Mini-Reviews in Organic Chemistry, **2011**, *8*, **427-433**

Free Radicals in Tobacco Smoke

Barry Dellinger*, Lavrent Khachatryan, Sofia Masko and Slawomir Lomnicki

Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803, USA

Abstract: A critical examination of the literature on gas-phase free radicals and TPM-associated EPFRs in cigarette smoke is presented. These radicals participate in catalytic cycles that generate ROS that can result in oxidative stress. It seems that primary radicals originate from the direct decomposition of tobacco constituents and are linked to the chemical structure of the constituents and the temperature of pyrolysis. On the other hand, secondary radicals are generated during aging of smoke. Their EPR spectra are indicative of hydroquinone and catechol. Lignin, chlorogenic acid, and proteins are major precursors of EPFRs in TPM, but combinations of components form more secondary radicals than the sum of the individual components, suggesting a synergistic role of TPM in the formation and the stabilization of secondary radicals. Matrix isolation EPR has identified cyclopentadienyl, phenoxyl, and semiquinone radicals as additional free radicals formed from phenolic precursors that may be responsible for gas-phase alkoxy radicals previously identified using spin-trapping techniques. EPFRs are found to initiate catalytic cycles that generate ROS, and the overall concentrations of ROS generated by EPFRS are expected to be much higher than that generated by hydroquinones/quinones.

Keywords: Free Radicals, Tobacco Smoke, Hydroquinones.

INTRODUCTION

A burning cigarette is a virtually unique, highly complex, and dirty combustion source. Controlled combustion sources whose purpose is to generate energy or destroy hazardous wastes are designed to have relatively uniform reaction atmospheres. In these sources, fuel and air are well mixed under stoichiometric or oxidizing conditions, and the temperature is high enough and the reaction time is long enough to destroy 99-99.99 % of the fuels and byproducts. In contrast, cigarettes are designed to deliver various chemicals to the smoker including nicotine and total particulate matter (TPM) that provide the desirable taste. This results in a wide range of burning conditions in a cigarette including temperatures ranging from room temperature to over 1000 C and both oxygen-rich and oxygen-starved burning and distillation conditions [1].

Chemicals of concern in cigarette smoke include polycyclic aromatic hydrocarbons (PAH), nitroso-amines, catechols, and carbon monoxide [1, 2]. The source of these chemicals is an active area of research, and methods to reduce their concentrations are under development. However, it is also known that burning cigarettes produce free radicals. Combustion research has not addressed exposure to free radicals because they have been considered to be unstable, transient species that would not survive long enough to result in human exposure. However, a smoker inhales the smoke so quickly that free radicals may be a concern. There are two reasons for this concern: 1) the initially generated, reactive free radicals can participate in cycles which generate secondary free radicals with a significant stationary state concentration and 2) some free radicals appear to be stabilized by association with TPM such that they have sufficiently extended lifetimes to result in exposure to the smoker or a by-stander. These longer lived free radicals have been referred to as Persistent Free Radicals (PFRs) or Environmentally Persistent Free Radicals (EPFRs) [3].

LITERATURE BACKGROUND

The conventional view of the source of free radicals in cigarette smoke is depicted in Fig. (**1**). Both gas-phase and TPM-phase radical formation are depicted.

The gas-phase radicals are formed by partial decomposition of polyphenolic and related structures in the tobacco to form simple gas-phase radicals such as methoxy and other oxy-alkyl radicals. These radicals then participate in catalytic cycles similar to those commonly accepted to occur in air pollution chemistry to generate peroxides, peroxy nitrites, peroxy nitrates, and nitroso derivatives. The reactants are sufficiently concentrated that the cycle can continue for several seconds and result in exposure to the smoker or second hand exposure to a by-stander. Upon exposure, the radicals produce still more radicals by interaction with lipids and the bodies defense mechanisms. These radicals are thought to cause acute responses such as coughing, or in extreme cases, asthma.

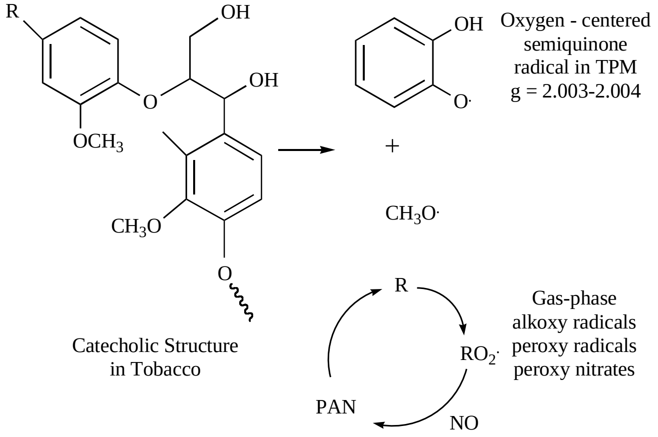


Fig. (1). Conventional view of free radical formation in cigarettes.

In a highly oversimplified mechanism of the formation of TPM-associated radicals, catecholic containing structures in components of tobacco partially decompose to form semiquinone-type radicals of catechol, hydroquinone, and their derivatives. Association of the radical with the TPM apparently imparts stability to the radical such that it can survive in air for long periods, possibly indefinitely. These EPFRS are of special concern because semiquinone radicals are know to generate reactive oxygen species (ROS) *in vivo* which can lead to oxidative stress and chronic diseases such as cardiopulmonary disease and cancer.

A review of the literature indicates that very little is known about the actual chemical structure of these EPFRs, their sources, and, especially, the reason they are so persistent. The seminal work on these radicals was performed by Pryor and associates. (cf. Table **1**) As early as 1970, radicals were detected in tobacco, char, and TPM. Spin trapping experiments resulted in the observation of alkoxyl (RO) type radicals. In the journal *Science* in1983, based on the non-Curie-Weiss behavior of EPR spectra of TPM, Pryor reported EPR spectra that were assigned as a donor-acceptor complex of quinone and hydroquinone. However, it was subsequently dis-

---

*Address correspondence to this author at the LSU Superfund Research Center, Louisiana State University, Baton Rouge, LA 70803, USA, Tel: 001 578-6759; Fax: 001 25 578-0276; E-mail: barryd@lsu.edu

1570-193X/11 $58.00+.00

Table 1. Summary of Key Publications by Pryor and Associates

Results	References
1. Radicals detected in tobacco, char, and TPM	Pryor, W.A. *Sci. Am.*, 223, 2, 70 **1970**.
2. Spin trapping, observed 3 gas-phase radicals: Type I were RO and ArCO$_2^{\cdot}$ **,** Type II and III were not typical spectra of spin adducts (secondary reactions). Conclusions were tentative.	Pryor, W.A., Terauchi, K-I., Davis, W.H, Jr. *Environ. Health Persp.,* 16, 161, **1976**.
3. Based on non-Curie-Wiess of EPR, donor-acceptor Q/QH2 in a tar matrix. (Retracted in IBM App Notes, 1985)	Pryor, W.A., Hales, B.J., Premovic, P.I., Church, D.F. *Science*, 220, 425, **1983**.
4. MS and SS smoke, broad singlet (g=2.003). Alcohol extract/addn. of base generated semiquinone radicals (2% of TPM). Spin trapped alkoxyl in gas-phase.	Pryor, W.A., Prier, D.G., Church, D.F. *Environ. Health Persp*., 47, 345, **1983**.
5. Q/QH2 principal radical complex held in tarry matrix and is capable of reducing molecular oxygen to produce superoxide.	Church, D.F., Pryor, W.A. *Environ. Health Persp.*, 64, 111, **1985**.
6. The aqueous extract of TPM was fractionated. The fraction containing radicals caused DNA damage.	Pryor, W.A., Stone, K., Zang, L-Y., Bermudez, E. *Chem. Res. Toxicol.*, 11, 441, **1998**.

Table 2. Key Literature Concerning Persistent Radicals in Cigarette Smoke

1. Delocalized e$^-$ on large PAH molecule (g = 2.0030). Suggests multiple radicals.	Austen, D.E.G., Ingram, D.J.E., Tapley, J.G. *Trans. Faraday Soc.*, 54, 400, **1958**.
2. Variety of active centers (g = 2.0030).	Forbes, W.F., Robinson, J.C., Wright, G.F. *Can. J. Biochem.*, 45, 1087, **1966**.
3. 4+ centers: phosphorous, graphitic (g=2.0028) , PAH (g=2.0026), and ~ 80 % HQ/Q (g=2.0035)	Pryor, W.A., Hales, B.J., Premovic, P.I., Church, D.F. *Science*, 220, 425, **1983**.
4. TPM (**g = 2.0029)**. Extracts: Benzene (g=2.0035), Dichloromethane (g=2.0037), DMSO (g=2.0038)	Church, D.F., Pryor, W.A. *Environ. Health Perspect.*, 64, 111, **1985**.
5. Benzene extract of TPM (g = 2.0035). Assigned as semiquinone.	Valavanidis, A., Haralambous, E. *Redox Rep.*, 6, 3, 161, **2001**.
6. TPM and extracts into hexane, benzene, isopropanol or mixture of these solvents. (g = 2.003). Poor reproducibility.	Baum, S.L., Anderson, I.G.M., Baker, R.R., Murphy, D.M., Rowlands, C.C. *Anal. Chim. Acta*, 481, 1-13, **2003**.
7. No correlation between HQ and radicals (benzene extract of TPM).	Blakley, R. L., Henry, D.D., Smith, C. *J. Food Chem. Toxicol.*, 39, 401-406, **2001**.

covered that the EPR signal was saturated, and this result was retracted in the journal *IBM Application Notes*. Later in 1983, Pryor reported both main stream and side stream smoke exhibited an EPR spectrum with a broad singlet with a g-value of 2.003. Sequential extractions, with the final extraction in alcohol, followed by the addition of base, generated an EPR spectrum identical to the semiquinone of hydroquinone in basic solution. The calculated concentration of semiquinone was 2% of the TPM. This has been erroneously interpreted in the literature, *vide infra*, that TPM contains 2% semiquinone radical, when in fact the observed semiquinone radical was probably formed from a hydroquinone or catechol in basic solution. Subsequent publications on the environmental implications of semiquinone radicals demonstrated that semiquinones in TPM were capable of reducing molecular oxygen to superoxide radical anion and induce DNA damage. These results have been generally supported by other reports [4-6], with some exceptions. (cf. Table **2**).

DELLINGER STUDIES OF TPM-ASSOCIATED AND GAS-PHASE FREE RADICALS

We have recently performed controlled studies of the formation of gas-phase radicals using the technique of Low Temperature Matrix Isolation (LTMI) trapping of cigarette generated radicals and more controlled studies of formation of EPFRs in TPM using a pyroprobe to pyrolyze or oxidize various types of tobaccos over a range of sequential reaction temperatures. These techniques had the advantage that TPM with less complex compositions were formed at each reaction temperature, and the resulting EPR spectra were simplified. This allowed for improved interpretation of the spectra (cf. Table **3**, **4**).

In these experiments, samples of bright tobacco were subject to pyrolysis and oxidative pyrolysis at temperatures ranging from 200 to 700 ^{o}C. The resultant TPM was collected on Cambridge filters, backed by a glass fiber filter, and analyzed by EPR. The resultant spectra were quantified and analyzed. Fig. (**2**) summarizes the results.

IDENTIFICATION OF TPM RADICALS

Two types of EPFRs were observed in the initially collected TPM (cf. Fig. **2**). Above 380 C, a narrow singlet spectrum with EPR parameters of g = 2.0035 and ΔHp-p = 8.5 G was observed (referred to as Type II, primary radicals). This is the same EPR signal observed by Pryor and other researchers. Although Pryor stops short of identifying this signal as a semiquinone radical in his original paper, other researchers and Pryor erroneously refer to this signal as that of a semiquinone radical in subsequent publications. This error is attributable to assignment of the radical structure as the same radical observed by Pryor following solvent extraction of TPM and addition of base.

The g-value of 2.0035 was actually consistent with that of a carbon-centered radical whose g-value has been shifted from the typical value of a hydrocarbon radical (<2.003) by spin-orbit cou-

Table 3. Dellinger Studies of TPM Radicals

1. Pyrolysis of tobacco as a function of temperature: Carbon-centered radicals and carbon-centered radicals with nearby oxygen-containing functional group. (g = 2.0034 -2.0045, ΔHp-p = 3.5 – 8.8 G decreased with increasing temperature.)	Maskos, Z., Khachatryan, L., Cueto, R., Pryor, W.A., Dellinger, B. *Energy Fuels*, 19, 791, **2005**.
2. Gas phase alkoxyl radical observed by spin trapping.	Maskos, Z., Khachatryan, L., Cueto, R., Pryor, W.A., Dellinger, B. *Energy Fuels*, 19, 791, **2005**.
3. Type I primary radicals in TPM formed at < 380^{o}C identified as tyrosyl radical (5-line EPR spectrum with g = 2.064, overall width = 42 G)	Maskos, Z., Khachatryan, L., Dellinger, B. *Energy Fuels*, 19, 791, **2005**.
4. Type II primary radicals in TPM formed > 380^{o}C. Carbon-centered radicals with unpaired electron vicinal to an oxygen - containing functional group (g = 2.0035-2.0040 and ΔHp-p = 8.8 G)	Maskos, Z., Khachatryan, L., Dellinger, B. *Energy Fuels*, 22, 1027, **2008**. Maskos, Z., Khachatryan, L., Dellinger, B. *Energy Fuels*, 19, 2466, **2005**.
5. Secondary radicals in TPM formed during aging of TPM. Oxygen-centered semiquinone radicals (g = 2.0053).	Maskos, Z. and Dellinger, B. *Energy Fuels*, 22, 382, **2008**.

Table 4. Dellinger Matrix Isolation Studies of Gas-Phase Radicals

1. Cyclopentadienyl Radical (CPD) identified from the gas-phase pyrolysis of hydroquinone, catechol, and phenol. 0.3 torr pyrolysis and Low Temperature Matrix Isolation identified CPD radical (g = 2.0043, 6 lines, intensity distribution 1:5:10:10:5:1, hsc=6 G,	Khachatryan, L., Adounkpe, J., Maskos, Z., Dellinger, B. **2006**, *ES&T*, 40, 5071-5076.
2. Radicals from the gas-phase pyrolysis of phenol. CPD radicals from 700 to 1000 C, g = 2.0043. Trace quantities of phenoxyl radical observed, g = 2.0060.	Khachatryan, L., Adunkpe, J., Dellinger, B. *J. Phys Chem.* A, **2008**, 112, 481-487.
3. Radicals from the gas-phase pyrolysis of hydroquinone. p-semiquinone (p-SQ) radicals at 350-725 C (g=2.0043), CPD radicals at 850-975 C (g=2.0042), and mixture of p-SQ and CPD radicals at 720-850 C (g=2.0048).	Adunkpe, J., Khachatryan, L., Dellinger, B. *Energy Fuels*, **2008**, 22, 2986-2990.
4. Radicals from the Gas-Phase Pyrolysis of Hydroquinone. Traces of alkyl peroxy radicals observed when oxygen was added to the reaction atmosphere (g = 2.0101).	Khachatryan, L., Adunkpe, J., Dellinger, B. *Energy Fuels*, **2008**, 22, 3810-3813.
5. Radicals from the Atmospheric Pressure Pyrolysis and Oxidative Pyrolysis of Hydroquinone, Catechol and Phenol CPD radicals from three compounds at higher temperatures, 750-1000 oC. Phenoxyl (g=2.0060) and peroxy (g=2.0100) radicals from oxidative pyrolysis of phenol (500-1000 °C)	Adunkpe, J., Khachatryan, L., Dellinger, B., and Ghosh, M. *Energy Fuels*, **2009**, 23, 1551-1554.
6. Radicals from the Gas Phase Pyrolysis of Catechol. 1. Ipso-Catechol and o-Semiquinone Radicals A doublet EPR line with a g-value of 2.0052 was identified as o-semiquinone radical. Intramolecular H-transfer in Catechol molecule by formation of *alpha-* , *ipso-* Catechol isomers as well as ipso-catechol radicals were justified by *ab-initio* calculations	Khachatryan, L., Adunkpe, J., Asatryan, R., Dellinger, B. *J. Phys. Chem. A.*, **2010**, 114, 6, 2306-2312

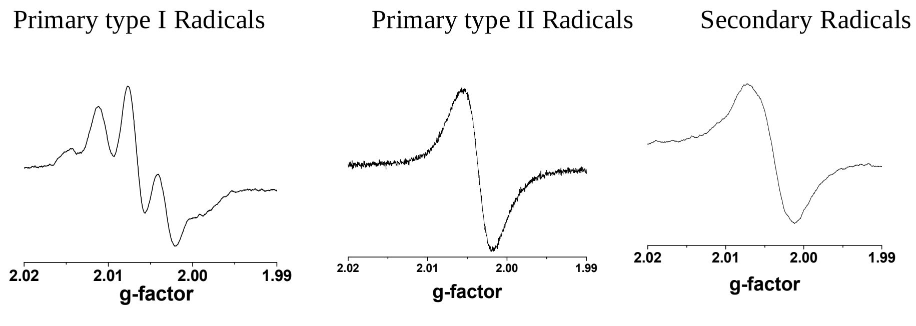

Primary type I Radicals
Primary type II Radicals
Secondary Radicals

Fig. (2). Radicals observed from fractional pyrolysis of Bright tobacco.
Primary Type I: Tyrosyl radicals, initial TPM, 200 - 380 °C, g = 2.0064, Htotal = 42 G. Primary Type II: "Pryor" radicals, g = 2.0035, ΔHp-p = 8.5 G. Secondary: Semiquinone radicals, aged TPM, Tmax = 280 °C, g = 2.0053, ΔHp-p = 13 G.

pling with a near-by oxygen-containing functional group. It is likely that Type II primary radicals are polymeric or oligomeric phenols with the unpaired electron largely located on an aromatic carbon. From 200-380 ^{o}C, an entirely different multi-line, EPR signal was observed that was very wide, with an overall width of 42 G with a very high g-value of 2.0064 (Type I, primary radicals). The high g-value suggests an oxygen-centered radical such as semiquinone. However, the slightly asymmetric 42 G width is inconsistent with semiquinone radicals. Based on comparison to known spectra in the literature and composition of tobacco, this signal has been assigned as the radical of tyrosyl. Although the tyrosyl radical is relatively un-reactive, it is relatively thermally fragile and decomposes above 380 ^{o}C. These two signals were the only ones consistently observed in the initially produced TPM from pyrolysis or oxidation of tobacco.

However, atmospheric aging of TPM in air produced a new radical (Secondary Radicals, cf. Fig. **2**). The EPR signal of these radicals was a narrow singlet with ΔHp-p of 13 G and a high EPR g-value of 2.0053. This signal is consistent with of a true semiqui-

Table 5. Radicals from the Pyrolysis of Bright Tobacco, Individual tobacco Components, and a Mixture of Components

%	Precursor	Primary Radicals	Secondary Radicals	Weighted Contribution to Total Radicals[#]	
				Primary Radicals	Secondary Radicals
13	Cellulose	<0.01[*]	2.7	<0.01	0.35
11	Pectin	<0.01	3.4	<0.01	0.37
7	Protein	280	325	19.6	22.7
2	Lignin	76	680	1.5	13.6
>1	Chlorogenic Acid	405	-	6.0	-
<1	Rutin	<0.01	2.8	<0.01	0.02
Weighted Sum of Components				27	37
Experimental Mixture				<0.01	250
Bright Tobacco				38	262

[*] All entries are spins x 10^{14} /g of sample. [#]Weighted based on % mass of component in Bright tobacco.

none-type radical which has lost resolution of the quintet observed in solution due to environmental interactions with the TPM. The partial decomposition of tobacco constituents by pyrolysis or oxidative pyrolysis can result in the release of catechol and hydroquinone-type compounds which are a component of the TPM. Upon exposure to air and moisture, these molecular species are converted to semiquinone-type radicals by oxidizing agents contained in the TPM.

QUANTIFICATION AND SOURCES OF TPM RADICALS

In an attempt to identify the major sources of radicals in TPM, additional studies were conducted in which various constituents of tobacco were pyrolyzed and the total yields of radicals were determined. (cf. Table **5**). These results indicate that while some of the sources of radicals in TPM can be identified, there are still many uncertainties. The data in the last row of the table indicates a total of 38 x 10^{14} primary radicals and 262 x 10^{14} secondary radicals were produced from the pyrolysis of Bright tobacco. This is about 10x the concentration reported for smoking a cigarette under standard conditions. Based on the data in Table **5**, protein contributes 19.6 x 10^{14} spins/g of the 38 x 10^{14} spins/g of primary radicals produced from pyrolysis of bright tobacco. Protein also contributes 22.7 x 10^{14} spins/g of the 262 x10^{14} spins/g of secondary radicals. The contribution of the tested tobacco constituents to the yield of primary radicals are in the order:

Protein > chlorogenic acid> lignin >> cellulose ~ pectin.

For secondary radicals the order is:

Protein > lignin >> pectin ~ cellulose >> rutin.

However, the individual components do not account for all the observed yield of radicals, and there are some notable anomalies.

The sum of radical yields for the six tested components accounts for 27x10^{14} spins/g of the 38x10^{14} spins/g of radicals from bright tobacco. However, a mixture of the six components produced <0.01x10^{14} spins/g of radicals indicating an inhibitory effect of some component of the mixture. In stark contrast, the sum of the weighted components to the yield of secondary radicals was only 37x10^{14} spins/g versus the yield from bright tobacco of 262x10^{14} spins/g. However, a mixture of the components increased the total radical yield to 250x10^{14}spins/g. This suggests a promoting component within the mixture for the formation of secondary radicals.

GAS-PHASE RADICALS

Studies of gas phase radicals in cigarette smoke have focused on radicals that are apparently easy to spin trap, including peroxy, peroxynitrates/nitrites, and nitroso substituted radicals. It is believed that these radicals are not initially produced by the burning tobacco but are formed by secondary reactions and cycles involving O_2 and NO that are very similar to reactions involved in formation of atmospheric pollution. Using known components of tobacco smoke, recent studies using matrix isolation techniques have identified additional organic radicals which may be formed from burning tobacco (cf. Table **4**).

Cylopentadienyl radicals have been observed via low temperature matrix isolation from the pyrolysis of phenol from 700-1000 ^{o}C, from hydroquinone (720-975 ^{o}C), and catechol (750-1000 ^{o}C). Semiquinone radicals were observed from pyrolysis of hydroquinone from 350-850 ^{o}C. Phenoxyl was observed in trace yields from the pyrolysis of phenol from 500-1000 ^{o}C. Alkyl peroxyl radicals were only observed in trace quantities and when oxygen was added to the reaction atmosphere.

While these studies confirmed the presence of alkyl peroxyl radicals reported by others, they also suggest that cyclopentadienyl, semiquinone, and phenoxyl radicals are primary, gas-phase radicals. All are resonance stabilized, and semiquinone and phenoxyl radical are relatively resistant to further oxidation. While cyclopentadienyl may actually be the most stable (resistant to decomposition), it should be more susceptible to reaction with oxygen. Hence, it may be a major precursor for alkylperoxy and other secondary radicals reported by other researchers. Matrix isolation studies of TPM generated from burning Virginia, Burley, and Oriental tobacco resulted in spectra too complex to identify individual radicals. However, these studies indicated that the ratio of yields of radicals from Burley, Virginia, and Oriental tobacco were 2:1:1 per gram of TPM produced.

MECHANISMS OF EPFR FORMATION AND STABILIZATION

Gas-phase alkoxy, peroxy, and their nitro derivatives can regenerate in catalytic cycles and have appreciable steady state concentrations for a few seconds in concentrated cigarette smoke. Resonance stabilized radicals such as semiquinones, phenoxyls, cyclopentadienyls are stable and resistant to oxidation, and they may have lifetimes in air of milliseconds. However, some radicals associated with TPM have lifetimes of days and may actually increase in concentration with time. Association with TPM apparently imparts environmental persistence to these radicals.

Fig. (**3**) depicts an experimentally demonstrated mechanism of formation and stabilization of substituted phenoxyl radicals including semiquinones on a copper oxide surface [7]. In this pathway,

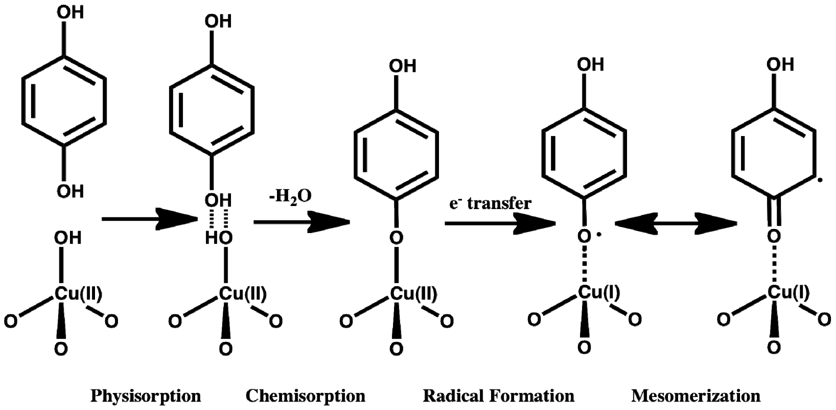


Fig. (3). Mechanism for formation of environmentally persistent p-semiquinone radical from hydroquinone on a copper hydroxide surface.

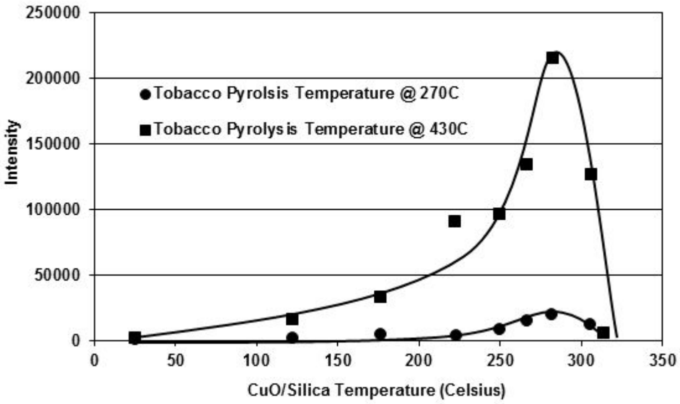


Fig. (4). Radical yield versus collection temperature for TPM collected on CuO/siica beads.

molecular precursors physisorb by hydrogen bonding, chemisorb by elimination of water, and form free radicals by oxidation by copper (II) which is concomitantly reduced to Cu(I). Physisorption and chemisorption have been demonstrated using FTIR; reduction of Cu(II) has been demonstrated using x-ray spectroscopy and formation of the radicals has been demonstrated using EPR. Association of the radical with the surface and forcing of the oxygen-centered, rather than the carbon-centered, structure is believed to be responsible for the lack of reactivity of the radicals [7-9]. A similar metal catalyzed formation and stabilization mechanism may be active in tobacco smoke.

Experiments were conducted in which tobacco was pyrolyzed at 270 ^{o}C (optimum temperature for Primary Type I and Secondary radicals) and 430 ^{o}C (optimum temperature for Primary Type II radicals). TPM was collected on silica beads and 5% CuO on silica held at various temperatures ranging from 25 to 320 ^{o}C. EPFRs were observed at both tobacco pyrolysis temperatures in TPM collected on CuO/silica beads at 5 to 10 times the yield for TPM collected on silica beads. The radical yield was at a maximum when the CuO/silica beads were at a temperature of 280 ^{o}C (cf. Fig. **4**). This study demonstrated that addition of an oxidizing agent increased the concentration of EPFRs and suggests that oxidizing agents, such as Cu(II)O, which are in trace quantities in TPM, may be responsible for Primary as well as Secondary Radical formation.

A MODEL OF RADICAL FORMATION FROM BURNING TOBACCO

Based on the available results, the current model of radical formation can be made more detailed. Some precursors, intermediates, formation reactions, and mechanisms of stabilization of TPM-associated EPFRs can now be incorporated into the model. (cf. Fig. **5**). Type I Primary Radicals are tyrosyl radicals formed from partial decomposition of protein at lower temperatures. Cell wall biopolymer components of tobacco are known to contain proteins including tyrosine. The ether type linkages in the protein are thermally fragile and can readily cleave at temperatures less than 380 ^{o}C to form tyrosyl radicals. The tyrosyl radical is partially oxygen-centered and stabilized through delocalization through the aromatic ring and the polymeric protein. It is moderately resistant to oxidation, but its concentration decreases with increasing oxygen concentration due to lack of an additional stabilization mechanism other than delocalization of the electron. At higher temperatures, the radical decomposes or the protein precursors decompose via new pathways to form other products.

Type II Primary Radicals are carbon-centered phenolic polymers. As the lignin and cellulose components of the tobacco degrade, they form a polymeric oil that consists of long chains of linked aromatic moieties with hydroxyl, alkoxyl, and carbonyl substituents. A delocalized electron is associated with the polymer that is largely carbon-centered; however, the oxygen-containing substituents shift the EPR spectrum and imparts additional stability to the radical. The polymer is the least volatile component of the TPM and is formed by molecular growth reactions as the bulk constitutents of the tobacco continue to degrade at temperatures greater than 380 C. The Type II Primary Radical is the highest concentration radical associated with TPM and dominates the EPR spectrum of TPM. However, because it is carbon-centered and resonance stabilized it is probably not the most biologically-active or relevant radical in TPM.

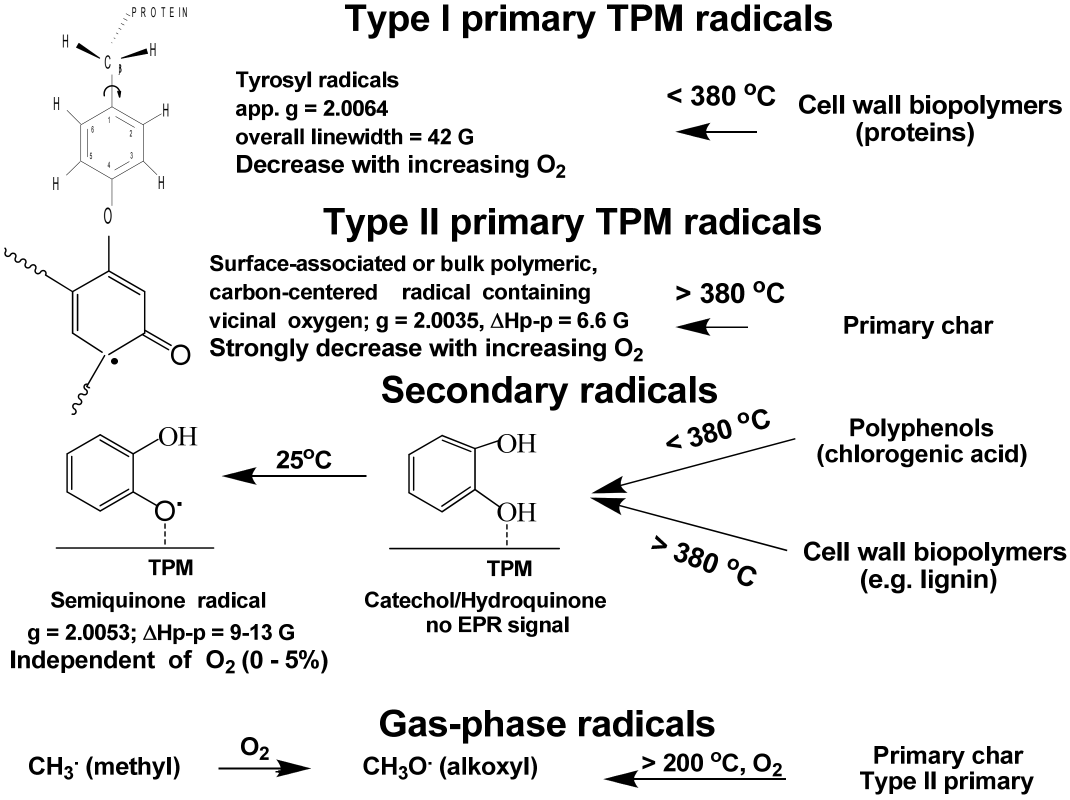


Fig. (5). Model of free radical generation from oxidative pyrolysis of tobacco.

Secondary TPM Radicals are the semiquinone-type radicals, rather than Type II Primary Radicals as commonly assumed in the literature. They are formed by oxidation (in the electrochemical sense) of molecular catechols and hydroquinones associated with TPM. The catechol and hydroquinone precursors are formed below 380 C by decomposition of lignin in the cell wall biopolymers of tobacco and above 380 C by decomposition of polyphenols such as chlorogenic acid. The surface-associated catechols and hydroquinones are slowly oxidized by copper oxide, or other oxidizing agents in TPM, to form semiquinone-type EPFRs. The oxygen-centered structure of the resultant radical and association with the TPM impart stability and resistance to further oxidation and reaction of the radical such that it can exist, apparently indefinitely, in tobacco smoke.

Gas-Phase Radicals that are commonly observed by spin trapping are oxy, peroxy, peroxynitrate, and nitroso radicals formed by secondary reaction cycles involving initially formed hydrocarbons radicals. These hydrocarbon radicals are decomposition products of any of the tobacco constituents. However, matrix isolation EPR has identified initial organic radicals more complex than the simple alkyl radicals typically thought to be responsible for gas-phase radicals. These radicals include cyclopentadienyl, phenoxyl, and semiquinones.

POTENTIAL FOR TOXICOLOGICAL AND ENVIRONMENTAL IMPACT OF FREE RADICALS

Although a link between exposure to free radicals and health has been long suspected, their potential health impacts were not recognized because they were thought to be "inaccessible to cells and too unstable to play any part in carcinogenesis" until the publication of a series of papers by Pryor demonstrating the viability of catalytic cycles involving semiquinone radicals. Some of the health impacts of smoking have been attributed to oxidative stress induced by redox cycles that produce biologically damaging ROS such as peroxide, superoxide, and hydroxyl radical.

A "quinone hypothesis" of the cause of oxidative stress induced by cigarette smoke has been suggested [2, 10]. EPR analysis has confirmed the presence of semiquinone and other radicals at concentrations of 1.4-14 x 10^{14} radicals/cigarette (0.1-1.0 x 10^{14} radicals/g of TPM), that may co-exist with hydroquinone/quinones. However, ROS generation has not correlated with the measured concentrations of molecular catechols, hyroquinones, and quinones (the actual species could not be determined).

A catalytic cycle in which ROS can be generated by semiquinone radicals is presented in Fig. (**6**). In this cycle, hydroquinones chemisorb to Cu(II) and are reduced forming a semiquinone radical anion and Cu(I). Semiquinone radical anion reduces molecular oxygen to form superoxide. The superoxide is converted to hydroxyl radical by the Cu(I)-catalyzed Fenton-Type Reaction and biological reducing equivalents such as NADPH. Reduction of the resulting quinone regenerates semiquinone radical, the cycle continues, and more hydroxyl radical is generated. Although both quinones/hydroquinones and semiquinone radicals are present in TPM, it is commonly believed that the higher concentration of molecular quinone/hydroquinoness drives the ROS generation cycle. However, contrary to common belief, the overall rate of ROS production is higher for a cycle that is initiated by the SQ radical than a molecular quinone or hydroquinone, strongly suggesting that SQ radicals are the dominant source of ROS production (Fig. **7**).

CONCLUSIONS

In summary, critical examination of the archival literature supports the existence of gas-phase free radicals and TPM-associated EPFRs in cigarette smoke. These radicals participate in catalytic cycle that generate ROS that can result in oxidative stress in exposed organisms that can ultimately lead to various diseases. Specific observation and conclusions include:

- Primary radicals originate from the direct decomposition of tobacco constituents and their nature depends on the chemical structure of the constituents and the temperature of py-

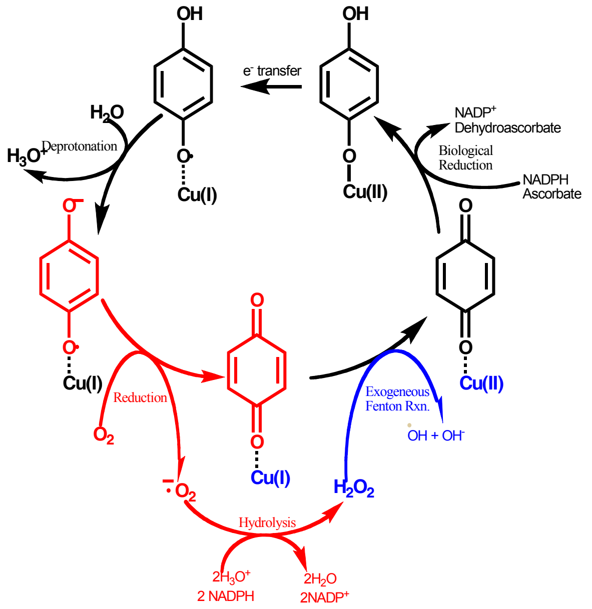
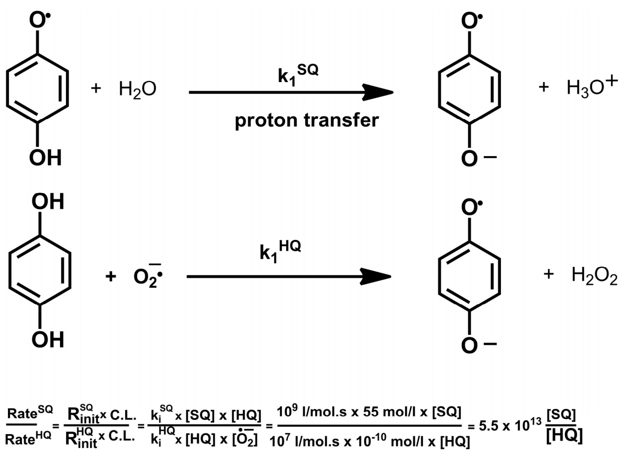


Fig. (6). Model for generation of ROS and oxidative stress by a SQ-CuO particle system. The reactions marked in blue represent the traditional Fenton Reaction chemistry for generation of hydroxyl radical from hydrogen peroxide and a reduced metal (in this example Cu^{+1} rather than the usual Fe^{+2}). The reactions and species marked in red (along with the blue Fenton chemistry) denote the principal reactions proposed for semiquinone-induced generation of O_2.- and .OH. The remaining reactions and species marked in black (along with the blue and red reaction cycles) complete the cycle in which chemisorbed semiquinone radicals generate O_2.-, H_2O_2, and a reduced metal, the latter catalyzing the conversion of hydrogen peroxide to hydroxyl radical and regenerating the original SQ-Cu(II)O particle system.

rolysis, i.e. Primary Type 1: tyrosyl and Type 2: polyphenol.

- Secondary radicals are generated during aging of TPM. Their behavior and EPR spectra are very similar indicating similar structures and similar precursors, i.e. hydroquinone and catechol produce Secondary semiquinones.
- Lignin, chlorogenic acid, and proteins are major precursors of EPFRs in TPM, but combinations of components form more secondary radicals than the sum of the individual components suggesting a synergistic role of TPM in the formation and the stabilization of secondary radicals.
- Copper oxide catalyzes the formation of semiquinone-type radicals from catechol, hydroquinone, phenol, and TPM.
- Matrix isolation EPR has identified cyclopentadienyl, phenoxyl, and semiquinone radicals as additional free radicals formed from phenolic precursors that may be responsible for gas-phase alkoxy radicals previously identified using spin-trapping techniques.
- EPFRs initiate catalytic cycles that generate ROS and the overall concentrations of ROS generated by EPFRS is expected to be much higher than that generated by hydroquinones/quinones.



$$\frac{Rate^{SQ}}{Rate^{HQ}} = \frac{R_{init}^{SQ} \times C.L.}{R_{init}^{HQ} \times C.L.} = \frac{k_i^{SQ} \times [SQ] \times [HQ]}{k_i^{HQ} \times [HQ] \times [\dot{O_2^-}]} = \frac{10^9 \text{ l/mol.s} \times 55 \text{ mol/l} \times [SQ]}{10^7 \text{ l/mol.s} \times 10^{-10} \text{ mol/l} \times [HQ]} = 5.5 \times 10^{13} \frac{[SQ]}{[HQ]}$$

Fig. (7). Comparison of the rate of ROS generation for cycles initiated by a HQ molecule versus an SQ radical. The initiation step by SQ radical involves deprotonation of SQ radical to form the SQ radical-ion. As proposed in the literature, the initiation step by a HQ molecule is the abstraction of a hydrogen atom by O_2.- or O_2. After the initiation step, the reactions follow identical paths with the same presumed chain lengths (C.L.). Using literature rates for these or similar reactions, the ratio of the overall rate of ROS production by SQ radical versus an HQ molecule is 5.5×10^{13} [SQ]/[HQ]. Thus the rates will only be comparable when the HQ concentration is 10^{13} x that of SQ. Our measurements indicate that the concentration of chemisorbed SQ radicals significantly exceed the concentration of molecular HQ/Q. Similar calculations where the rate of initiation by HQ is due to reaction with molecular oxygen, rather than superoxide, gives a ratio of 10^7, which still indicates dominance of the SQ radical initiation pathway.

REFERENCES

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

[2] Halliwell, B.B.; Poulsen, H.E., Ed. *Cigarete Smoke and Oxidative Stress.* Springer-Verlag Berlin: Heidelberg, **2006**.

[3] Dellinger, B.; Lomnicki, S.; Khachatryan, L.; Maskos, Z.; Hall, R.; Adounkpe, J.; McFerrin. C.; Truong, H. Formation and stabilization of persistent free radicals. *Proc. Comb. Inst.* **2007**, *31*, 521-528.

[4] Nalbandyan, A.B., Mantashyan, A.H. *The Elementary Processes in Slow Gas-Phase Reactions*. Inst. Chem. Phys. NA of Armenia, Yerevan, Armenia, **1975**.

[5] Carlier, M.; Pauwels, J.P.; Sochet, L-R. Application of ESR techniques to the study of gas-phase oxidation and combustion phenomena. *Oxid. Commun*. **1984**, *6*, 141-156.

[6] Davidson, I.M.T.; Ellis, A.M.; Mills, G.P.; Pennington, M.; Povey, I.M.; Raynor, J.B.; Russell, D.K.; Saydam, S.; Workman, A.D. Mechanism of pyrolysis of organometallic deposition precursors. *J. Mater. Chem.* **1994**, *4*, 13.

[7] Lomnicki, S.; Truong, H.; Vejerano, E.; Dellinger, B. Copper oxide-based model of persistent free radical formation on combustion-derived particulate matter. *Environ. Sci. Technol.* **2008**, *42*, 4982-4988.

[8] Alderman, S.L.; Farquar, G. R.; Poliakoff, E. D.; Dellinger, B. An infrared and X-ray spectroscopic study of the reactions of 2-chlorophenol, 1,2-dichlorobenzene, and chlorobenzene with model CuO/silica fly ash surfaces. *Environ. Sci. Technol.* **2005,** *39*, 7396-7401.

[9] Alderman, S.L.; Farquar, G.R.; Poliakoff, E.D.; Dellinger, B. FTIR Investigation of 2-chlorophenol Chemisorption on Cu(II)O/SiO_2. *Environ. Sci. Technol.* **2003**, *37*, 935

[10] Zang, L.-Y.; Stone, K.; Pryor, W.A. Detection of free radicals in aqueous extracts of cigarette tar by electron spin resonance. *Free Radic. Biol. Med.* **1995**, *19*, 161-167.

Received: March 18, 2010 Revised: August 08, 2010 Accepted: October 02, 2010