# This student paper was written as an assignment in the graduate course

## *Free Radicals in Biology and Medicine*

**(77:222, Spring 2005)**

**offered by the**

**Free Radical and Radiation Biology Program**
**B-180 Med Labs**
**The University of Iowa**
**Iowa City, IA 52242-1181**
**Spring 2005 Term**

**Instructors:**
**GARRY R. BUETTNER, Ph.D.**
**LARRY W. OBERLEY, Ph.D.**

**with guest lectures from:**
**Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann**

**The Fine Print:**

Because this is a paper written by a beginning student as an assignment, there are no guarantees that everything is absolutely correct and accurate.

In view of the possibility of human error or changes in our knowledge due to continued research, neither the author nor The University of Iowa nor any other party who has been involved in the preparation or publication of this work warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for the results obtained from the use of such information. Readers are encouraged to confirm the information contained herein with other sources.

# Environmental Xenobiotic: Cigarette Smoke

By

**Disha Dayal**

Free Radical and Radiation Biology Graduate Program
The University of Iowa,
Iowa City, IA-52242-1181

For: 077:222, Spring 2005
Date: 03-14-05

**Abbreviations:**

| | |
|---|---|
| CS | Cigarette smoke |
| CSC | Cigarette smoke condensate |
| EPR | Electron paramagnetic resonance |
| ETS | Environmental tobacco smoke |
| $NO^{\bullet}$ | Nitric oxide |
| $O_2^{\bullet-}$ | Superoxide anion radical |
| PD | Parkinsons disease |
| Q | Quinone |
| $QH^{\bullet}$ | Semiquinone |
| $QH_2$ | Hydroquinone |
| RNS | Reactive nitrogen species |
| ROS | Reactive oxygen species |
| SOD | Superoxide dismutase |
| Vit | Vitamin |

# Index



**1. Abstract**

Cigarette smoke (CS) is a potent xenobiotic. Its deleterious effects are not limited to the smoker alone, but affect the environment and passive smokers as well. Most of the toxic effects of CS arise from its several constituent free radicals. It is reported that a single puff of a cigarette may have as many as $10^{15}$ free radicals. The other toxic product of cigarette combustion is the particulate tar. Tar also contains dangerous free radicals in addition to several carcinogens. Due to the widespread harmful effects of CS and tar, they have been extensively studied. This report summarizes the radicals in CS, various techniques available to study them, and their myriad of effects as environmental xenobiotics.

## 2. Introduction

There is a large body of evidence to suggest that cigarette smoke (CS) produces free radicals [1]. CS can be separated into two phases, the particulate tar phase and the gas phase. The smoke can further be divided into mainstream smoke (that which is taken in through the mouthpiece of the cigarette during puffing) and sidestream smoke (that which is emitted from the smouldering cigarette in between puffs). Although the components of a cigarette vary from brand to brand, the three essential components are a tobacco rod wrapped in cigarette paper, a porous plugwrap and a filter (**Figure 1**).

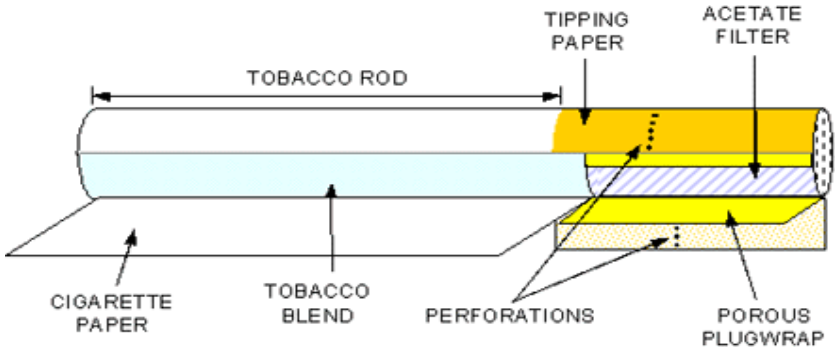



**Figure 1:** Essential parts of a typical cigarette. The filter provides some protection to the smoker, against harmful radicals. *

It has been estimated that there are about $10^{15}$ radicals per puff [2]. The type of radicals in tar and smoke are different. The tar consists of moieties like quinone (Q), semiquinone radical ($QH^{\bullet}$) and hydroquinone ($QH_2$), whereas the smoke consists of reactive oxygen species (ROS), *e.g.* $O_2^{\bullet -}$, $HO^{\bullet}$ and reactive nitrogen species (RNS), *e.g.* $NO^{\bullet}$ and the much more reactive $NO_2^{\bullet}$ [1]. The RNS can react with isoprene, a conjugated diene that occurs in smoke, to form more reactive radicals [1]. There can be several reactions between ROS and RNS to produce oxidizing free radicals, which can cause significant biological damage.

* http://www.gallahergroup.com/corporate/relationship_gallaheruk_07.asp (Acessed_02-26-05)

### 3. RNS and ROS reactions in cigarette smoke

RNS and ROS that are the predominant constituents of cigarette smoke that react with each other to produce a variety of radicals. Although in case of cigarette smoke these reactions occur in gas phase, most of these reactions have been studied in aqueous phase using pulse radiolysis. Some of these reactions are summarized below [2].

$$HO^{\bullet} + NO_2^- \longrightarrow OH^- + NO_2^{\bullet} \quad k = 1.3 \times 10^9 \text{ L M}^{-1}\text{s}^{-1}$$

$$2NO_2^{\bullet} \longrightarrow N_2O_4 \quad k = 9 \times 10^8 \text{ L M}^{-1}\text{s}^{-1}$$

$$e_{aq}^- + NO_3^- \longrightarrow NO_3^{\cdot 2-} \quad k = 1 \times 10^{10} \text{ L M}^{-1}\text{s}^{-1}$$

### 4. Analysis of cigarette smoke

The separation between tar and smoke is usually done by a Cambridge filter which can retain 99.9% of the particulate matter of the smoke.

4.1 EPR analysis of cigarette smoke

Free radicals produced in CS can be analyzed using electron paramagnetic resonance (EPR). Cigarette smoke is generated and collected as shown in **Figure 2** [3]. N-*tert*-butyl-2-phenyl nitrone (PBN) is usually used as the spin trapping agent or free radical scavenger.

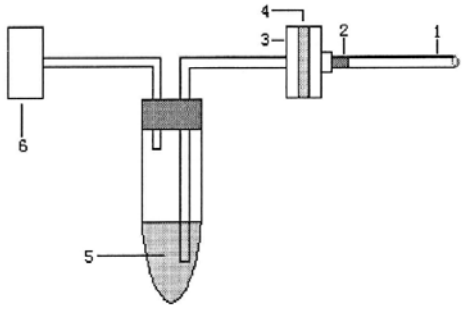



**Figure 2:** Detection of free radicals in CS using EPR. 1-cigarette, 2-filter, 3-hold, 4-Cambridge filter, 5-spin trapping solution, 6-pump. The Cambridge filter (4) is used to separate the CS from tar. This smoke passes through the spin trap solution(5) and is analyzed by the EPR (6). EPR thus provides an elegant method for detection of free radicals in cigarette smoke [2].

Free radicals in tar can also be analyzed using the EPR. **Figure 3** shows an EPR spectrum obtained from adducts of mainstream cigarette tar [4].

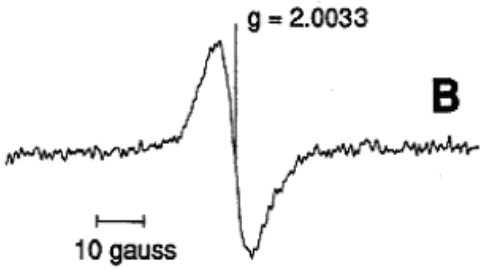



**Figure 3:** EPR spectrum of a radical from tar extracted in phosphate buffer. Different radicals in smoke or tar can be identified based on their characteristic peak in the EPR spectrum. This g-value corresponds to semiquinone radical [3].

4.2 Detection of oxygen radicals

Production of ROS ($O_2^{\bullet-}$, $HO^{\bullet}$) in leukocytes of a smoker can be monitored using luminol-dependent chemiluminescence. The leukocytes have to be first stimulated by zymosan before chemiluminescence can be measured. As a control, the samples are monitored in the presence of superoxide dismutase (SOD), which diminishes the signal [5].

**5. Constituents of cigarette**

The ingredients of the cigarette vary from brand to brand. Some of the common constituents of a typical cigarette are listed in **Table I** [6]. Apart from the compounds listed in Table I, CS may also contains susbstituted guaiacols [7], cyclopentenones [8], phenol derivates, polycyclic aromatic hydrocarbons, and trace amounts of polychlorinated

**Table I:** Constituents of a typical cigarette. Table adapted from Pyror *et al.* (1997) [4]

| **Chemical composition**** | **Amount / %** |
|---|---|
| Cigarette mass (g) | 0.996 |
| Cigarette humidity (%) | 11.22 |
| pH | 6.43 |
| Nicotine (%) | 1.36 |
| Total nitrogen (%) | 2.86 |
| Proteins (%) | 8.21 |
| Soluble sugars (%) | 7.97 |
| Potassium (mg/g) | 19.78 |
| Calcium (mg/g) | 37.29 |
| Magnesium (mg/g) | 7.33 |

** This table does not include all compounds within a cigarette. Composition may vary with brand.

hydrocarbons, which are otherwise used as pesticides [4]! The addiction causing compound in cigarettes, namely, nicotine, is found in greater quantities in the sidestream smoke than the mainstream smoke [9].

**6. Cigarette smoke as a xenobiotic**

6.1 Oxidative stress

Formation of free radicals leads to oxidative stress in smokers. Exposure to CS *in vivo* leads to an increase in pulmonary glutathione level. This suggests that there is an adaptive response to the increased oxidative load brought about by smoking [1]. The concentration of protein mixed disulfides is found to be higher in smokers [10]. The lungs of long-term smokers are darker in color compared to non-smokers. This is due to deposition of particulate tar and also because of iron deposition in the form of ferritin and homosiderin. These iron deposits are probably due to long term oxidative insult to the lung tissue [1]. However, vitamin E, a major antioxidant in the body is shown to offer protection against oxygen radicals. Vitamin E deficient mice show higher mortality rate than those, which are given vitamin E supplements. Vitamin C is involved indirectly by helping in regeneration of vitamin E. Smokers however, have reduced levels of vitamin E [1].

6.2 DNA damage

Reports suggest that CS can potentiate DNA damage, which can be assessed by the hydroxylation of DG (deoxyguanosine) to 8-oxoDG (8-hydroxydeoxyguanosine) [11]. The superoxide anion radical produced by CS

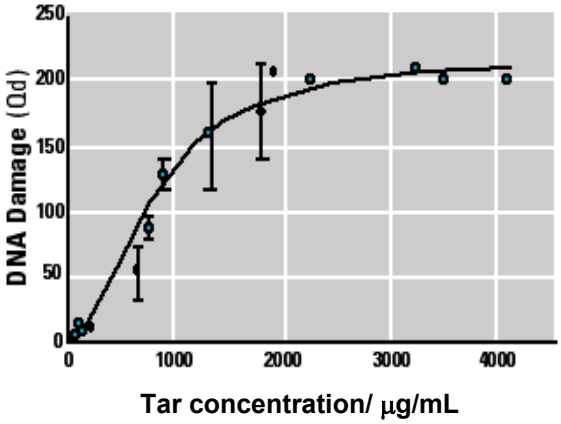



**Figure 4:** The DNA damage varies directly with tar concentration up to a certain point. It reaches a plateau at high concentration.

can give rise to sister chromatid exchanges and chromosomal aberrations. The DNA damage is directly proportional to tar concentration (**Figure 4**) [12]. These effects can be suppressed by antioxidant molecules and enzymes [13]. The schematic shown in **Figure 5** illustrates ways of DNA damage by various radicals in cigarette tar and smoke [3].

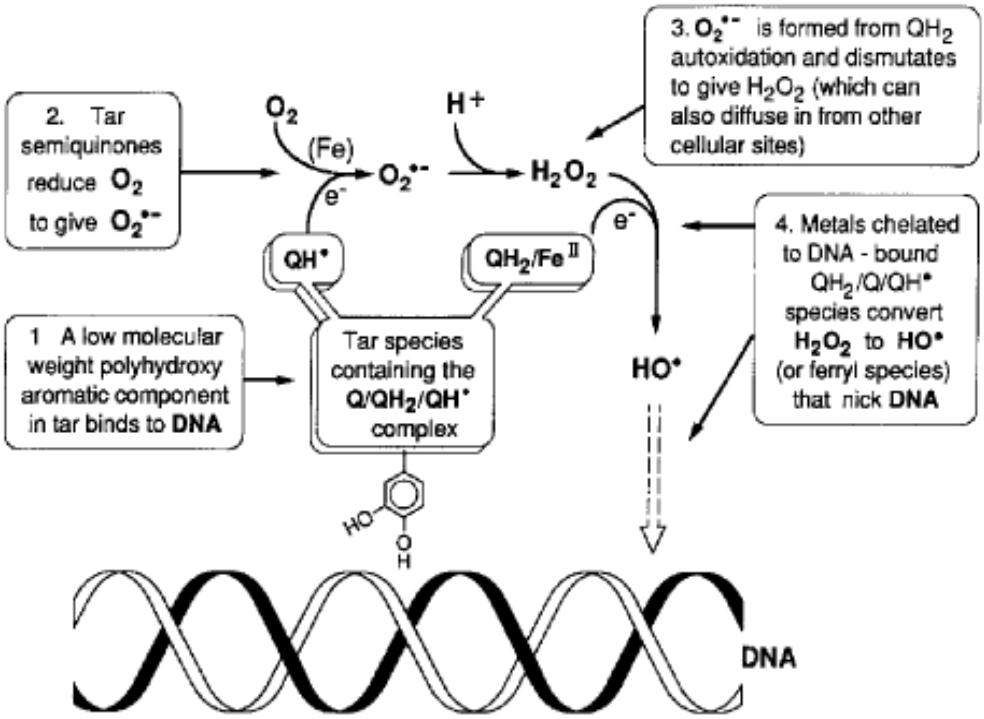



**Figure 5:** DNA damage by cigarette tar. The quinone and seminquinone radicals formed by superoxide and other ROS, can cause a nick in the DNA.

6.3 Coronary diseases

Numerous reports have demonstrated the increased risk of coronary problems in smokers. One of the major reasons of these heart diseases is the thickening of the intimal lining of arteries and plaque development [14]. CS has been demonstrated to contribute in this thickening *via* iNOS [9]. Apart from coronary diseases, CS can also cause blockage of pulmonary airway, emphysema *etc.* [15].

6.4 Carcinogenesis

Cigarette smoke contains several well-known potent carcinogens. These are present in both mainstream and sidestream smoke [3]. Some of these carcinogens in CS are catechols, *N*'-nitrosonornicotine, *N*-nitrosodimethylamine, *N*-nitrosopyrrolidine, *N*-nitrosopiperidine *etc.*[7]. Most of the carcinogens of CS are polycyclic aromatic hydrocarbons. Structures of some these is shown in **Figure 6.** Cigarette smoking has been associated with increased risk of acute myelocytic leukemia, lung cancer, cancer of reproductive organs, throat cancer *etc*. [16].

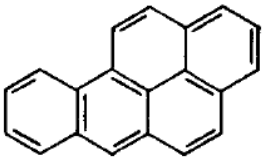
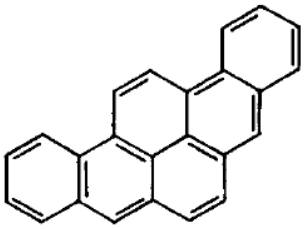
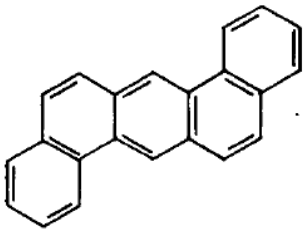
| Compound | Structure | Carcino-genicity | Amount reported, μg/1000 cigarettes |
|---|---|---|---|
| 1. Benzo(a)pyrene |  | ++++ | 16 (ave. of 10 reports) |
| 2. Dibenzo(a,i)pyrene |  | ++++ | 0.02-10 (2 reports) |
| 3. Dibenzo(a,h)anthracene |  | ++ | 4 (1 report) |

**Figure 6**[***] **:** Polycyclic aromatic hydrocarbons found in cigarettes and the carcinogenicity associated with each. Some of the most potent carcinogens are present in high amounts.

**7. Passive smoking and environmental issues**

A major concern associated with smoking is the effect CS has on non-smokers. People

*** http://www.cdc.gov/tobacco/sgr/sgr_1964/1964%20SGR%20Chapter%206.pdf (Acessed_03-12-05)

who are exposed to CS due to their association with smokers but do not smoke themselves are called passive smokers. Since the smoke inhaled by passive smokers does not pass through a filter, its effects are more direct and dangerous. It has been reported that non-smokers married to smokers have a 20-50% higher risk of lung cancer than non-smokers married to non-smokers [17]. The breath of smokers and smoke emitted by the cigarette itself, contribute to high levels of nicotine in environmental tobacco smoke (ETS). Thus, a person maybe at a risk of getting lung cancer if the ETS in the area is high even if they have no direct association with a smoker [18].

**8. Summary**

Cigarette smoke and tar contain various long-lived and short-lived radicals. These cause damage to humans at molecular level (*e.g.* oxidative stress, DNA damage) and at physiologic level (*e.g.* coronary heart diseases and carcinogenesis). Thus, cigarette smoking is indeed injurious to health. However, the only possible advantage of this potentially suicidal addiction is the protection that CS offers from Parkinsons disease (PD). There are reports which suggest that both men and women smokers show less incidence of PD [19]. However, the deleterious effects of cigarette smoking clearly outnumber its advantages to human health.

**9. References**

[1] Church DF, Pryor WA. (1991) The lung scientific foundation Chapter 7.6.3 Raven Press Ltd. NY.

[2] Forni LG, Mora-Arellano VO, Packer JE, Wilson RL. (1986) Nitrogen dioxide and related free radicals: Electron-transfer reactions with organic compounds in solution containing nitrite or nitrate. *J Chem Soc Perkin Trans.* **2:** 1-6.

[3] Zhang D, Tao Y, Gao J, Zhang C, Wan S, Chen Y, Huang X, Sun X, Duan S, Schonlau F, Rhodewold P, Zhao B. (2002) Pycnogenol® in cigarette filters scavenges free radicals and reduces mutagenicity and toxicity of tobacco smoke *in vivo*. *Toxicology and Industrial Health* **18:** 215-224.

[4] Pryor WA**.** (1997) Cigarette smoke radicals and the role of free radicals in chemical carcinogenicity. *Environ Health Perspect*. **4:** 875-82.

[5] Kalra J, Chaudhary AK, Prasad K. (1991) Increased production of oxygen free radicals in cigarette smokers. *Int J Exp Path*. **72:** 1-7.

[6] Radavanovic BS, Misic Z. (1999) Gas chromatographic analysis of some toxic organic compounds in mainstream cigarette smoke. *Working and living environmental protection.* **1:** 59-65.

[7] Arnap J, Bielawski J, Dahlin BM, Dahlman O, Enzell CR, Petersson T. (1989) Tobacco smoke chemistry 2. Alkyl and akenyl substituted guaiacols found in cigarette smoke condensate. *Acta Chem Scand*. **43:** 44-50.

[8] Arnap J, Enzell CR, Petersson K, Petersson T. (1986) Tobacco smoke chemistry 1. A chemical and mass spectrometric study of tobacco smoke alkyl 2-hydroxy-2-cyclopentenones. *Acta Chem Scand*. **40:** 839-854.

[9] Adams JD, Kathleen J, Adams MO, Hoffman D. (1987) Toxic and carcinogenic agents in undiluted mainstream smoke and sidestream smoke of different types of cigarettes. *Carcinogenesis*. **8:** 729-731.

[10] Muscat JE, Kleinman W, Colosimo S, Muir A, Lazarus P, Park J, Richie JP Jr. (2004) Enhanced protein glutathiolation and oxidative stress in cigarette smokers. *Free Radic Biol Med.* **36:** 464-470.

[11] Leanderson P, Tagesson C. (1989) Cigarette smoke potentiates the DNA–damaging effects of manmade mineral fibers. *Am J Indus Med.* **16:** 697-706.

[12] Bermudez E, Stone K, Carter KM, Pyror WA. (1994) Environmental tobacco smoke is just as damaging to DNA as mainstream smoke. *Environ Health Prospect.* **102**: 870-874.

[13] Lee CK, Brown BG, Rice WY, Doolittle DJ. (1989) Role of oxygen free radicals in the induction of sister chromatid exchanges by cigarette smoke. *Environmental and molecular mutagenesis.* **13:** 54-59.

[14] Anazawa T, Dimayuga PC, Li H, Tani S, Bradfield J, Chyu KY, Kaul S, Shah PK, Cercek B. (2004) *Arterioscler Thromb Vasc Biol*. **24:** 1652-1658**.**

[15] Dayton CS, Wilson JS. (1999) Smoking cessation. *P and T news.* **19:** 41-48.

[16] Severson RK. (1987) Cigarette smoking and leukemia. *Cancer.* **60:** 141-144.

[17] Lee PN. (1987) Passive smoking and lung cancer association: A result of bias? *Human Toxicol.* **6:** 517-524.

[18] Saracci R, Riboli E. (1989) Passive smoking and lung cancer: current evidence and ongoing studies at the International Agency for Research on Cancer. *Mutation Research.* **222:** 117-127.

[19] Mazio EA, Kolta MG, Reams R, Soliman KFA. (2005) Inhibitory effects of cigarette smoke on glial inducible nitric oxide synthase and lack of protective properties against oxidative neurotoxins *In Vitro. Neuro toxicol*. **26**: 49-62.