by GSH S-transferases. GSH S-conjugates are catabolized to their

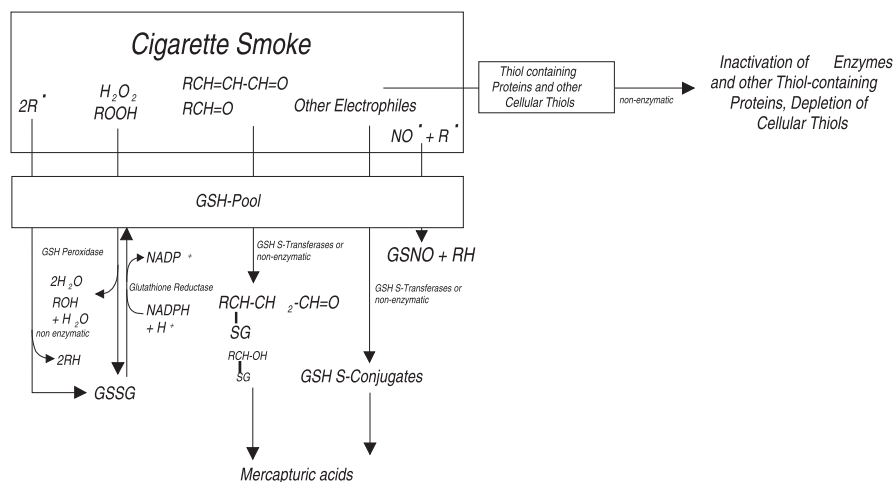


Fig. 2.3 Cigarette smoke-induced depletion of cellular thiols

corresponding mercapturic acids, which are subsequently excreted into the urine. 3-Hydroxypropylmercapturic acid (3-HPMA) is a urinary metabolite of acrolein and can be used as biomarker of cigarette smoke exposure (Mascher et al. 2001). 3-HMPA excretion in smokers as compared with nonsmokers is about three to four times higher (Martin and Tricker 2004).

2.3 Tobacco Leaf Constituents Affecting Smoke Chemistry and Toxicity

2.3.1 Phenolic Compounds

Phenolic compounds are an important class of chemicals that form during the thermal decomposition of biomass (Achladas 1991; Amen-Chen et al. 1997) and tobacco (Schlotzhauer and Chortyk 1987). Their formation, identification and quantification in cigarette smoke has been extensively studied and reviewed (Arrendale et al. 1984; Brunneman et al. 1976; Chen and Moldoveanu 2003; Counts et al. 2005; Crouse et al. 1963; Forehand et al. 2000; Klus and Kuhn 1982; Risner and Cash 1990; Yang and Wender 1962). The most abundant phenolic constituents in tobacco smoke are phenol, dihydroxybenzenes, and their methyl-substituted derivatives. Hydroquinone, catechol, and their methyl-substituted derivatives have been shown by us (see Sect. 4.3) and others (Smith et al. 2002a) to be highly cytotoxic.

Most of the research on phenolic compounds has focused on the formation of catechol and phenol from tobacco, tobacco extracts, and selected tobacco constituents such as polyphenols and lignin (Patterson et al. 1976; Sakuma et al. 1982; Schlotzhauer and Chortyk 1981, 1987; Schlotzhauer et al. 1967, 1982, 1992; Sharma et al. 2000; Spears et al. 1965; Zane and Wender 1963). Despite numerous papers addressing the formation of phenolic compounds from tobacco, there are few data available on their temperature of formation or the contribution of specific tobacco constituents to the yield of phenolic compounds in cigarette smoke TPM (Carmella et al. 1984; Schlotzhauer and Chortyk 1981; Schlotzhauer et al. 1969; Torikaiu et al. 2005). Such information is essential to understand the apportionment of phenolic compounds from tobacco leaf constituents and to develop strategies to reduce the yield of these cytotoxic agents.

We have systematically studied the formation of phenolic compounds from heated tobacco and tobacco leaf constituents in our laboratory. Pyrolysis experiments were carried out in a tube furnace in the heating range from 250 to 600 °C (McGrath et al. 2003). The effect of pyrolysis temperature and water extraction on the formation of phenolic compounds was investigated. Smoking experiments were carried out under Federal Trade Commission smoking conditions (Federal Register 1967, 1980), using cigarettes made from the three individual types of tobacco found in typical American blend cigarettes: bright, burley, and oriental tobaccos. Two reference cigarettes, 2R4F (Chen and Moldoveanu 2003) and IM17 (an industry monitor), containing a representative blend of these tobaccos, were also studied. The 2R4F and IM17 cigarettes have the same blend composition, but the 2R4F cigarette has ventilation holes in the filter tip, whereas the IM17 cigarette does not.

2.3.1.1 Phenolic Compound Yields in TPM

The chemical structures of the ten phenolic compounds in our study (hydroquinone, catechol, resorcinol, 3-methyl catechol, 4-methyl catechol, guaiacol, phenol, *o*-, *m*-, and *p*-cresol) are shown in Fig. 2.4. Quantitative yields of phenolic compounds from smoking and pyrolysis experiments were determined by gas chromatography mass spectrometry (GC/MS) and high-performance liquid chromatography (HPLC). The yield of phenolic compounds was calculated using a calibration curve obtained from the analysis of standard solutions and the yields are reported as the average of three independent measurements. The yields of phenolic compounds in the TPM from the five cigarettes are shown in Fig. 2.5, expressed as micrograms of phenol per milligram of TPM. The TPM yields per cigarette were 7.3 ± 0.7 , 14.2 ± 0.2 , 16.3 ± 1.0 , 8.6 ± 1.3 , and 9 ± 1.2 mg, respectively, for the 2R4F, IM17, bright, burley and oriental cigarettes.

Of the ten phenolic compounds measured, hydroquinone and catechol are the most abundant in the TPM of all five cigarettes. The two reference cigarettes gave relatively similar yields of all phenols. Except for the yields of catechol, phenol, and 4-methylcatechol, the yields of phenolic compounds from all three single-component blend cigarettes were quite similar. The 100% bright cigarette gave the highest yield of hydroquinone and 4-methylcatechol. The 100% burley cigarette yielded approximately 47% less catechol as compared with the bright and oriental cigarettes. The trend in the yields of hydroquinone and catechol obtained from the three single-component blend cigarettes followed the order: bright \rightarrow oriental \rightarrow burley.

Tobacco polyphenols such as chlorogenic acid and rutin have previously been shown to be precursors of phenolic compounds in cigarette smoke (Carmella et al. 1984; Sakuma et al. 1982; Schlotzhauer et al. 1967, 1982; Sharma et al. 2000; Zane and Wender 1963). The polyphenol content of cigarette tobacco filler ranges from ca. 2.2 mg per cigarette for 100% burley tobacco to ca. 14.22 mg per cigarette for 100% bright tobacco (Table 2.1). Comparison of the polyphenol content of the tobacco filler and the yield of phenolic compounds in the TPM (Fig. 2.5) reveals that the yield of phenolic compounds in the TPM is not directly proportional to the polyphenol content of the tobacco filler. Thus, other tobacco constituents in addition to the polyphenols must contribute to the overall yield of phenolic compounds in cigarette smoke. (see Section 2.3.1.4)

Table 2.1 Polyphenol contents (milligrams per cigarette) for tobacco filler from 2R4F, IM17, and single-tobacco component cigarettes

Cigarette type	Chlorogenic acid	Rutin	Scopoletin	Quinic acid	Caffeic acid	Gentisic acid
2R4F	5.1	1.8	0.01	1.2	0.06	0.01
IM17	6.7	2.2	0.06	1.8	0.13	0.01
Bright	9.7	2.3	0.13	1.9	0.19	0.02
Burley	0.4	0.4	<0.01	1.4	<0.01	<0.01
Oriental	9.0	2.1	<0.01	1.2	0.15	0.01

Polyphenol contents determined from an acetone/water extraction of respective tobacco fillers and quantified by liquid chromatography/mass spectrometry/mass spectrometry

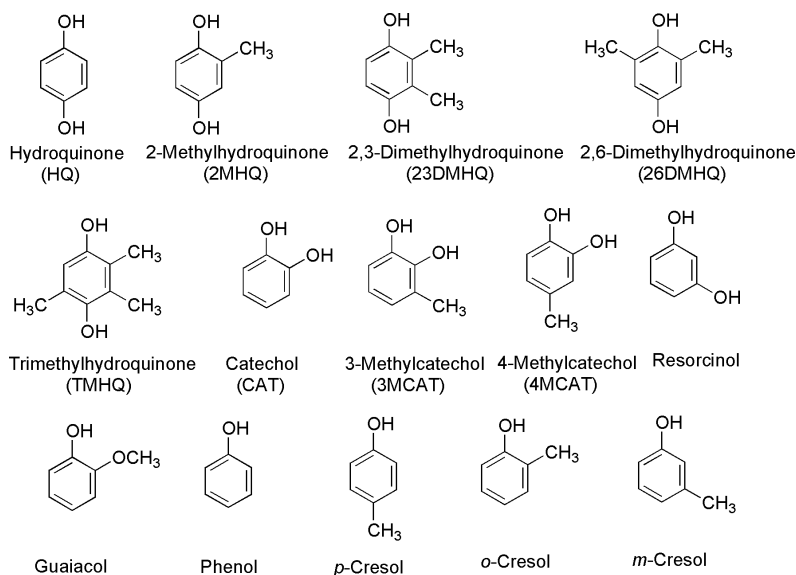


Fig. 2.4 Chemical structures of phenolic compounds found in the total particulate matter (TPM) of mainstream tobacco smoke

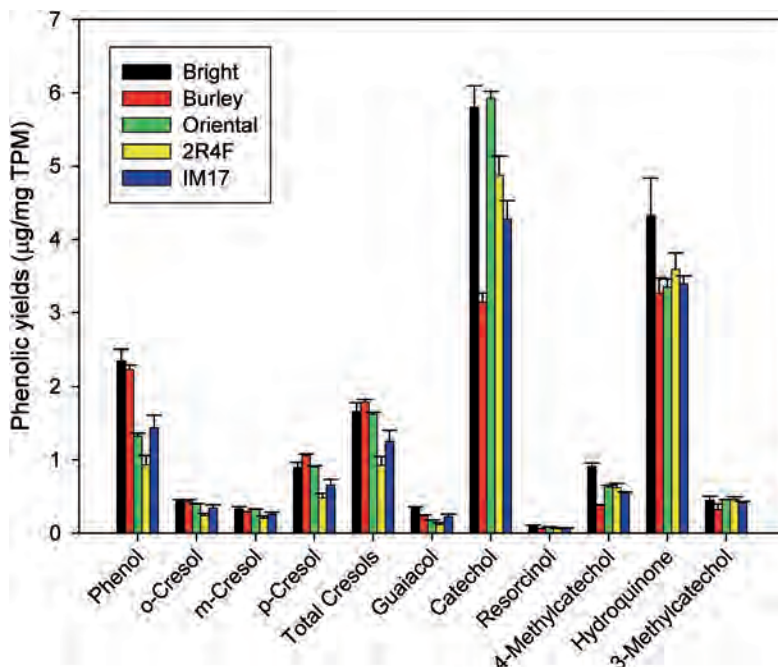


Fig. 2.5 Yields of phenolic compounds in the total particulate matter (TPM) of mainstream smoke from several cigarettes smoked under Federal Trade Commission conditions. The cigarettes were three single-component cigarettes containing bright, burley, or oriental tobacco and two reference cigarettes containing the typical American blend of tobaccos (2R4F and IM17)

2.3.1.2 Effect of Temperature on Phenolic Compound Yields

We also investigated the effect of pyrolysis temperature on the formation of phenolic compounds from heated bright tobacco lamina. Samples were first heated at 350 °C for 10 min under helium and the smoke condensate collected and analyzed. The precharred tobacco sample was then heated to 600 °C and held at this temperature for a total of 10 min under helium. The phenolic compounds found in the low-temperature TPM ([LT-TPM] 25–350 °C) and the high-temperature TPM ([HT-TPM] 350–600 °C) were characterized using GC/MS. The constituents of the LT-TPM fraction have previously been described (McGrath et al. 2005). Nicotine is the dominant constituent of the LT-TPM. Furans, furanones, phenols, pyranones, benzenediols, indoles, pyridines, fatty acids, vitamin E, and long-chain hydrocarbons are also present. The HT-TPM fraction was dominated by phenol, mono-, di-, and trimethyl phenols. Indole and methyl indole were also major products, followed by methyl pyridines, substituted pyrroles, methylpyridoindole, stigmaterol, and cholesterol acetates. It is interesting to note that approximately 86% of the total amount of TPM collected by this two-step process forms over the 25–350 °C temperature region.

The relative yields of phenols produced over the two temperature regions of 25–350 °C and 350–600 °C, expressed as a percentage of the total yields formed at 600 °C, are shown in Fig. 2.6. Hydroquinone (96%), catechol (97%), guaiacol (95%), 3-methylcatechol

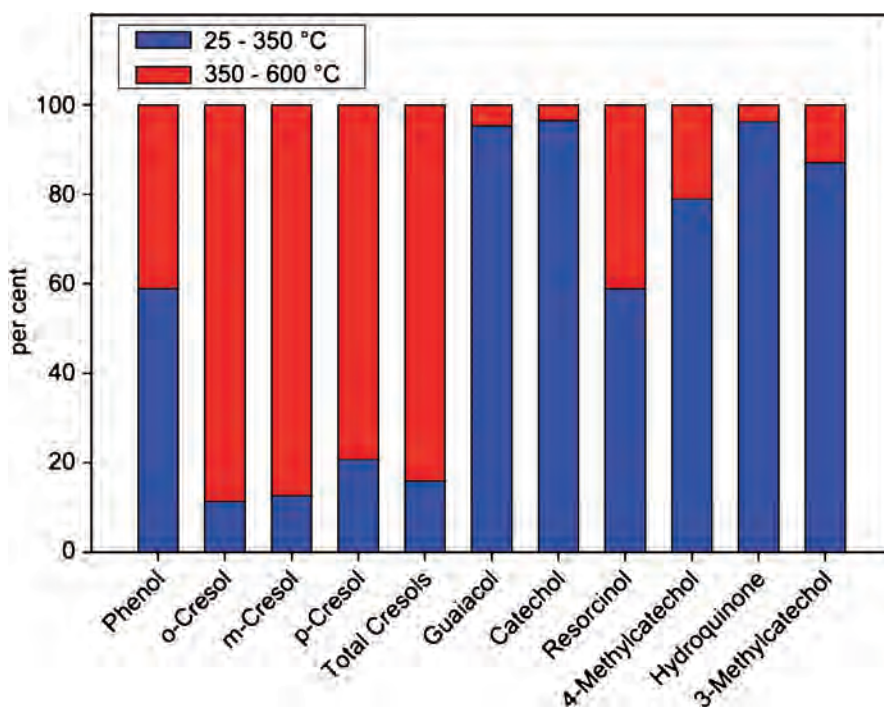


Fig. 2.6 Temperature formation range for phenolic compounds in the total particulate matter (TPM) of bright tobacco heated in a tube furnace under flowing helium for 10 min

(87%) and 4-methylcatechol (79%) are formed predominantly in the LT-TPM fraction, whereas *o*-cresol (89%), *m*-cresol (87%), and *p*-cresol (79%) form predominantly in the HT-TPM fraction. Formation of phenol (59%:41%) and resorcinol (59%:41%) appears to span the two temperature regions. The total yield of the ten phenolic compounds studied accounts for approximately 4% of the total weight of TPM formed.

2.3.1.3 Effect of Water Extraction on Phenolic Compound Yields

To investigate the effect of removing or concentrating potential phenolic precursors from tobacco on the yields of phenolic compounds in cigarette smoke, we extracted samples of bright, burley, and oriental lamina with water. Tobacco polyphenols and brown pigments can be removed and isolated from tobacco by extraction with water, methanol, acetone, and water/methanol solutions (Chortyk et al. 1966; Schlotzhauer and Chortyk 1981; Schlotzhauer et al. 1972, 1969, 1992; Wright et al. 1960, 1964; Zane and Wender 1963). Extraction with water leads to about a 50, 40, and 53% reduction in sample weight of the bright, burley, and oriental lamina, respectively. Water extraction removes the more polar constituents such as inorganic salts, organic salts, polyphenols and alkaloids, while concentrating (on a per-unit weight basis) various carbohydrate, lignin, and lipophilic constituents such as waxes, fatty acids, and high-molecular-weight sterols.

The water-extracted lamina samples were heated at 600 °C under helium, and the yield of phenolic compounds formed were compared with the nonextracted samples. Significant reductions in the yields of hydroquinone (50–60%), catechol (37–41%) and phenol (50–55%) on a per-unit weight basis were observed in the TPM of heated bright and oriental tobacco. A 40% reduction in the yield of hydroquinone was also observed for the TPM of extracted burley tobacco, but, by comparison, there was only a slight reduction in the yield of phenol (21%) and cresols (9%), and there was a significant increase of catechol (85%). When the yields of phenolic compounds from the water-extracted tobacco are normalized to the total amount of material extracted, larger reductions are observed. Decreases of 63–82% for hydroquinone, 53–74% for phenol, and 35–57% for cresols were observed for the burley, oriental, and bright samples, respectively. Whereas the catechol also decreased to around 71% for both bright and oriental tobaccos, the yield of catechol for burley increased slightly by 11%.

Consistent with previous work carried out on the formation of phenol and catechol from extracted tobacco, we found that extraction of tobacco lamina with water removes precursors of hydroquinone, catechol, and phenol from bright and oriental tobacco. Extraction of burley lamina also removes hydroquinone precursors, but significantly concentrates catechol precursors. Because of the longer curing times employed for burley tobacco, precursors to catechol such as chlorogenic acid may be polymerized via enzymatic reactions to water insoluble polymeric precursors (Kameswararo and Gopalachari 1965; Wright et al. 1960, 1964).

2.3.1.4 Phenolic Compound Formation from Tobacco Constituents

To identify possible tobacco precursors of hydroquinone and catechol, we pyrolyzed a number of polyphenolic, carbohydrate, and lignin samples at 600°C under helium for 10 min and analyzed the collected TPM condensate for hydroquinone, catechol, phenol, and cresols (sum of *o*-, *m*-, and *p*-cresols) by HPLC. The chemical structures of the polyphenols (gentisic, quinic, chlorogenic, and caffeic acids, scopoletin, and rutin) are shown in Fig. 2.7. The yields of hydroquinone, catechol, phenol, and cresols produced from the pyrolysis of these compounds added to bright tobacco under helium gas are shown in Fig. 2.8. The increase in the yield of hydroquinone from the addition of gentisic acid was ca. 6 times higher than that from quinic acid and ca. 17 times higher than from chlorogenic acid on a per-unit weight basis.

The yields of hydroquinone, catechol, phenol, and cresols from the 600°C pyrolysis of the individual tobacco cell wall constituents are shown in Fig. 2.9. Comparable yields of hydroquinone, catechol, and phenol were formed from cellulose, xylan, glucose, and fructose, with slightly lower amounts of hydroquinone being formed from the pectin sample. The yield of cresols was very similar for cellulose and pectin, with slightly lower yields being formed from glucose and fructose. Although the yield of hydroquinone from the model lignin sample is comparable to the carbohydrates pyrolyzed, the yields of catechol, phenol, and cresols are approximately 9, 11, and 13 times higher, respectively, compared with the cell wall carbohydrates.

Among the 11 tobacco constituents studied, gentisic, quinic, and chlorogenic acids were found to be the most significant precursors of hydroquinone. Caffeic, chlorogenic, and quinic acids are major precursors to catechol, followed by lignin and then the carbohydrates. Lignin yields significantly more catechol compared with the cell wall carbohydrates, (Carmella et al. 1984; Schlotzhauer et al. 1982) but significantly less in comparison to chlorogenic or caffeic acid (ca. 4 and 17 times lower, respectively, on a per-unit weight basis).

To estimate the contribution of each tobacco leaf constituent to the overall yield of the phenolic compounds from the pyrolysis of tobacco, we normalized the yield of phenolic compounds from each of the precursors studied to the amount of each precursor reported in bright tobacco. The estimated level of phenolic compounds from each precursor in bright tobacco leaf lamina is given in Table 2.2. For the normalization step, glucose was used to represent the total reducing sugars, and amounts of cellulose, pectin, hemicellulose, reducing sugars, and lignin were taken from the work of Leffingwell (1999) and Bokelman and Ryan (1985). The amounts of free quinic, caffeic, chlorogenic, and gentisic acids were obtained experimentally from the liquid chromatography mass spectrometry (LC/MS) analysis of an acetone/water extract of bright tobacco.

The carbohydrates and lignin constituents in tobacco were found *not* to be major precursors to hydroquinone. Even though they make up ca. 46% of the weight of tobacco, together they only account for 6–8% of the overall yield of hydroquinone. The polyphenols, which account for less than 2% of the weight of tobacco, account for ca. 17% of the overall hydroquinone yield. Chlorogenic acid and the estimated free quinic acid level account for ca. 11 and 6%, respectively.

From the pyrolysis of the individual tobacco constituents at 600°C presented in Figs. 2.8 and 2.9, we found that caffeic, chlorogenic, and quinic acids are the major pre-

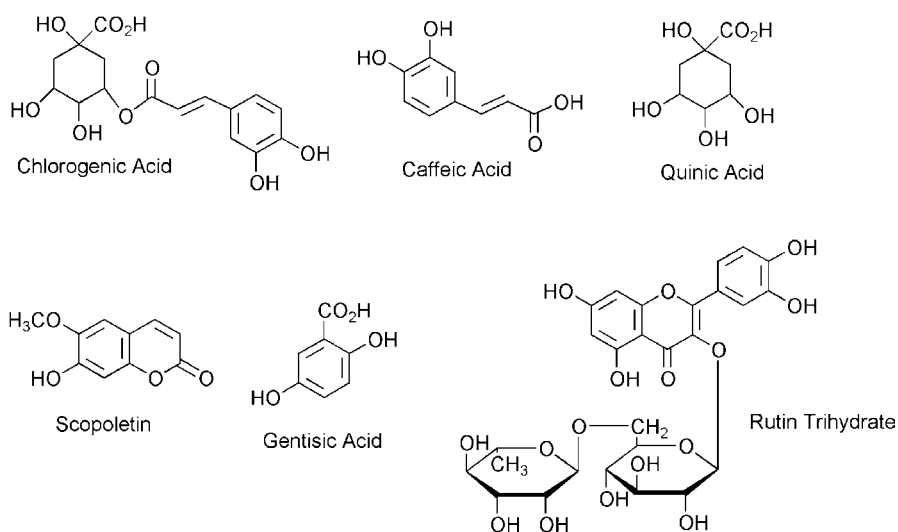


Fig. 2.7 Chemical structures of phenolic precursor compounds found in bright tobacco

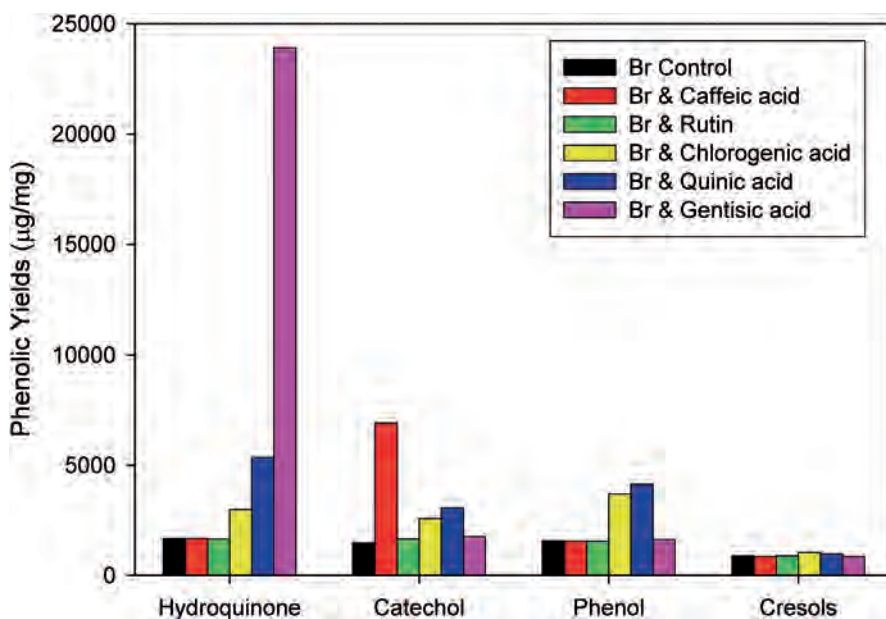


Fig. 2.8 Yield of phenolic compounds from 1 g bright tobacco mixed with 60 mg of the indicated phenolic precursors and heated at 600°C for 10 min under flowing helium in a tube furnace

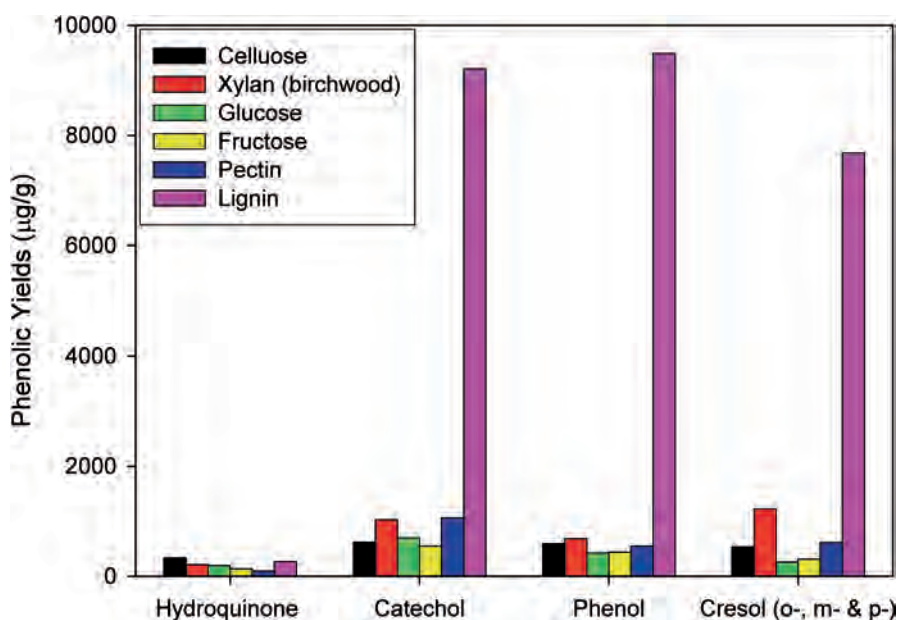


Fig. 2.9 Yield of phenolic compounds from several tobacco leaf constituents heated at 600°C under flowing helium for 10 min

cursors to catechol, followed by rutin, lignin and, to a lesser extent, the polysaccharides. Upon normalization of the yields, the carbohydrate and lignin together were found to account for approximately one third of the overall catechol yield. Although the carbohydrate content in tobacco is generally approximately five times higher than that of lignin, lignin accounts for 11% of the total yield of catechol, followed by hemicellulose (6.1%), pectin (6.6%), glucose (6%), and cellulose (3.7%). Of the polyphenols, chlorogenic acid (11%), quinic acid (2.7%), caffeic acid (1.2%), and rutin (0.8%) contribute ca. 16% towards the overall yield of catechol. The individual contributions from the pyrolysis for chlorogenic acid, glucose, cellulose, and rutin to the overall yield of catechol reported here are very similar to those previously reported by Carmella et al. (1984).

Based on the assumptions employed for the normalization, we found that ca. 24% of the overall hydroquinone yield and ca. 49% of the overall catechol yield can be accounted for by the 11 tobacco constituents examined. It should be noted that the addition of potassium nitrate and potassium acetate to pure cellulose (1% [w/w] potassium levels) led to a threefold increase in the yields of hydroquinone and catechol. Calcium (in the form of CaCO_3) also significantly increases the yield of catechol when added to cellulose. The influence of the two most abundant endogenous inorganic cations (potassium and calcium) can potentially increase the overall contribution of tobacco carbohydrates to catechol yields.

Table 2.2 Estimated source apportionment for hydroquinone and catechol in the TPM of smoke from 100% bright tobacco heated at 600 °C for 10 min under helium

	Wt%	HQ yield ^a	Percentage of total ^b	CAT yield ^a	Percentage of total
Carbohydrates					
Cellulose	10	38	2.4	62	3.7
Glucose	14	34	2.1	100	6.0
Pectin	10	13	0.8	110	6.6
Hemicellulose	10	20	1.2	102	6.1
Lignin	2	5	0.3	184	11.0
Polyphenols					
Chlorogenic acid	0.84	183	11.0	155	10.5
Rutin	0.41	1	0.1	12	0.8
Quinic acid	0.15	92	5.5	39	2.7
Caffeic acid	0.02	0	0.0	18	1.2
Gentisic acid	0.0015	6	0.4	0	0.0

Wt% Estimated weight percentage of each component in bright tobacco lamina (Bokelman and Ryan 1985)

^aYields of hydroquinone (HQ) and catechol (CAT), respectively, for each tobacco constituent normalized to the amount of each constituent found in a methanol/water extract of bright tobacco

^bContribution to the total yield

2.3.2 Trace Heavy Metals

The yields reported in the literature of trace metals that transfer from the cigarette tobacco to the mainstream cigarette smoke vary widely. For example, Purkis et al. (2003) reported the yields of several trace metals in the TPM from three different cigarettes (5-, 8-, and 12-mg tar delivery), tested by five independent laboratories under the same smoking regime. For the 8-mg product, the yield (nanograms per cigarette) for cadmium was 20.6–35.3; for lead, 8.8–16.8; for mercury, 0.4–3.5; for arsenic, 1.9–2.2; for chromium, 2–8.7; for nickel, 2–5.3; and for selenium, 0.8–6. Although the Cambridge filter is the most common method of collecting organic compounds from cigarette smoke condensate, it is not suitable for the collection of tar from mainstream cigarette smoke for trace metal analysis because of the trace metal impurities in the pad. Instead, quartz glass filters have been used for collection of inorganic compounds in tobacco smoke, because their background contamination is relatively low. Although cold traps and jet impaction traps have been employed, electrostatic precipitation into quartz tubes has become the preferred technique for collection of cigarette smoke condensate for trace metal analysis. Samples must be handled with meticulous care to avoid potential sources of metal contamination from the laboratory environment (Counts et al. 2004, 2005; Gregg et al. 2004; Roemer et al. 2004). In general, the use of an isolation clean room is required for accurate trace metal analysis.

A variety of analytical techniques has been employed for determining trace metals in mainstream cigarette smoke. In the most recently reported measurements, the two

Table 2.3 Trace metal analysis for 1R4F research cigarettes and three commercial products reported by different labs under different smoking regimes

Reference	Cigarette	Smoking Conditions	Tar (mg/cig.)	Cd (ng/cig.)	Pb (ng/cig.)	Hg (ng/cig.)	As (ng/cig.)	Cr (ng/cig.)	Ni (ng/cig.)	Se (ng/cig.)	Method	No. labs/rep.
Gregg et al. 2004	1R4F	ISO ^a	9	63.2	39.3	4.6	6.1	— ^b	— ^b	— ^b	ICP-MS	2 labs (average)
Torrence et al. 2002	1R4F	FTC ^c	9	64.2±6.3 ^d	38.2+/-1.8	— ^e	6.9±0.5	— ^e	— ^e	— ^e	ICP-MS	20 cigarettes
Chang et al. 2002	1R4F	FTC	9	— ^e	— ^e	5±0.4	— ^e	— ^e	— ^e	— ^e	CV-AAS	20 cigarettes
Chen and Moldoveanu 2003	1R4F	ISO	9.38	55.1	42.5	5.4	12.2	57.7	6.4	34.9	Various	1–4 labs
Counts et al. 2004	1R4F	ISO	9.1±0.4	64.4±2.9	36.7±1.5	5.5±0.5	4.7±0.8	— ^b	— ^b	— ^b	GF/CV-AAS	20 cigarettes/replicates
Counts et al. 2004, 2005	1R4F	MDPH ^f	19.1±0.8	143.6	69.5	10	11.2	— ^b	— ^b	— ^b	GF/CV-AAS	20 cigarettes/replicates
	1R4F	HC ^g	26.3±1.4	160.1	89	10.1	11.9	— ^b	— ^b	— ^b	GF/CV-AAS	20 cigarettes/replicates

Cd cadmium, Pb lead, Hg mercury, As arsenic, Cr chromium, Ni nickel, Se selenium, mg/cig: milligrams per cigarette, ng/cig: nanograms per cigarette, labs/rep: laboratories/replicates, ISO International Organization for Standardization, ICP-MS inductively coupled plasma mass spectrometry, FTC Federal Trade Commission, CV-AAS cold-vapor atomic absorption spectrometry, GF/CV-AAS graphite-furnace atomic absorption spectrometry, MDPH Massachusetts Department of Health, HC Health Canada

^aISO puffing conditions: 35-ml volume, 60-s interval, 2-s duration

^bBelow detection limit or too low to quantify

^cFTC puffing conditions: 35-ml volume, 60-s interval, 2-s duration

^dMean±SD

^enot measured

^fMDPH puffing protocol: 45-ml volume, 30-s interval, 2-s duration, 50% filter ventilation blocking

^gHC puffing protocol: 55-ml volume, 30-s interval, 2-s duration, 100% filter ventilation blocking

most common techniques were either inductively coupled plasma mass spectrometry (ICP-MS), or graphite-furnace (GF-) or cold-vapor atomic absorption spectrometry (CV-AAS). Because of its higher volatility, mercury is usually measured by cold-vapor atomic absorption spectrometry (Chang et al. 2002; McDaniel et al. 2001).

The yields of several trace metals in the mainstream smoke collected under International Organization for Standardization (ISO) smoking machine conditions from 48 commercial brands of filtered cigarettes were reported by Counts et al. (2004). The tar yield in the various brands ranged from 0.9 to 14.4 mg per cigarette. To a very good approximation, the amount of cadmium, lead, mercury, and arsenic in the smoke condensate was found to be proportional to the yield of tar per cigarette. The overall yield of trace metals showed a dependence on the cigarette brand, design, and the smoking machine conditions employed (Counts et al. 2005). For the 48 brands tested, the range of the average yield per cigarette for each metal was cadmium, 1.6–101.1 ng (48 brands); lead, 13.0–31.4 ng (5 brands); mercury, 1.5–4.7 ng (40 brands); and arsenic, 3.9–5.5 ng (3 brands). For the brands not included in this summary, the levels of these metals in the cigarettes were either below the detection limits of the analytical methods, or too low to quantify. A similar survey of cigarette brands sold in the United Kingdom was made by Gregg et al. (2004). In both studies, some of the variation in trace metal yields between cigarettes was likely because of the variation in the trace metal composition of regional cigarette tobaccos. Other reviews of metal ions in cigarette smoke include (Baker 1999; Hoffmann et al. 2001; IARC Monographs 1986; Smith et al. 1997). In some of these reports, single values are given for the yield of particular metals without indicating the cigarette design, TPM yield, or smoking regimen (see examples cited by Baker [1999] and Stohs et al. [1977]). Such reports are not very meaningful in light of the large range in tar yields reported for different commercial products.

The remaining toxic elements that have been assayed in commercial cigarette products are nickel, chromium, and selenium. The yields of these elements in the most recent studies with commercial filtered cigarettes are either below the detection limit of the methods or too low to quantify (<2 ng per cigarette), over a range of cigarette tar yields. (See, for example, the results of Counts et al. [2004, 2005], Gregg et al. [2004], and the summary for 1R4F research cigarettes given in Table 2.3.) In a systematic comparison of the analyses of 1R4F research cigarettes by four different laboratories, Chen and Moldoveanu (2003) reported high values of 57.7, 6.4, and 34.9 ng per cigarette for chromium, nickel, and selenium, respectively (Table 2.3). The authors noted that different analytical limits of quantification between laboratories contributed to uncertainties in several reported yields. Other potential contributors to these analytical variations are the laboratory environmental or apparatus contaminants addressed by Torrence et al. (2002). Thus, appropriate analysis precautions appear necessary to ensure that accurate data are available for health, regulatory, and tobacco science groups.

2.4 Free Radicals, ROS, and RNS

Cigarette smoke contains a large amount of free radicals (Pryor et al. 1983a, c) and constituents that readily produce free radicals (Cosgrove et al. 1985; Pryor et al. 1983a). The free radicals in cigarette smoke can be classified into two categories: (1) free radicals that form during the burning of tobacco and the smoking process and (2) free radicals that are *not* initially present in the smoke, but are generated either when the gas phase or the

TPM constituents are oxidized in the smoke aerosol, or when they dissolve in oxygenated aqueous solutions or biological media. The first category includes the radicals in the TPM and the gas phase, whereas the second category includes semiquinone radicals, ROS, and RNS.

2.4.1 Particulate-Phase Free Radicals

The TPM free radicals from cigarette smoke are known to be stable and last for an indefinitely long period of time (Chouchane et al. 2005; Pryor et al. 1983a, c, 1998; Zang et al. 1995). They can be detected using EPR either directly on the filter used to collect the TPM or in solution by extraction of the TPM. A cellulose filter is employed because the Cambridge pad exhibits a background EPR signal. Figure 2.10 shows the EPR signal of cigarette smoke TPM free radicals detected directly on a cellulose filter (Chouchane et al. 2005). The EPR signal is characterized by a broad singlet with a g -factor equal to 2.0028. The EPR spectrum in Fig. 2.10 is very similar to previously reported spectra (Pryor et al. 1983a; Pryor et al. 1983c). The concentration of these radicals in TPM can be as great as 10^{17} radicals per gram of TPM depending on the tobacco, cigarette type, and the smoking regime. The chemical nature of the radicals in cigarette smoke TPM has never been fully characterized. The accepted view of TPM radicals is that they consist primarily of semiquinone radicals in a polymeric tarry matrix. (Pryor et al. 1983a; Pryor et al. 1983c). Semiquinones undoubtedly account for part of the radicals, but recent findings suggest that the TPM radicals are not simply semiquinone radicals, but can be distinguished as oxygen-centered radicals or carbon-centered radicals (Chouchane et al. 2005).

Using bright tobacco cigarette filler heated in a tube furnace under helium atmosphere, we observed that the free radical yield in TPM increases with the heating temperature, as shown in Fig. 2.11. Maskos et al. (2005) made similar observations and showed that the TPM radicals from bright tobacco filler heated at 200–400 °C exhibit g -factors that vary from 2.0039 to 2.0050, characteristic of oxygen-centered radicals, whereas radicals from tobacco heated at ca. 600 °C exhibit a g -factor equal to 2.0028, characteristic of carbon-centered radicals. Experiments in our laboratory showed that TPM radicals from both cigarette smoke and pyrolyzed tobacco undergo an aging process when exposed to air, exhibiting an increase in the intensity of the EPR signals and a shift in the g -factors after the TPM is aged for more than 24 h, as shown in Fig. 2.12. The results of Maskos et al. (2005) and our own suggest that a significant fraction of the cigarette smoke radicals trapped on the filter is initially carbon-centered radicals that convert to oxygen-centered radicals on exposure to molecular oxygen in air.

In experiments utilizing a smoking machine, we measured the yield of free radicals in the TPM from several cigarettes containing different amounts of polyphenolic compounds in the tobacco filler, as shown in Fig. 2.13 (Chouchane et al. 2005). The cigarettes used in our study were the same cigarettes employed in the phenolic compound analysis (see Sect. 2.3.1). We found that the yield of free radicals generated in the TPM of the smoke from these cigarettes was *not* directly related to the total amount of polyphenolic compounds in the tobacco leaf filler. For example, a cigarette containing 100% bright tobacco, which contains a significantly higher amount of polyphenolic compounds in comparison to burley tobacco (Table 2.1), did not generate the highest amount of free radicals in the TPM. However, with the exception of the bright cigarettes, a slight trend was observed between the TPM radicals and the dihydroxybenzenes in the TPM

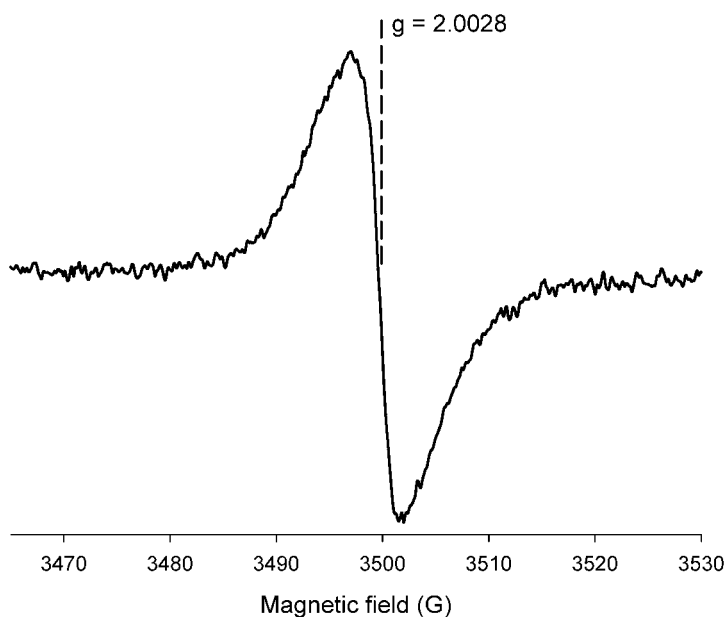


Fig. 2.10 EPR signal of free radicals in the total particulate matter (TPM) of mainstream smoke from a single 2R4F cigarette. The spectrum of the fresh TPM was measured directly on the cellulose collection filter (Chouchane et al. 2005)

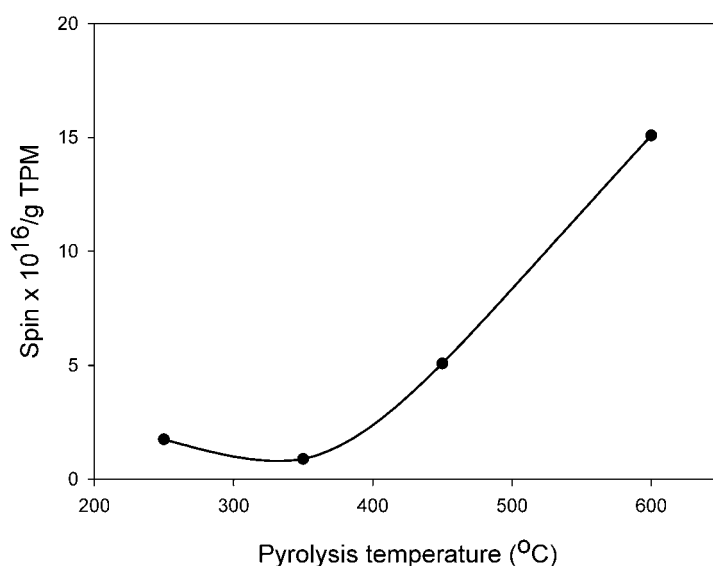


Fig. 2.11 Effect of pyrolysis temperature on the yield of free radicals in the total particulate matter (TPM) from bright tobacco. The tobacco was heated for 10 min in a tube furnace at the indicated temperatures under a helium atmosphere

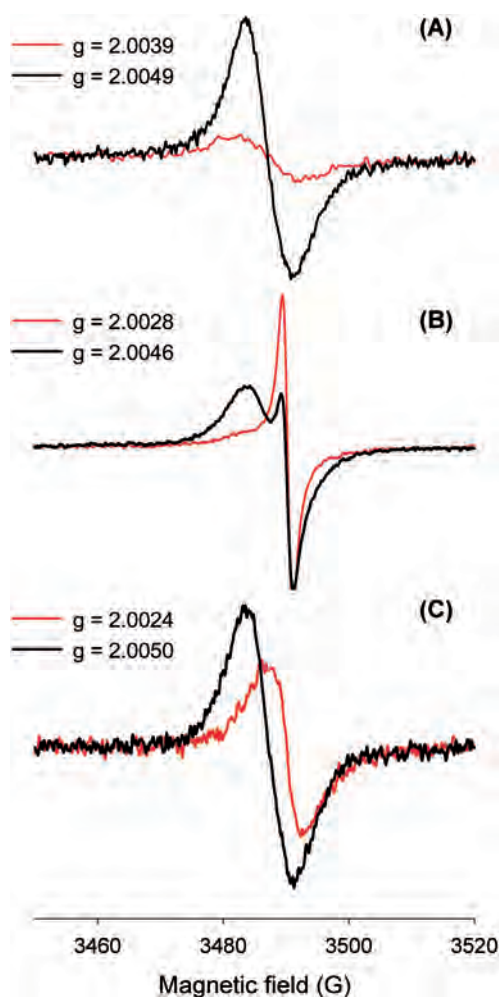


Fig. 2.12 Effect of aging on the free radicals in the total particulate matter (TPM) of smoke from 100% bright tobacco cigarettes or bright tobacco heated under helium in a tube furnace. EPR spectra of free radicals in fresh (.....) and aged (—) TPM for a week from **a** tobacco pyrolyzed in at tube furnace at 450°C, **b** tobacco pyrolyzed at 600°C in a tube furnace, and **c** a smoked cigarette

(Fig. 2.14), suggesting that these phenolic compounds do contribute to the formation of the TPM radicals. This result differs, however, from the data previously reported by Blakley et al. (2001) that showed that, there is no relationship between the radicals and the yield of phenolics in the TPM.

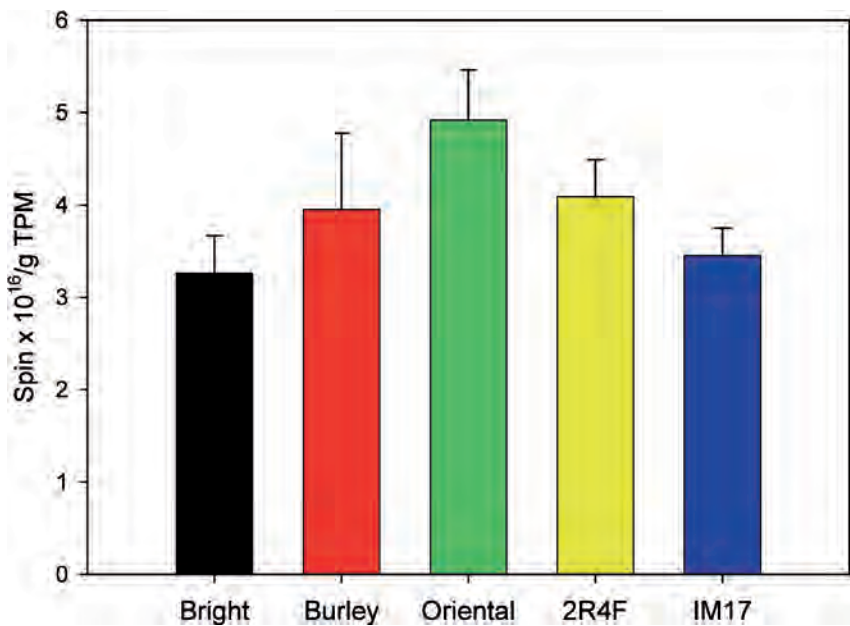


Fig. 2.13 Yield of free radicals in fresh total particulate matter (*TPM*) of mainstream smoke of different cigarettes (Chouchane et al. 2005)

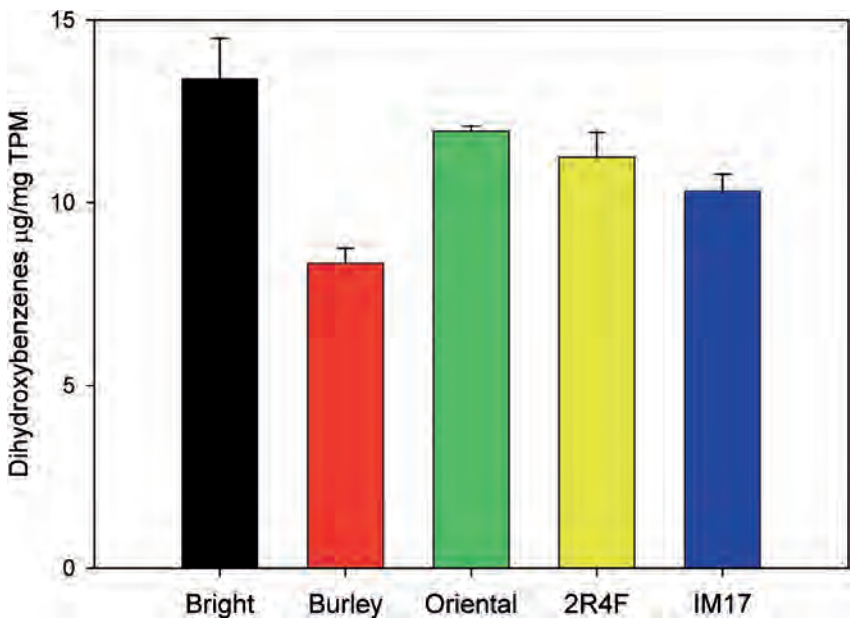


Fig. 2.14 Yield of dihydroxybenzenes in the total particulate matter (*TPM*) of mainstream from different cigarette smoke (Chouchane et al. 2005)

2.4.2 Gas-Phase Free Radicals

The radicals in the gas-vapor phase of cigarette smoke are oxidizing, and they are generally more reactive than are the TPM radicals. EPR spin-trapping techniques are usually used to detect the free radicals in the gas phase of cigarette smoke, typically using the spin trap PBN in benzene solution (Bluhm et al. 1971). Figure 2.15 shows the resulting EPR signal of the gas phase of cigarette smoke, separated from the TPM by a Cambridge filter, and bubbled into a benzene solution of 100 mM PBN (Chouchane et al. 2005). The measured hyperfine coupling constants of the radical adducts (confirmed by spectral simulation) are $a_N = 13.9$ G and $a_H = 2.0$ G, and the g-factor is 2.00565. These parameters are in agreement with previously reported hyperfine coupling constants for the PBN adducts of alkoxy radicals (Pryor et al. 1983a, c). Quantification of the gas-phase radicals from different cigarettes shows that the yield of radicals per cigarette falls in the order: 2R4F > IM17 > burley > oriental > bright (Fig. 2.16) (Chouchane et al. 2005).

$\text{NO}\cdot$ is known to be the predominant precursor of other cigarette smoke gas-phase radicals (Sect. 2.2.1). Nitrate, amino acids, ammonium salts, and other nitrogen-containing compounds can potentially produce $\text{NO}\cdot$ in the gas phase of cigarette smoke by thermolytic decomposition. Notably, however, gas-phase free radicals can also be produced by heating tobacco leaf constituents that do not contain nitrogen, e.g., cellulose (Pryor et al. 1983a). Im et al. (2003) showed that the evolution of $\text{NO}\cdot$ from heated tobacco occurs in two distinct temperature regimes from primarily two sources. Between 275 and 375°C, under pyrolytic or combustion conditions, the source of $\text{NO}\cdot$ was attributed to the decomposition of nitrate. At a higher temperature range (425–525°C), $\text{NO}\cdot$ is produced only in an oxidative environment. In this thermal regime, the $\text{NO}\cdot$ was attributed to the oxidation of the char, which contains nitrogen originating from the decomposition of amino acids and protein at lower temperatures.

2.4.3 Cytotoxicity of TPM Constituents

The TPM of cigarette smoke is a very complex mixture that contains numerous substituted phenolic compounds (Smith et al. 2002a). Among these compounds, the dihydroxybenzenes are notable because they can act either as prooxidants or antioxidants. In this section, we present evidence for their possible involvement in the cytotoxicity of cigarette smoke TPM. As discussed in Sect. 3.1.1, the most abundant phenolic compounds found in the TPM from blended or single tobacco cigarettes are phenol, dihydroxybenzenes, and their methyl-substituted derivatives, with hydroquinone and catechol exhibiting the highest yields (see Fig. 2.5). Hydroquinone and catechol are abundant in the smoke of commercial cigarettes (Baker 1999; Counts et al. 2004; Roemer et al. 2004). They are known to generate semiquinone and superoxide radicals via the redox cycling mechanism in aqueous solutions (Samuni et al. 2003; Squadrito et al. 2001), and they have been shown to induce damage in physiological systems (DeCaprio 1999; Deisinger et al. 1996; do Céu Silva et al. 2003; McCue et al. 2003).

We have measured the *in vitro* cytotoxicity of hydroquinone, catechol, and their methyl-substituted derivatives. The results, given in Table 2.4 (Chouchane et al. 2004), are reported in terms of the 50% effective concentration (EC_{50}), the effective concentration required to kill 50% of mouse embryo BALB/c 3T3 cells in the NRU assay. We found that the methyl-substituted dihydroxybenzenes exhibited a higher cytotoxicity

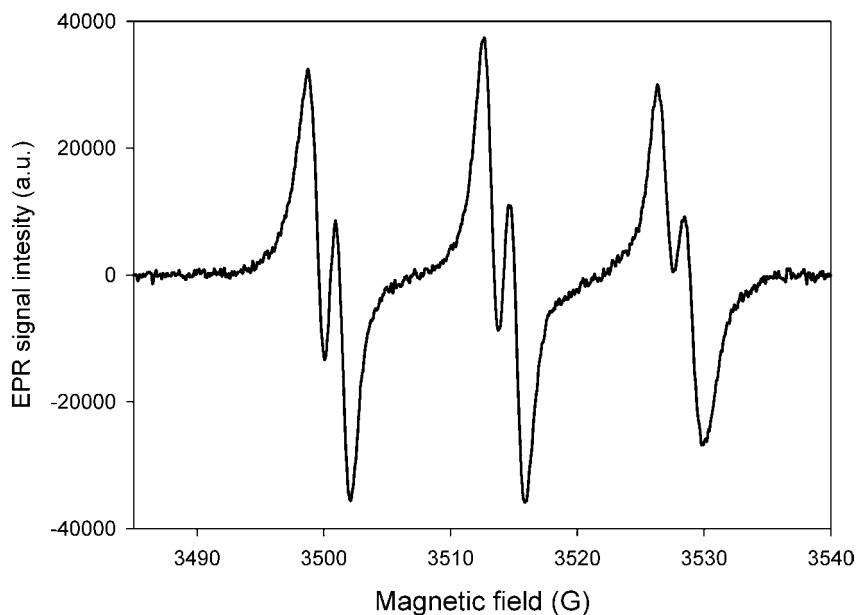


Fig. 2.15 EPR signal of spin adducts of radicals in the gas phase of cigarette smoke. The gas phase was separated from the total particulate matter (TPM) using a Cambridge pad and bubbled into a solution of benzene containing a 100-mM α -phenyl-*N*-tert-butyl nitron (PBN) spin trap. The sample was degassed and analyzed by EPR spectroscopy (Chouchane et al. 2005)

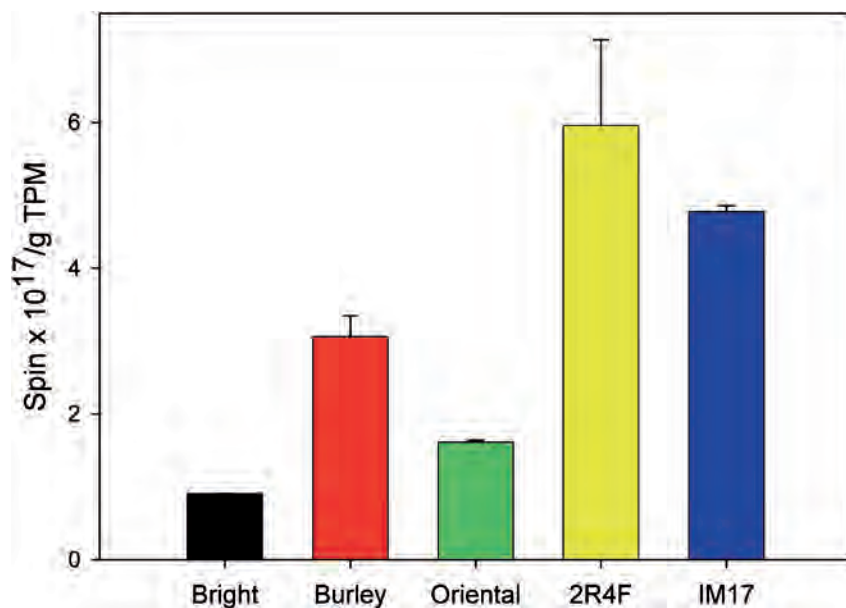


Fig. 2.16 Yield of free radicals found in the gas phase of mainstream smoke from different cigarettes (Chouchane et al. 2005)

than did their unsubstituted parent compounds. Similar results were reported for substituted phenols by Moridani et al. (2003), who attributed the difference in cytotoxicity to their higher lipophilic character and their lower redox potentials. The dihydroxybenzenes shown in Fig. 2.4 are present in the TPM of cigarette smoke, together with many other substances in a complex matrix. The EC_{50} values of the pure compounds, however, do not necessarily reflect the cytotoxicity of such a mixture. The cytotoxicity of the matrix depends on a number of factors such as the structure, concentration, and redox potentials of the individual dihydroxybenzenes. Moreover, molecular interactions between the dihydroxybenzenes (and their oxidation products) and other TPM constituents can affect the cytotoxicity of a mixture of TPM constituents.

Table 2.4 Yield of dihydroxybenzenes in the total particulate matter from 2R4F cigarettes and their specific in vitro cytotoxicity expressed as the 50% effective concentration (EC_{50}) determined by the neutral red uptake assay (Chouchane et al. 2004)

Dihydroxybenzenes	EC_{50} (mM)	Yield ($\mu\text{g/cig.}$)
Hydroquinone	0.021 \pm 0.004	39.3 \pm 1.26 ^a
2-Methylhydroquinone	0.011 \pm 0.001	4.02 \pm 0.03 ^b
2,3-Dimethylhydroquinone	0.015 \pm 0.005	1.37 \pm 0.07 ^b
2,6-Dimethylhydroquinone	0.016 \pm 0.002	0.49 \pm 0.04 ^b
Trimethylhydroquinone	0.026	1.83 \pm 0.03 ^b
Catechol	0.33	45.3 \pm 0.52 ^c
3-Methylcatechol	0.036 \pm 0.005	5.3
4-Methylcatechol	0.052 \pm 0.013	4.4
Total		102.0

EC_{50} 50% effective concentration, $\mu\text{g/cig.}$ micrograms per cigarette

^aMean \pm SD, $n=3$

^bMean \pm SD, $n=4$

^cMean \pm SD, $n=10$

When dissolved in the cell culture medium employed in the cytotoxicity assay (e.g., Dubelcco's Modified Eagle's Medium (DMEM), we found that the dihydroxybenzenes generate significant amounts of the corresponding semiquinone radicals, as represented by their EPR spectra shown in Fig. 2.17. However, the yield of semiquinone radicals depends on the structure of each dihydroxybenzene and its redox potential. Standard one-electron reduction potentials for the redox couple $Q/Q^{\cdot-}$ at 25°C and pH 7.0 have previously been reported for several dihydroxybenzenes found in TPM including hydroquinone, 78 mV; methylhydroquinone, 23 mV; 2,3-dimethylhydroquinone, -74 mV; 2,6-dimethylhydroquinone, -80 mV; and trimethylhydroquinone, -165 mV (Wardman 1989). The reduction potential decreases with methyl substitution. Among the most abundant dihydroxybenzenes in TPM, hydroquinone is the most potent semiquinone radical generator (Fig. 2.18), and it has a high reduction potential. The reduction potentials of the dihydroxybenzenes increase with their capacity to undergo autooxidation and generate semiquinone radicals, as shown in Fig. 2.19. An association between the cytotoxicity of quinones and their reduction potential has been previously proposed. Nemeikaite-Ceniene et al. (2002), for example, observed that the toxicity of natural hydroxyanthraquinones increases at pH 7 with an increase of their reduction potential, pointing to an oxidative stress mechanism.

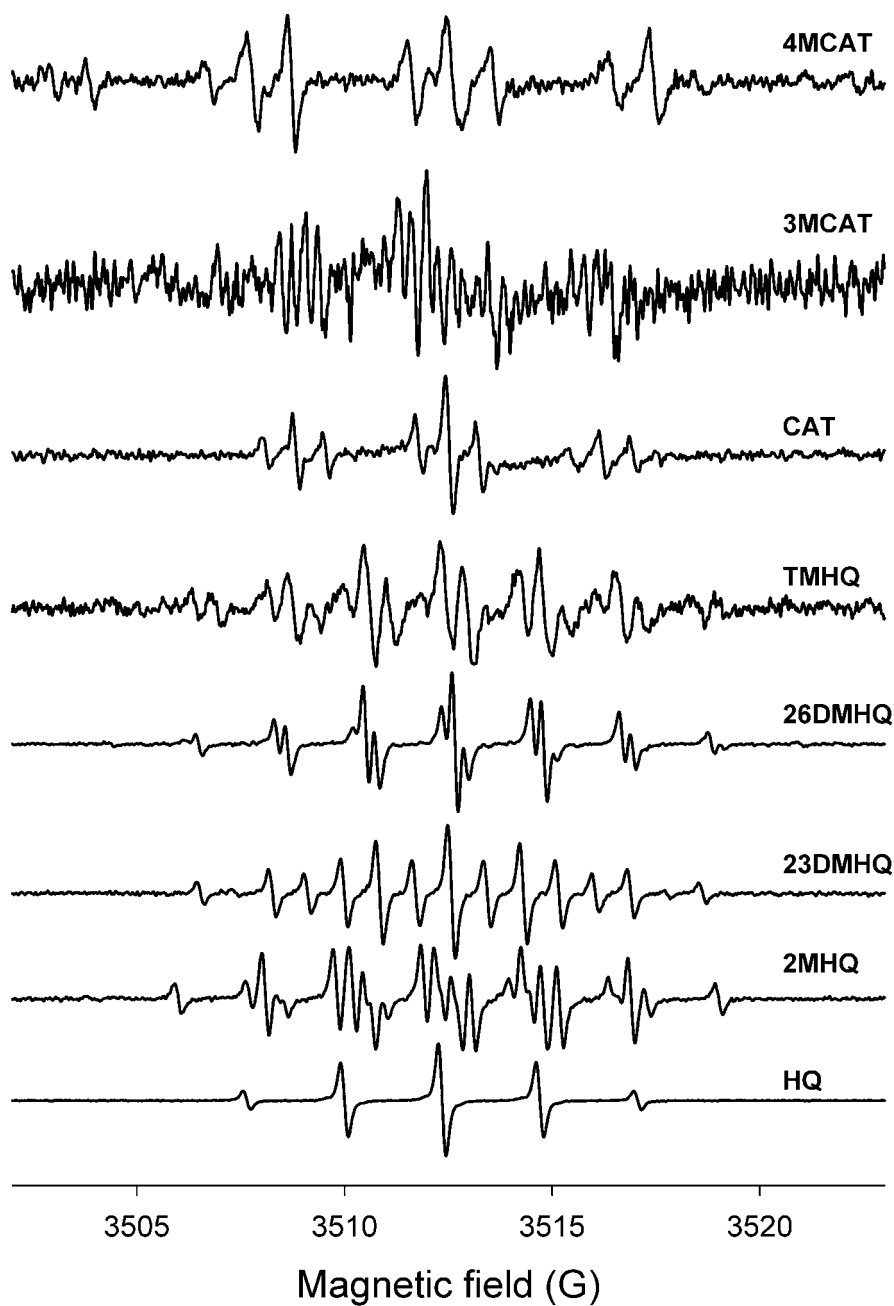


Fig. 2.17 EPR spectra of semiquinone radicals observed in 1-mM solutions of dihydroxybenzenes in DMEM (Chouchane et al. 2004)

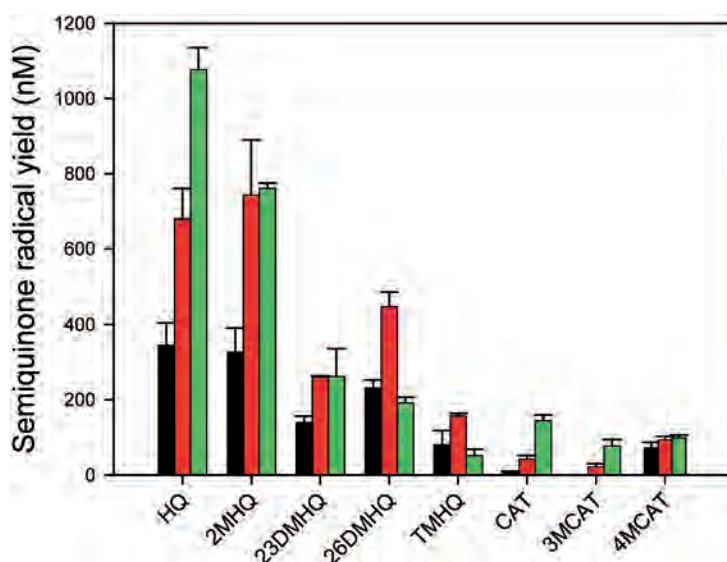


Fig. 2.18 Yield of semiquinone radicals obtained when 1-mM dihydroxybenzenes were dissolved in cell culture medium DMEM (Chouchane et al. 2004)

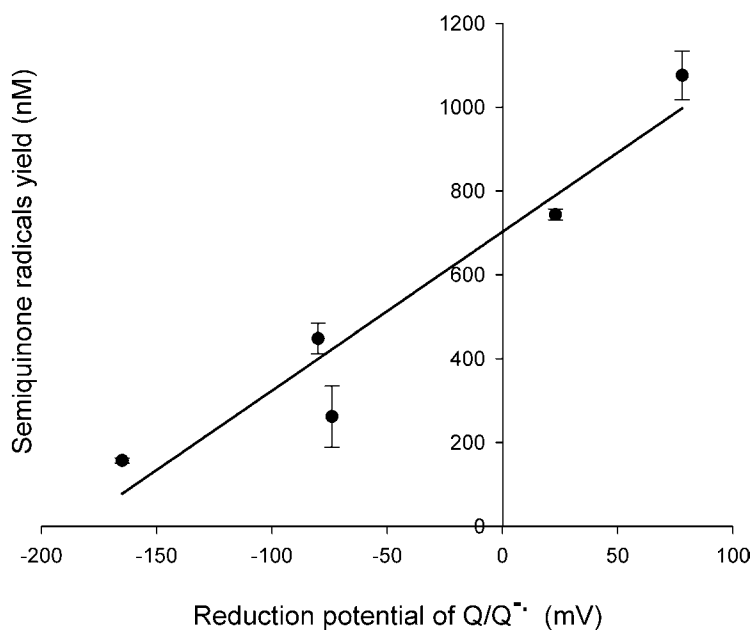


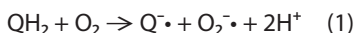
Fig. 2.19 Yield of semiquinone radicals formed in 1-mM solutions of dihydroxybenzenes dissolved in DMEM versus their respective reduction potentials to the semiquinone radical ($Q/Q^{\bullet-}$) (Chouchane et al. 2004)

2.4.4 ROS and RNS in Aqueous Solutions of Cigarette Smoke

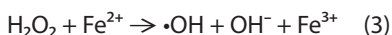
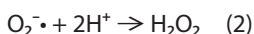
The examination of the chemistry of cigarette smoke-bubbled aqueous solutions is important on two counts. First, immediately after a smoker takes a puff, smoke enters an environment of high humidity, and the smoke deposits in the epithelial lining fluid (ELF) of the respiratory tract. Second, reactive species can form in aqueous solutions of cigarette smoke that are not generated inside the burning cigarette, but rather form in solution by reaction between smoke constituents, or by reactions of smoke constituents with dissolved oxygen.

2.4.4.1 Superoxide and Hydroxyl Radicals

Several cigarette smoke constituents have been suggested to be responsible for the generation of reactive oxygen species. Among these constituents, dihydroxybenzenes are good candidates. Oxidation of hydroquinone (QH₂) by molecular oxygen in aqueous solution generates the semiquinone (Q^{•−}) and superoxide radicals following reaction 1:



Spontaneous disproportionation of the superoxide radical anion, or catalytic disproportionation *in vivo* by SOD, generates H₂O₂ (reaction 2). In the presence of transition metal ions, H₂O₂ can undergo disproportionation to generate hydroxyl radical, a powerful oxidant (reaction 3).



Superoxide and hydroxyl radicals have been shown to form in aqueous extracts of cigarette smoke (Pryor et al. 1998; Zang et al. 1995). It was also shown that aqueous extracts of TPM produce hydroxyl radicals that can be spin-trapped with 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO). The hydroxyl radicals arise from the metal mediated decomposition of H₂O₂, as shown above (Cosgrove et al. 1985; Pryor 1992).

The DMPO-superoxide radical adduct is unstable, with a half-life of 80 s at pH 6 and 35 s at pH 8. The superoxide spin adduct slowly decays to form the DMPO-hydroxyl radical adduct (Buettner and Oberley 1978; Finkelstein et al. 1979, 1980, 1982). The Pryor group observed superoxide radicals in ACT in a high-pH solution, but not hydroxyl radicals. In the same study, superoxide radicals were detected in ACT by employing a much higher concentration of DMPO (ca. 1 M) (Zang et al. 1995). With the development of new spin-trap molecules for reactive oxygen species, particularly superoxide radical, it is possible to overcome some of the problems encountered with the use of DMPO. 5-Diethoxyphosphoryl-5-methyl-1-pyrroline-*N*-oxide (DEPMPO), for example, can trap both superoxide and hydroxyl radicals. The DEPMPO-superoxide radical adduct has a half-life of 13 min (Frejavi et al. 1995), allowing its detection by EPR within a shorter period in comparison with the DMPO adduct. As shown in

Fig. 2.20, when whole cigarette smoke is passed through a phosphate buffered solution pH 7.4 containing DEPMPO (100 mM), both superoxide radical and hydroxyl radical are trapped. Under the same experimental conditions using DMPO, only superoxide radicals are trapped.

If superoxide is involved in the adverse effects of cigarette smoke, a strategy aimed at the elimination of excess superoxide might minimize these effects. For example, acute exposure to cigarette smoke is known to induce the infiltration of neutrophils into the airways in guinea pigs, a phenomenon associated with a defensive response to oxidative stress. This response is manifest by activation of the nuclear factor-kappaB (NF- κ B) transcription factor and increased expression of interleukin 8 (IL-8) mRNA (Nishikawa et al. 1999). Prior treatment of the guinea pigs with SOD, to reduce the accumulation of superoxide, inhibited neutrophil accumulation and reduced both NF- κ B activation and IL-8 mRNA expression. Another example was reported by (Smith et al. 2002b): Intra-tracheal instillation of a SOD-mimetic (AEOL 10150, a manganese porphyrin) into the airways of rats was shown to provide a marked protective effect against cigarette smoke-induced inflammation and damage.

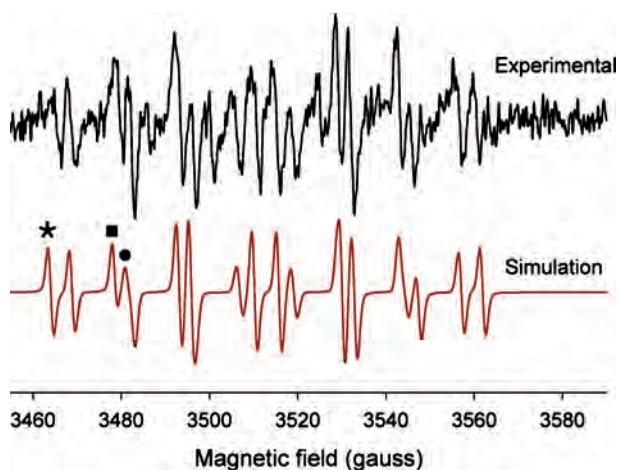


Fig. 2.20 EPR signal of spin adducts of superoxide radicals and hydroxyl radicals trapped in a phosphate buffer pH 7.4 containing 5-diethoxyphosphoryl-5-methyl-1-pyrroline-*N*-oxide (DEPMPO) 100 mM bubbled with whole mainstream smoke. ■ DEPMPO-superoxide radical adduct, ● DEPMPO-hydroxyl radical adduct, * DEPMPO-carboxyl radical adduct

2.4.4.2 Hydrogen Peroxide

There are relatively few reports of H_2O_2 measurements in cigarette smoke under any conditions. One difficulty in analyzing for H_2O_2 is its transient nature, and the established methods of analysis are slow and laborious. Both a polarographic method developed for the analysis of H_2O_2 in TPM (Nakayama et al. 1989) and a previously reported colorimetric method developed to analyze whole smoke (Nakayama et al. 1984) require

the removal of the organic phase by chromatography or solvent extraction as a preliminary step in the analysis. Another approach to measure H_2O_2 in cigarette tar is to use an oxygen electrode, but the oxygen electrode is not specific to H_2O_2 , and the addition of catalase to the analyte solutions is required to confirm that the oxygen adsorbed species is because of H_2O_2 (Stone et al. 1995).

By application of the polarographic method, Nakayama et al. (1989) found that the aqueous extracts of cigarette tar produce H_2O_2 for prolonged periods up to 24 h and longer. The dihydroxybenzenes in tar, i.e., hydroquinone, catechol, etc., provide an ample reservoir for the formation of semiquinone radicals via autooxidation. The semiquinone radicals generate superoxide radical anions in oxygenated solutions for extended periods, producing H_2O_2 by dismutation. Other pathways of H_2O_2 formation may exist, but they have not been clearly delineated as the semiquinone pathway. The primary source of H_2O_2 in cigarette tar is believed to be superoxide formed by the reduction of atmospheric oxygen by semiquinone radicals in the tar (Tanigawa et al. 1994).

In a series of experiments by Nakayama et al. (1989) on experimental and commercial cigarettes, extracts of TPM in phosphate buffer were bubbled with oxygen for 1 min and incubated in the dark. The yield of H_2O_2 after 4 h ranged from 37 to 123 μM per cigarette. The study included both filtered (with and without ventilation) and nonfiltered cigarettes, and the yield of H_2O_2 varied depending on the cigarette design. In general, nonfiltered cigarettes yielded higher amounts of H_2O_2 than do filtered cigarettes. The results were not normalized to the amount of TPM delivered, but the authors showed that the overall yield of H_2O_2 in most cigarettes is proportional to the yield of TPM.

Recently, a fluorometric method was developed that detects the oxidized form of Amplex Red, a fluorescent dye, in the presence of horseradish peroxidase and H_2O_2 (Yan et al. 2005). This method has several advantages over the polarographic method: (1) it only requires a 2-min incubation time and is therefore faster than are previous methods, (2) the H_2O_2 does not have to be separated from the tar matrix, (3) it can be applied to small samples, and (4) the method is readily adapted for automation. Applying their method, Yan et al. (2005) bubbled five puffs of whole smoke from 1R4F or 2R4F research cigarettes into phosphate buffered saline solution (PBS) containing the fluorescent dye, using a smoking machine. Concentrations of 3–8 micromolar H_2O_2 were found in the whole smoke bubbled samples, while there was negligible H_2O_2 formation from gas-phase smoke bubbled samples. Others have developed electrochemical means of detecting hydrogen peroxide. H_2O_2 is an electrochemically active species that will disproportionate at the surface of a metallic electrode. For example, an amperometric detection principle similar to the oxygen detection using the Clark electrode has been utilized. Hydrogen peroxide is selectively detected by an electrode after passing through a H_2O_2 permeable membrane. This method can compliment the fluorometric approach with direct quantitative measurement in biological samples in the low nM range.

2.4.4.3 NO \cdot and Peroxynitrite

NO \cdot and peroxynitrites are the major reactive nitrogen species derived from cigarette smoke. The yields of NO \cdot from 49 commercial cigarettes smoked on a smoking machine under ISO conditions were reported to be 78–487 μg per cigarette (Counts et al. 2005). As described in Sect. 2.2.1, NO \cdot forms in the cigarette gas phase from the burning of different tobacco constituents. Im et al. (2003) showed that NO \cdot from heated tobacco is

produced in two distinct temperature ranges, a low-temperature range (275–375 °C) in an oxygen-free atmosphere, and a high-temperature range (425–525 °C) that requires an oxygen-containing atmosphere. Nitrates were determined to be the source of NO \cdot formation in the low-temperature ranges, and amino acids and proteins were suggested to be the sources of NO \cdot at the higher-temperature ranges. The individual contribution of these precursors to the overall yield of NO \cdot in cigarette smoke has yet to be determined.

Peroxynitrite forms by the reaction of NO \cdot and superoxide radicals. In smoke-bubbled aqueous solutions, smoke constituents that can reduce molecular oxygen to superoxide, e.g., hydroquinone, continuously generate superoxide, which reacts rapidly with NO \cdot from the gas phase to give peroxynitrite. Alkyl peroxynitrites can also form by reaction of NO \cdot and peroxy radicals (Halliwell and Gutteridge 1999), which are presumed to form in the gas phase of cigarette smoke (Pryor et al. 1983a, 1984, 1985). Peroxynitrite in cigarette smoke extract has been shown to react with and inactivate the α_1 -proteinase inhibitor (Moreno and Pryor 1992). Peroxynitrite has also been identified as an oxidative stress-inducing constituent of aqueous cigarette smoke fractions. After depletion of intracellular GSH by electrophilic aldehydes, peroxynitrite interferes with specific target molecules, resulting in the activation of stress signal transduction and stress gene expression in cigarette smoke-treated cells in vitro (Müller and Gebel 1994, 1998; Müller et al. 1997).

2.5 Summary

Cigarette smoke has considerable potential for inducing oxidative modifications and depletion of antioxidants. In cigarette smoke-exposed aqueous solutions, ROS and RNS form and subsequently act as potent oxidants. Oxidative damage to lipids, proteins, and DNA by cigarette smoke-derived ROS and RNS has been extensively demonstrated both in vitro and in vivo. In many cases, the initially generated reactive intermediates convert cellular constituents into second-generation reactive intermediates (e.g., acrolein, 4-hydroxynonenal) capable of inducing further cytotoxic and genotoxic damage. When free radicals react with nonradicals (e.g., lipids), new radicals can form that may result in a chain reaction of free radicals. Thus, relatively short-lived free radicals may propagate their damaging effects beyond the limits set by their short half-lives and limited diffusion times. ROS and RNS activate numerous redox sensitive signaling pathways that modulate cellular responses, such as inflammation, which may itself result in the formation of endogenous oxidative species. Therefore, the oxidative damage resultant from cigarette smoke exposure is complex and likely mediated by both the oxidative potential of cigarette smoke and indirect biological responses.

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References

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- Achladas GE (1991) Analysis of biomass pyrolysis liquids: separation and characterization of phenols. *J Chromatogr A* 542:263–275
- Amen-Chen C, Pakdel H, Roy C (1997) Separation of phenols from Eucalyptus wood tar. *Biomass Bioenergy* 13:25–37
- Arrendale RF, Severson RF, Chortyk OT (1984) The application of capillary gas chromatography to the analyses of acidic constituents of tobacco leaf and smoke. *Beitr Tabak* 12:186–197
- Ault JG, Lawrence DA (2003) Glutathione distribution in normal and oxidatively stressed cells. *Exp Cell Res* 285:9–14
- Baker RR (1999) Smoke chemistry. In: Davis LD, Nielson MT (eds) *Tobacco: production, chemistry and technology*. Blackwell, London, pp 308–439
- Baker RR (2002) The development and significance of standards for smoking-machine methodology. *Beitr Tabak* 20:23–41
- Bal W, Kasprzak KS (2002) Induction of oxidative DNA damage by carcinogenic metals. *Toxicol Lett* 127:55–62
- Barchowsky A, O'Hara KA (2003) Metal-induced cell signaling and gene activation in lung diseases. *Free Radic Biol Med* 34:1130–1135
- Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA (1990) Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc Natl Acad Sci USA* 87:1620–1624
- Blakley RL, Henry DD, Smith CJ (2001) Lack of correlation between cigarette mainstream smoke particulate phase radicals and hydroquinone yield. *Food Chem Tox* 39:401–406
- Bluhm AL, Weinstein J, Sousa JA (1971) Free radicals in tobacco smoke. *Nature* 229:500
- Bokelman GH, Ryan WS Jr (1985) Analysis of bright and burley tobacco laminae and stems. *Beitr Tabak Int* 13:29–36
- Bolton JL, Trush MA, Penning RM, Dryhurst L, Monks TJ (2000) Role of quinones in toxicology. *Chem Res Toxicol* 13:135–160
- Bosio A, Knorr C, Janssen U, Gebel S, Haussmann HJ, Müller T (2002) Kinetics of gene expression profiling in Swiss 3T3 cells exposed to aqueous extracts of cigarette smoke. *Carcinogenesis* 2002 23:741–748
- Briede JJ, Godschalk RWL, Emans MTG, De Kok TMCM, Van Agen E, Van Maanen J, Van Schooten FJ, Kleinjans JCS (2004) In vitro and in vivo studies on oxygen free radical and DNA adduct formation in rat lung and liver during benzo[a]pyrene metabolism. *Free Radic Res* 38:995–1002
- Brunmark A, Cadenas E (1989) Redox and addition chemistry of quinoid compounds and its biological implications. *Free Radic Biol Med* 7:435–477
- Brunneman KD, Lee H, Hoffman D (1976) Chemical studies on tobacco smoke. XLVII. On the quantitative analysis of catechols and their reduction. *Anal Lett* 9:939–955
- Buettner GR, Oberley LW (1978) Considerations in the spin trapping of superoxide and hydroxyl radical in aqueous systems using 5,5-dimethyl-1-pyrroline-1-oxide. *Biochem Biophys Res Commun* 83:69–74
- Cahours X, Renault D, Dubois M, Marchand V, Dumery B (2004) Oxidative effects of cigarette smoke: assessment by fast cell-free method. Presentation at the CORESTA Congress, Kyoto, Japan
- Carmella SG, Hecht SS, Tso TC, Hoffman D (1984) Roles of tobacco cellulose, sugars, and chlorogenic acid as precursors to catechol in cigarette smoke. *J Agric Food Chem* 32:267–273
- Chang MJ, McDaniel RL, Naworal JD, Self DA (2002) A rapid method for the determination of mercury in mainstream cigarette smoke by two-stage amalgamation cold vapor atomic absorption spectroscopy. *J Anal Atomic Spec* 17:710–715

- Chen PX, Moldoveanu SC (2003) Mainstream smoke chemical analyses for 2R4F Kentucky reference cigarette. *Beitr Tabak* 20:448–458
- Chortyk OT, Schlotzhauer SW, Stedman RL (1966) Composition studies on tobacco XXIII-pyrolytic and structural investigations on the polyphenol-amino acid pigments of leaf. *Beitr Tabak* 3:422–429
- Chouchane S, Meruva NK, Brown AP, Wooten JB (2005) Precursors of the free radicals generated in the tar and gas phases of mainstream cigarette smoke. *American Chemical Society Division of Fuel Chemistry Preprints* 50:419–420
- Chouchane S, Müller B, Wittig A, Teweze F, Wooten JB (2004) Involvement of semiquinone radicals generated from dihydroxybenzenes in the in-vitro cytotoxicity of cigarette smoke tar. *Free Radic Biol Med* 37:S14–S14
- Church DF, Pryor WA (1985) Free-radical chemistry of cigarette smoke and its toxicological implications. *Environ Health Perspec* 64:111–126
- Cosgrove JP, Borish ET, Church DF, Pryor WA (1985) The metal-mediated formation of hydroxyl radical by aqueous extracts of cigarette tar. *Biochem Biophys Res Commun* 132:390–396
- Counts ME, Hsu FS, Laffoon SW, Dwyer RW, Cox RH (2004) Mainstream smoke constituent yields and predicting relationships from a worldwide market sample of cigarette brands: ISO smoking conditions. *Regul Toxicol Pharmacol* 39:111–134
- Counts ME, Morton MJ, Laffoon SW, Cox RH, Lipowicz PJ (2005) Smoke composition and predicting relationships for international commercial cigarettes smoked with three machine-smoking conditions. *Regul Toxicol Pharmacol* 41:185–227
- Crouse RH, Garner JW, O'Neill HJ (1963) Determination of phenolic constituents of cigarette smoke by gas chromatograph. *J Gas Chromatogr* 1:18–22
- DeCaprio AP (1999) The toxicology of hydroquinone—relevance to occupational and environmental exposure. *Crit Rev Toxicol* 29:283–330
- Deisinger PJ, Hill TS, English JC (1996) Human exposure to naturally occurring hydroquinone. *J Toxicol Environ Health* 47:31–46
- Dellinger B, Pryor WA, Cueto R, Squadrito GL, Hegde V, Deutsch WA (2001) Role of free radicals in the toxicity of airborne fine particulate matter. *Chem Res Toxicol* 14:1371–1377
- Denicola A, Radi R (2005) Peroxynitrite and drug-dependent toxicity. *Toxicology* 208:273–288
- do Céu Silva M, Gaspar J, Duarte ID, Leão D, Rueff J (2003) Mechanisms of induction of chromosomal aberrations by hydroquinone in V79 cells. *Mutagenesis* 18:491–496
- Dube MFR, Green CR (1982) Methods of collection of smoke for analytical purposes. *Rec Adv Tob Sci* 8:42–102
- Eiserich J, Vossen V, O'Neill CA, Halliwell B, Cross CE, van der Vliet A (1994) Molecular mechanisms of damage by excess nitrogen oxides: nitration of tyrosine by gas-phase cigarette smoke. *FEBS Lett* 353:53–56
- Eiserich JP, van der Vliet A, Handelman GJ, Halliwell B, Cross CE (1995) Dietary antioxidants and cigarette smoke-induced biomolecular damage: a complex interaction. *Am J Clin Nutr* 62:1490S–1500S
- Ercal N, Gurer-Orhan H, Aykin-Burns N (2001) Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. *Curr Top Med Chem* 1:529–539
- Federal Register, August 1, 1967. Cigarettes: testing for tar and nicotine content, vol. 32, no. 147, p. 11178
- Federal Register, July 10, 1980. Cigarettes and related matters: carbon monoxide, tar and nicotine content of cigarette smoke; description of new machine and methods to be used in testing, vol. 45, no. 134, pp 46483–46487
- Finkelstein E, Rosen GM, Rauckman EJ (1980) Spin trapping of superoxide and hydroxyl radical: practical aspects. *Arch Biochem Biophys* 200:1–16
- Finkelstein E, Rosen GM, Rauckman EJ (1982) Production of hydroxyl radical by decomposition of superoxide spin-trapped adducts. *Mol Pharmacol* 21:262–265

- Finkelstein E, Rosen GM, Rauckman EJ, Paxton J (1979) Spin trapping of superoxide. *Mol Pharmacol* 16:676–685
- Flicker TM, Green SA (1998) Detection and separation of gas-phase carbon-centered radicals from cigarette smoke and diesel exhaust. *Anal Chem* 70:2008–2012
- Flicker TM, Green SA (2001) Comparison of gas-phase free-radical populations in tobacco smoke and model systems by HPLC. *Environ Health Perspect* 109:765–771
- Forehand JB, Dooly GL, Moldoveanu SC (2000) Analysis of polycyclic aromatic hydrocarbons, phenols and aromatic amines in particulate phase cigarette smoke using simultaneous distillation and extraction as a sole sample clean-up step. *J Chromatogr A* 898:111–124
- Frejaville C, Karoui H, Tuccio B, Le Moigne F, Culcasi M, Pietri S, Lauricella R, Tordo P (1995) 5-(Diethoxyphosphoryl)-5-methyl-1-pyrroline-*N*-oxide: a new efficient phosphorylated nitrone for the in vitro and in vivo spin trapping of oxygen-centered radicals. *J Med Chem* 38:258–265
- Gaisch H, Nyffeler U (1976) The measurement of thiol reactivity in cigarette smoke. *Beitr Tabak Int* 8:399–403
- Gregg E, Hill C, Hollywood M, Kearney M, McAdam K, McLaughlin D, Purkis S, Williams M (2004) The UK smoke constituents testing study. Summary of results and comparison with other studies. *Beitr Tabak Int* 21:117–138
- Hagedorn H-W, Gilch G, Janket D, Scherer G (2003) Mechanistically-based tests for toxicity of tobacco smoke. Presentation at the CORESTA Congress, Freiburg, Germany
- Halliwell B, Gutteridge JMC (1999) Free radicals in biology and medicine. Oxford University Press, New York
- Hirakawa K, Oikawa S, Hiraku Y, Hirose I, Kawanishi S (2002) Catechol and hydroquinone have different redox properties responsible for their differential DNA-damaging ability. *Chem Res Toxicol* 15:76–82
- Hoffmann D, Hoffmann I, El Bayoumy K (2001) The less harmful cigarette: a controversial issue. a tribute to Ernst L. Wynder. *Chem Res Toxicol* 14:767–790
- Huie RE, Padmaja S (1993) The reaction of NO with superoxide. *Free Radic Res Commun* 18:195–199
- IARC Monographs (1986) The evaluation of the carcinogenic risk of chemicals to humans: tobacco smoking, vol. 38, p. 83
- Im H, Rasouli F, Hajaligol MR (2003) Formation of nitric oxide during tobacco oxidation. *J Agric Food Chem* 51:7366–7372
- Kameswararo BV, Gopalachari NC (1965) Polyphenolic constituents tobacco—a review. *Indian J Appl Chem* 28:165–172
- Kasprzak KS (2002) Oxidative DNA and protein damage in metal-induced toxicity and carcinogenesis. *Free Radic Biol Med* 32:958–967
- Klus H, Kuhn H (1982) Distribution of various tobacco smoke constituents in main- and side-stream smoke (a review). *Beitr Tabak Int* 11:229–265
- Knaapen AM, Borm PJA, Catrin A, Schins RPF (2004) Inhaled particles and lung cancer. Part A: mechanisms. *Int J Cancer* 109:799–809
- Koppenol WH (1998) The basic chemistry of nitrogen monoxide and peroxynitrite. *Free Radic Biol Med* 25:385–391
- Kwon NS, Stuehr DJ, Nathan CF (1991) Inhibition of tumor cell ribonucleotide reductase by macrophage-derived nitric oxide. *J Exp Med* 174:761–767
- Lakritz L, Stanke-Labesque F, Baker MS, Stedman RL (1972) Composition studies on tobacco XLV. Use of cigarette additives to alter the composition and reducing the properties of cigarette smoke. *Beitr Tabak* 6:120–123
- Lau SS, Hill BA, Highet RJ, Monks TJ (1988) Sequential oxidation and glutathione addition to 1,4-benzoquinone: correlation of toxicity with increased glutathione substitution. *Mol Pharmacol* 34:829–836

- Leffingwell JC (1999) Leaf chemistry: basic chemical constituents of tobacco leaf and differences among tobacco types. In: Davis LD, Nielson MT (eds) Tobacco: production, chemistry and technology. Blackwell, London, pp 265–303
- Lepoivre M, Flaman JM, Bohe P, Lemaire G, Henry Y (1994) Quenching of the tyrosyl free radical of ribonucleotide reductase by nitric oxide. Relationship to cytostasis induced in tumor cells by cytotoxic macrophages. *J Biol Chem* 269:21891–21897
- Leuchtenberger C, Leuchtenberger R, Zbinden I (1974) Gas vapour phase constituents and SH reactivity of cigarette smoke influence lung cultures. *Nature* 247:565–567
- Leuchtenberger C, Leuchtenberger R, Zbinden I, Schleh E (1976) SH Reactivity of cigarette smoke and its correlation with carcinogenic effects on hamster lung cultures. *Soz Präventivmed* 21:47–50
- Li YB, Trush MA (1993) Oxidation of hydroquinone by copper: chemical mechanism and biological effects. *Arch Biochem Biophys* 300:346–355
- Li Y, Kuppusamy P, Zweier JL, Trush MA (1995) ESR evidence for the generation of reactive oxygen species from the copper-mediated oxidation of the benzene metabolite, hydroquinone: role in DNA damage. *Chem Biol Interact* 94:101–120
- Lougon-Moulin N, Zhang M, Gadani F, Rossi L, Koller D, Kauss M, Wagner GJ (2004) Critical review of the science and options for reducing cadmium in tobacco (*Nicotiana Tabacum* L.) and other plants. In: Sparks D (ed) Advances in agronomy. Academic, New York, pp 111–180
- Lyons MJ, Gibson JF, Ingram DJ (1958) Free-radicals produced in cigarette smoke. *Nature* 181:1003–1004
- Martin LC, Tricker AR (2004) Biological monitoring of urinary metabolites of acrolein, benzene, and 1,3-butadiene in smokers and non-smokers. Presentation at Sixth International Symposium on Biological Monitoring in Occupational and Environmental Health. Heidelberg September 6–8 2004
- Mascher DG, Mascher HJ, Scherer G, Schmid ER (2001) High-performance liquid chromatographic-tandem mass spectrometric determination of 3-hydroxypropylmercapturic acid in human urine. *J Chromatogr B* 750:163–169
- Maskos S, Khachatryan L, Cueto R, Pryor WA, Dellinger B (2005) Radicals from the pyrolysis of tobacco. *Energy Fuels* 19:791–799
- McCue JM, Lazis S, Cohen JJ, Modiano JF, Freed BM (2003) Hydroquinone and catechol interfere with T cell cycle entry and progression through the G(1) phase. *Mol Immunol* 39:995–1001
- McDaniel RL, Torrence KM, Self DA, Chang MJ (2001) Determination of mercury in mainstream cigarette smoke by conventional and amalgamation of vapor atomic absorption spectroscopy. *Beitr Tabak* 39:995–1001
- McGrath TE, Chan WG, Hajaligol MR (2003) Low temperature mechanism for the formation of polycyclic aromatic hydrocarbons from the pyrolysis of cellulose. *J Anal Appl Pyrolysis* 66:51–70
- McGrath TE, Wooden JB, Chan WG, Hajaligol MR (2005) Formation of polycyclic aromatic hydrocarbons (PAHs) from tobacco: the link between low temperature residual solid and PAH formation. *Food and Chemical Toxicology* (submitted).
- Meacher DM, Menzel DB (1999) Glutathione depletion in lung cells by low-molecular-weight aldehydes. *Cell Biol Toxicol* 15:163–171
- Monks TJ, Hanzlik RP, Cohen GM, Ross D, Graham DG (1992) Quinone chemistry and toxicity. *Toxicol Appl Pharmacol* 112:2–16
- Moreno JJ, Pryor WA (1992) Inactivation of alpha 1-proteinase inhibitor by peroxynitrite. *Chem Res Toxicol* 5:425–431
- Moridani MY, Siraki A, O'Brien PJ (2003) Quantitative structure toxicity relationships for phenols in isolated rat hepatocytes. *Chem Biol Interact* 145:213–223
- Müller T, Gebel S (1994) Heme oxygenase expression in Swiss 3T3 cells following exposure to aqueous cigarette smoke fractions. *Carcinogenesis* 15:67–72

- Müller T, Gebel S (1998) The cellular stress response induced by aqueous extracts of cigarette smoke is critically dependent on the intracellular glutathione concentration. *Carcinogenesis* 19:797–801
- Müller T, Haussmann HJ, Schepers G (1997) Evidence for peroxynitrite as an oxidative stress-inducing compound of aqueous cigarette smoke fractions. *Carcinogenesis* 18:295–301
- Nakayama T, Church DF, Pryor WA (1989) Quantitative analysis of the hydrogen peroxide formed in aqueous cigarette tar extracts. *Free Radic Biol Med* 7:9–15
- Nakayama T, Kodama M, Nagata C (1984) Generation of hydrogen peroxide and superoxide anion radical from cigarette smoke. *Gann* 75:95–98
- Nemeikaite-Ceniene A, Sergediene E, Nivinskas H, Cenas N (2002) Cytotoxicity of natural hydroxyanthraquinones: role of oxidative stress. *Z Naturforsch C* 57:822–827
- Nishikawa M, Kakemizu N, Ito T, Kudo M, Kaneko T, Suzuki M, Uda N, Ikeda H, Okubo T (1999) Superoxide mediates cigarette smoke-induced infiltration of neutrophils into the airways through nuclear factor-kappaB activation and IL-8 mRNA expression in guinea pigs in vivo. *Am J Respir Cell Mol Biol* 20:189–198
- Norman A (1999) Cigarette design and materials. In: Davis DL, Nielson MT (eds) *Tobacco production, chemistry and technology*. Blackwell, London, pp 353–97
- Patterson JM, Haider NF, Chen SP, Smith WT (1976) An investigation of some factors affecting phenol production in tobacco pyrolysis. *Tob Sci* 20:114–116
- Pedersen JA (2002) On the application of electron paramagnetic resonance in the study of naturally occurring quinones and quinols. *Spectrochim Acta A* 58:1257–1270
- Pryor WA (1992) Biological effects of cigarette smoke, wood smoke, and the smoke from plastics: the use of electron spin resonance. *Free Radic Biol Med* 13:659–676
- Pryor WA, Dooley MM, Church DF (1984) Inactivation of human alpha-1-proteinase inhibitor by gas-phase cigarette smoke. *Biochem Biophys Res Commun* 122:676–681
- Pryor WA, Hales BJ, Premovic PI, Church DF (1983a) The radicals in cigarette tar: their nature and suggested physiological implications. *Science* 220:425–427
- Pryor WA, Prier DG, Church DF (1983b) Electron-spin resonance study of mainstream and side-stream cigarette smoke: nature of the free radicals in gas-phase smoke and in cigarette tar. *Environ Health Perspect* 47:345–355
- Pryor WA, Tamura M, Dooley MM, Premovic P, Hales BJ, Church DF (1983c) Reactive oxy-radicals from cigarette smoke and their physiological effects. In: Greenwald R, Cohen G (eds) *Oxy-radicals and their scavenger systems: cellular and medical aspects*. Elsevier, New York, pp 185–192
- Pryor WA, Dooley MM, Church DF (1985) Mechanisms of cigarette smoke toxicity: the inactivation of human alpha-1-proteinase inhibitor by nitric oxide/isoprene mixtures in air. *Chem Biol Interact* 54:171–183
- Pryor WA, Stone K, Zang LY, Bermudez E (1998) Fractionation of aqueous cigarette tar extracts: fractions that contain the tar radical cause DNA damage. *Chem Res Toxicol* 11:441–448
- Purkis SW, Hill CA, Bailey IA (2003) Current measurement reliability of selected smoke analytes. *Beitr Tabak Int* 20:314–324
- Rahman I, MacNee W (1996a) Oxidant/antioxidant imbalance in smokers and chronic obstructive pulmonary disease. *Thorax* 51:348–350
- Rahman I, MacNee W (1996b) Role of oxidants/antioxidants in smoking-induced lung diseases. *Free Radic Biol Med* 21:669–681
- Reddy S, Finkelstein EI, Wong PS, Phung A, Cross CE, van der Vliet A (2002) Identification of glutathione modifications by cigarette smoke. *Free Radic Biol Med* 33:1490–1498
- Reiter CD, Teng RJ, Beckman JS (2000) Superoxide reacts with nitric oxide to nitrate tyrosine at physiological pH via peroxynitrite. *J Biol Chem* 275:32460–32466
- Risner CH, Cash SL (1990) A high-performance liquid chromatographic determination of major phenolic compounds in tobacco smoke. *J Chromatogr Sci* 28:239–244
- Rodriguez CE, Shinyashiki M, Froines J, Yu RC, Fukuto JM, Cho AK (2004) An examination of quinone toxicity using the yeast *Saccharomyces cerevisiae* model system. *Toxicology* 201:185–196

- Roemer E, Stabbert R, Rustemeier K, Veltel DJ, Meisgen TJ, Reininghaus W, Carchman RA, Gaworski CL, Podraza KF (2004) Chemical composition, cytotoxicity and mutagenicity of smoke from US commercial and reference cigarettes smoked under two sets of machine smoking conditions. *Toxicology* 195:31–52
- Roginsky VA, Barsukova TK, Stegmann HB (1999a) Kinetics of redox interaction between substituted quinones and ascorbate under aerobic conditions. *Chem Biol Interact* 121:177–197
- Roginsky VA, Pitz M, Bors W, Michel C (1999b) The kinetics and thermodynamics of quinone-semiquinone-hydroquinone systems under physiological conditions. *J Chem Soc Perkin Trans* 24:871–876
- Sakuma H, Matsushima S, Munakata S, Sugawara S (1982) Pyrolysis of chlorogenic acid and rutin. *Agric Biol Chem* 46:1311–1317
- Samuni AM, Chuang EY, Krishna MC, Stein W, DeGraff W, Russo A, Mitchell JB (2003) Semiquinone radical intermediate in catecholic estrogen-mediated cytotoxicity and mutagenesis: chemoprevention strategies with antioxidants. *Proc Natl Acad Sci* 100:5390–5395
- Schlottzhauer WS, Chortyk OT (1981) Pyrolytic studies on the origin of phenolic compounds in tobacco smoke. *Tob Sci* 25:6–10
- Schlottzhauer WS, Chortyk OT (1987) Recent advances in studies on the pyrosynthesis of cigarette smoke constituents. *J Anal Appl Pyrolysis* 12:193–222
- Schlottzhauer WS, Schmeltz I, Hickey LC (1967) Pyrolytic formation of phenols from some high-molecular-weight tobacco leaf constituents and related non-tobacco materials. *Tob Sci* 11:31–34
- Schlottzhauer WS, Chortyk OT, Higman HC, Schmeltz I (1969) Pyrolytic studies on fractions sequentially extracted from tobacco. *Tob Sci* 13:153–155
- Schlottzhauer WS, Higman EB, Schmeltz I (1972) *The chemistry of tobacco and tobacco smoke*. Plenum, New York
- Schlottzhauer WS, Martin RM, Snook ME, Williamson RE (1982) Pyrolytic studies on the contribution of tobacco leaf constituents to the formation of smoke catechols. *J Agric Food Chem* 30:372–374
- Schlottzhauer WS, Snook ME, Chortyk OT, Wilson RL (1992) Pyrolytic evaluation of low chlorogenic acid tobaccos in the formation of the tobacco smoke co-carcinogen catechol. *J Anal Appl Pyrolysis* 22:231–238
- Schmeltz I, Tosk J, Jacobs G, Hoffmann D (1977) Redox potential and quinone content of cigarette smoke. *Anal Chem* 49:1924–1929
- Seeman JJ, Lipowicz PJ, Piade JJ, Poget L, Sanders EB, Snyder JP, Trowbridge CG (2004) On the deposition of volatiles and semivolatiles from cigarette smoke aerosols: relative rates of transfer of nicotine and ammonia from particles to the gas phase. *Chem Res Toxicol* 17:1020–1037
- Seung SA, Lee JY, Lee MY, Park JS, Chung JH (1998) The relative importance of oxidative stress versus arylation in the mechanism of quinone-induced cytotoxicity to platelets. *Chem Biol Interact* 113:133–144
- Sharma RK, Hajaligol MR, Martoglio-Smith PA, Wooten JB, Baliga VL (2000) Characterization of char from pyrolysis of chlorogenic acid. *Energy Fuel* 14:1083–1093
- Smith CJ, Livingston SD, Doolittle DJ (1997) An international literature survey of “IARC Group I carcinogens” reported in mainstream cigarette smoke. *Food Chem Toxicol* 35:1107–1130
- Smith CJ, Perfetti TA, Morton MJ, Rodgman A, Garg R, Selassie CD, Hansch C (2002a) The relative toxicity of substituted phenols reported in cigarette mainstream smoke. *Toxicol Sci* 69:265–278
- Smith KR, Uyeminami DL, Kodavanti UP, Crapo JD, Chang LY, Pinkerton KE (2002b) Inhibition of tobacco smoke-induced lung inflammation by a catalytic antioxidant. *Free Radic Biol Med* 33:1106–1114
- Spears AW, Bell JH, Saunders AO (1965) The contribution of tobacco constituents to phenol yield of cigarettes. *Tob Sci* 19:19–19

- Spencer JP, Jenner A, Chimel K, Aruoma OI, Cross CE, Wu R, Halliwell B (1995) DNA damage in human respiratory tract epithelial cells: damage by gas phase cigarette smoke apparently involves attack by reactive nitrogen species in addition to oxygen radicals. *FEBS Lett* 375:179–182
- Squadrito GL, Cueto R, Dellinger B, Pryor WA (2001) Quinoid redox cycling as a mechanism for sustained free radical generation by inhaled airborne particulate matter. *Free Radic Biol Med* 31:1132–1138
- Squadrito GL, Pryor WA (1998) Oxidative chemistry of nitric oxide: the roles of superoxide, peroxynitrite, and carbon dioxide. *Free Radic Biol Med* 25:392–403
- Stohs SJ, Bagchi D (1995) Oxidative mechanisms in the toxicity of metal ions. *Free Radic Biol Med* 18:321–336
- Stohs SJ, Bagchi D, Bagchi M (1997) Toxicity of trace elements in tobacco smoke. *Inhal Toxicol* 9:867–890
- Stone K, Bermudez E, Zang LY, Carter KM, Queenan KE, Pryor WA (1995) The ESR properties, DNA nicking, and DNA association of aged solutions of catechol versus aqueous extracts of tar from cigarette smoke. *Arch Biochem Biophys* 319:196–203
- Tanigawa T, Yoshikawa T, Takahashi S, Naito Y, Kondo M (1994) Spin trapping of superoxide in aqueous solutions of fresh and aged cigarette smoke. *Free Radic Biol Med* 17:361–365
- Torikaiu K, Uwano Y, Nakamori T, Tarora W, Takahashi H (2005) Study on tobacco components involved in the pyrolytic generation of selected smoke constituents. *Food Chem Toxicol* 43:559–568
- Torrence KM, McDaniel RL, Self DA, Chang MJ (2002) Slurry sampling for the determination of arsenic, cadmium, and lead in mainstream cigarette smoke condensate by graphite furnace-atomic absorption spectrometry and inductively coupled plasma-mass spectrometry. *Anal Bioanal Chem* 372:72373
- Tso TC (1990) Production, physiology and biochemistry of tobacco plants. Ideal, Beltsville, MD
- Urios A, Lopez-Gresa MP, Gonzalez MC, Primo J, Martinez A, Herrera G, Escudero JC, O'Connor JE, Blanco M (2003) Nitric oxide promotes strong cytotoxicity of phenolic compounds against *Escherichia coli*: the influence of antioxidant defenses. *Free Radic Biol Med* 35:1373–1381
- USCPCSC (1993) U.S. Consumer Product Safety Commission in consultation with the U.S. Department of Health and Human Services. Toxicity testing plan 5
- Waisberg M, Joseph P, Hale B, Beyersmann D (2003) Molecular and cellular mechanisms of cadmium carcinogenesis. *Toxicology* 192:95–117
- Wardman P (1989) Reduction potentials of one-electron couples involving free radicals in aqueous solution. *J Phys Chem Ref Data* 18:1637–1755
- Winston GW, Church DF, Cueto R, Pryor WA (1993) Oxygen consumption and oxyradical production from microsomal reduction of aqueous extracts of cigarette tar. *Arch Biochem Biophys* 304:371–378
- Wright HE, Burton WW, Berry RC (1960) Soluble browning reaction pigments of aged Burley tobacco. I. The nondialyzable fraction. *Arch Biochem Biophys* 86:94–101
- Wright HE, Burton WW, Berry RC (1964) Soluble browning reaction pigments of aged Burley tobacco. II. The dialysable fraction. *Phytochemistry* 3:525–533
- Yan F, Williams S, Griffin GD, Jagannathan R, Plunkett SE, VoDinh H (2005) Near-real-time determination of hydrogen peroxide generated from cigarette smoke. *J Environ Monitor* 7:681–687
- Yang CH, Wender SH (1962) Free phenolic acids in cigarette smoke and tobacco. Paper chromatography: separation and identification. *J Chromatogr A* 8:82–89
- Zane A, Wender SH (1963) Pyrolysis products of rutin, quercetin, and chlorogenic acid. *Tob Sci* 7:21–23
- Zang LY, Stone K, Pryor WA (1995) Detection of free radicals in aqueous extracts of cigarette tar by electron spin resonance. *Free Radic Biol Med* 19:161–167

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