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ScienceDirect
Toxicology 236 (2007) 103–113
TOXICOLOGY
www.elsevier.com/locate/toxicol

Particle size-dependent radical generation from wildland fire smoke

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Received 29 September 2006; received in revised form 9 April 2007; accepted 10 April 2007
Available online 24 April 2007

Abstract

Firefighting, along with construction, mining and agriculture, ranks among the most dangerous occupations. In addition, the work environment of firefighters is unlike that of any other occupation, not only because of the obvious physical hazards but also due to the respiratory and systemic health hazards of smoke inhalation resulting from combustion. A significant amount of research has been devoted to studying municipal firefighters; however, these studies may not be useful in wildland firefighter exposures, because the two work environments are so different. Not only are wildland firefighters exposed to different combustion products, but their exposure profiles are different.

The combustion products wildland firefighters are exposed to can vary greatly in characteristics due to the type and amount of material being burned, soil conditions, temperature and exposure time. Smoke inhalation is one of the greatest concerns for firefighter health and it has been shown that the smoke consists of a large number of particles. These smoke particles contain intermediates of hydrogen, carbon and oxygen free radicals, which may pose a potential health risk.

Our investigation looked into the involvement of free radicals in smoke toxicity and the relationship between particle size and radical generation. Samples were collected in discrete aerodynamic particle sizes from a wildfire in Alaska, preserved and then shipped to our laboratory for analysis. Electron spin resonance was used to measure carbon-centered as well as hydroxyl radicals produced by a Fenton-like reaction with wildfire smoke. Further study of reactive oxygen species was conducted using analysis of cellular H_2O_2 generation, lipid peroxidation of cellular membranes and DNA damage. Results demonstrate that coarse size-range particles contained more carbon radicals per unit mass than the ultrafine particles; however, the ultrafine particles generated more $^{\bullet}OH$ radicals in the acellular Fenton-like reaction. The ultrafine particles also caused significant increases in H_2O_2 production by monocytes and lipid peroxidation. All particle sizes showed the ability to cause DNA damage. These results indicate that the radical generation and the damage caused by them is not only a function of surface area but is also influenced by changing chemical and other characteristics due to particle size.

Keywords: Free radicals; Firefighters; Particulate sampling; Smoke inhalation; Electron spin resonance

1. Introduction

Over 15,000 wildland firefighters are employed by the Federal government each year and exposed annually to

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doi:10.1016/j.tox.2007.04.008

extremely hazardous working conditions during fire suppression activities at wildland fires (Ward et al., 1989). Firefighting ranks among the most dangerous occupations, along with construction, mining and agriculture (Leigh, 1995; US Bureau of Labor Statistics, 2003). The work environment of firefighters is unlike that of any other occupation, not only because of the obvious physical hazards but also due to the respiratory and systemic health hazards from smoke inhalation resulting from combustion (Jankovic et al., 1991; Lees, 1995). Firefighters, both municipal (structural) and wildland, are known to have respiratory problems (Musk et al., 1982; Materna et al., 1992). Much research has been devoted to the deleterious effects of smoke exposure in municipal firefighters (Burgess et al., 2001; Chia et al., 1990; Scannell and Balmes, 1995; Bergstrom et al., 1997). Those results, however, may not be generalizable to wildland (grass, brush and forest) firefighters, given the difference in smoke composition, the longer duration of exposures for wildland firefighters and the fact that municipal firefighters routinely wear respiratory protection while wildland firefighters do not. Due to these differences the exposure levels of municipal and wildland firefighters to airborne contaminants can be expected to differ. Wildland firefighters are not likely to experience the extreme acute exposures that structural firefighters may encounter while working in confined spaces. However, they may spend several days or weeks working in smoke for shifts of 12 h or longer. Use of a self-contained breathing apparatus is not feasible, and respiratory protection consists primarily of bandannas tied over the nose and mouth (Sutton et al., 1990).

Previous studies of wildland firefighters showed cross-season changes in prevalence of one or more respiratory symptom(s) (Rothman et al., 1991; Liu et al., 1992; Betchley et al., 1997). Respiratory problems are estimated to affect 5–10% of wildland firefighters. Studies of forest firefighters have shown both short- and long-term effects on their pulmonary functions (Rothman et al., 1991; Serra et al., 1996). Another study (Jalava et al., 2006) found major differences between the coarse and ultrafine particles in proinflammatory cytokines production and apoptosis in RAW 264.7 cells. However, differences in MTT tests and NO production were relatively small. The study suggests that the aerosol smoke particles, which had undergone long-range transport, had chemically transformed during aging.

Smoke is the most obvious product of combustion and by far the most complex. While smoke can be viewed simplistically as partially combusted carbonaceous material, it is really a complicated array of organic and inorganic compounds originating from the combusted material, decomposed from the original material or formed from the combustion process (Large et al., 1990). Combustion chemistry predicts that the majority of the reactive intermediates generated in fires will be free radicals (Bizovi and Leikin, 1995; Lowry et al., 1985; MacFarland, 1968; Pryor et al., 1990). The chemical intermediates generated are usually in the form of hydrogen, carbon and oxygen free radicals (Tuve, 1976). These free radicals have been suggested to be involved in mechanisms involved in bronchopulmonary carcinogenesis, fibrogenesis, inflammation and toxicity (Shi et al., 1994; Leonard et al., 2004a,b). Previous investigations have identified wood smoke as a source of free radicals (Yamaguchi et al., 1992; Lowry et al., 1985; Leonard et al., 2000). Free radical mechanisms have been implicated in general toxicity, inflammation, fibrogenesis, bronchopulmonary carcinogenesis and atherosclerotic plaque formation (Vallyathan et al., 1995). It has been demonstrated that smoke generated in wood fires also generates carbon-centered free radical species. Carbon-centered free radicals have been shown to initiate reactions in the bronchopulmonary tree, where inhaled smoke particles deposit (Lachoki et al., 1989).

Investigations have suggested that oxygen-derived free radicals and their metabolites, reactive oxygen species (ROS), are important mediators of pulmonary injury, including asthma (Jarjour and Calhoun, 1994), adult respiratory distress syndrome (Gonzalez et al., 1996; McCord et al., 1994), pulmonary fibrosis and inflammation caused by exposure to asbestos (Kamp et al., 1992; Goodglick and Kane, 1986) or silica (Tuomala et al., 1992; Vallyathan et al., 1995). Earlier studies (Kimura et al., 1988; Traber et al., 1989; Brown et al., 1988) have suggested a role of free radicals in smoke inhalation injury but have not identified the types of free radicals involved or the biological mechanism of action. Wildfire smoke has been associated with chronic obstructive pulmonary disease (COPD) and bronchial asthma (Jalava et al., 2006; Mott et al., 2005).

In March 2004, a pilot study was initiated by NIOSH to assess the feasibility of collecting medical and environmental exposure data preseason, in a wildfire setting and post-season. The National Interagency Fire Center arranged for the National Park Service Alpine Interagency Hotshot Crew (IHC) of the Rocky Mountain National Park ($n = 20$ total firefighters) to participate in the pilot study. In the summer of 2004, NIOSH staff accompanied the Alpine IHC to the Boundary Fire in Fox, AK. The Boundary Fire was the fourth largest

fire of 2004 burning a total of 537,098 acres from 6/20/04 to 9/3/04 and we used this fire as a representative sampling of the possible types of smoke wildland firefighters can be exposed to and conditions they would deal with.

We hypothesize that free radicals are associated with wildfire smoke and that particle size plays a role in the type of radicals generated. Furthermore, these radicals are related to hydrogen peroxide generation, and have the ability to cause lipid peroxidation and DNA strand breaks. Our hypothesis will be tested by performing experiments related to the following specific aims: (1) determine and quantify particle-induced ROS associated with particle size using a cell-free model. (2) Define the mechanism and types of radicals involved in the process. (3) Determine if cellular exposure to wildland fire can cause activation of redox systems. (4) Determine if wildland fire smokes samples have the ability to cause DNA strand breaks associated with ROS. The major goals of the present study are focused on answering these questions.

2. Materials and methods

2.1. Reagents

Chelex 100 chelating resin was purchased from Bio-Rad Laboratories (Richmond, CA, USA). Phosphate-buffered saline (PBS) (KH_2PO_4 (1.06 mM), Na_2HPO_4 (5.6 mM), NaCl (154 mM), pH 7.4), was purchased from Biowhittaker Inc. (Walkersville, MD, USA). The PBS was treated with chelex 100 to remove transition metal ion contaminants. Dulbecco's modified Eagles medium (DMEM), 5,5-dimethyl-1-pyroline-oxide (DMPO), fetal bovine serum (FBS), $FeSO_4$, H_2O_2 and penicillin/streptomycin were purchased from Sigma Chemical Company (St. Louis, MO, USA). The spin trap, DMPO, was purified by charcoal decolorization and vacuum distillation and was free of ESR detectable impurities. Quartz sample tubes were purchased from Wilmad Glass (Buena, NJ, USA). DNA fragments, λ Hind III, were purchased from Invitrogen (Carlsbad, CA, USA). Vistra green nucleic acid stain was purchased from Amersham Biosciences (Piscataway, NJ, USA).

2.2. Cell culture

RAW 264.7 mouse peritoneal monocytes were purchased from American type culture collection (Rockville, MD). RAW 264.7 cells are commonly used and have been found to respond to particle exposure in a manner similar to primary alveolar macrophages (Hiura et al., 1999; Jalava et al., 2006; Leonard et al., 2000, 2004a,b; Li et al., 2003; Zhang et al., 2005). RAW 264.7 cells were cultured in DMEM with 10% FBS, 2 mM L-glutamine and 50 mg/ml pen/strep at 37 °C in a 5% CO_2 incubator. Cells were split after confluence approximately every 3 days.

2.3. Smoke sampling

Six sets of aerodynamically size-selected aerosol samples were collected with the Micro-Orifice Uniform Deposit Impactor (MOUDI) model# 110 with rotator (MSP, Inc., Minneapolis, MN, USA) at wildfire mop-up and back-burn operations over the course of 5 days. These operations are defined as—mop-up: to make a fire safe or reduce residual smoke after the fire has been controlled by extinguishing or removing burning material along or near the control line and back-burn: a fire set along the inner edge of a fire line to consume the fuel in the path of a wildfire and/or change the direction of force of the fire's convection column. The MOUDI is an 11-stage research-grade cascade impactor (including a final filter to collect particles <0.056 μm). Filters used were from Millipore Corp. (Billerica, MA, USA) 47 mm, 0.8 μm, PVC model PVC0847600. PVC was selected because it was previously demonstrated to have no effect in the analysis. The MOUDI substrates are normally coated with grease to ensure adherence of deposited particles and to avoid bounce of large particles to lower stages of the impactor. However, grease can alter the surface of collected aerosol particles and is not suitable for use in collecting samples for free radical analysis. Therefore, the cascade impactor was operated without grease substrates to collect and fractionate the smoke aerosol. This approach was based on the assumption that the tarry nature of smoke particles would negate major concerns for particle bounce or entrainment. The MOUDI samplers were placed as close to the fire as was permitted by the hotshot crews in regard to safety of the firefighters and the NIOSH personnel taking the samples. The samplers were supported on a tripod of steel legs in order to place the sampling inlet approximately 5 ft off the ground to simulate breathing space of a firefighter. The average sampling time was 3.5 h. The long sampling time was required in order to obtain sufficient mass within the time period of firefighter exposure. Particle bounce, particularly in the larger sizes, may have been an issue due to the long sampling time. The mass concentration of particulates ranged from 0.75 to 1.3 mg/m^3. Filters were immediately placed on dry ice and shipped to NIOSH for temporary storage at −80 °C until free radical analysis was performed.

Filter suspensions, which were used for H_2O_2, lipid peroxidation and DNA damage analysis, were prepared by splitting the filters into three size groups. For this study, the size groups were defined as ultrafine (0.042–0.24 μm), fine (0.42–2.4 μm) and coarse (4.2–24 μm) particles. The groups consisted of four filters each of which were placed in PBS and blended on ice into fine slurry using a Tissue Tearor (Biospec Products Inc., Racine, WI). Pre- and post-filter weights were used to calculate a final concentration of 1 mg/ml smoke suspension. The slurry was centrifuged, and the smoke suspension was decanted from the filter pellet. A clean control suspension was also prepared at a ratio of 4 filters/1 ml PBS.

2.4. Free radical measurements

ESR spin trapping was used to detect both carbon radicals and short-lived free radical intermediates. Carbon radicals were measured directly by placing the filter into a 5 mm quartz sample tube and placing it in the ESR cavity. Hydroxyl radicals were measured using the addition-type reaction of a short-lived radical with a paramagnetic compound (spin trap) to form a relatively long-lived free radical product (spin adduct), which can then be studied using conventional ESR. For hydroxyl radical measurements, reactants were mixed in test tubes at a final volume of 1.0 ml of PBS in the presence of 1 mM H_2O_2. The reaction mixture was then transferred to a flat cell for ESR measurement. Experiments were performed at room temperature and under ambient air.

The concentrations given in the figure legends are final concentrations. The intensity of the signal is used to measure the amount of short-lived radicals trapped, and the hyperfine couplings of the spin adduct are characteristic of the original trapped radicals. Spin trapping is the method of choice for detection and identification of free radical generation due to its specificity and sensitivity. All ESR measurements were conducted using a Bruker EMX spectrometer (Bruker Instruments Inc., Billerica, MA 01821, USA) and a flat cell assembly. Hyperfine couplings were measured (to 0.1 G) directly from magnetic field separation using potassium tetraperoxochromate (K_3CrO_8) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) as reference standards (Janzen and Blackburn, 1968; Buettner, 1987). The relative radical concentration was estimated by multiplying half of the peak height by $(\Delta H_{pp})^2$, where ΔH_{pp} represents peak-to-peak width. The Acquisit program was used for data acquisitions and analyses (Bruker Instruments Inc., Billerica).

2.5. Scanning electron microscopy

For scanning electron microscopy (SEM), the filters were trimmed into pie-shaped sections and mounted onto aluminum stubs with double-stick carbon tape. The samples were then coated with gold/palladium and viewed on a JEOL 6400 scanning electron microscope (Tokyo, Japan) at 20 kV. Elemental analysis of particles was done using a Princeton Gamma-Tech IMIX system (Princeton, NJ, USA).

2.6. H_2O_2 measurements

H_2O_2 production was monitored using a Bioxytech quantitative hydrogen peroxide assay kit (H_2O_2-560, Oxis International Inc., Portland, OR, USA). Measurements were made on a system containing 5×10^6 RAW 264.7 mouse peritoneal monocytes per millilitre in pH 7.4 PBS and exposing them to the size groupings (refer to filter methods) of wildfire smoke solution. Cells were exposed to wildland fire smoke solution [100 μg/ml] for 30 min in a 37 °C incubator. Absorbance was monitored at a wavelength of 560 nm using a Spectra Max 250 multi-well plate reader (Molecular Devices, Sunnyvale, CA, USA).

2.7. Lipid peroxidation

Lipid peroxidation of RAW 264.7 mouse peritoneal monocytes was measured by using a colormetric assay for malondialdehyde (LPO-586 Oxis International Inc., Portland). A reaction mixture contained various size groups (refer to filter methods) of wildfire smoke samples [100 μg/ml], H_2O_2 (1 mM) and 1×10^7 cells in a total volume of 1.0 ml PBS (pH 7.4). A Fenton reaction, $FeSO_4$ (1 mM), H_2O_2 (1 mM) and 1×10^7 cells, was also carried out as a positive control. The mixtures were exposed for 1 h in a shaking water bath at 37 °C. The measurement of lipid peroxidation is based on the reaction of a chromogenic reagent with malonaldehyde at 45 °C (Brambilla et al., 1989). The absorbance of the supernate was measured at 586 nm.

2.8. DNA damage

The DNA strand break assay was carried out according to methods described earlier (Daniel et al., 1993). Briefly, reactions were performed in phosphate-buffered saline (pH 7.4) in 1.5 ml polypropylene tubes at 37 °C. A positive control reaction between $FeSO_4$ and H_2O_2 was used to generate hydroxyl radicals. A reaction mixture contained various size groups (refer to filter methods) of wildfire smoke samples [100 μg/ml], 10 μg DNA (λ Hind III fragments) and H_2O_2 (1 mM). This solution was allowed to react for 30 min at 37 °C in a shaking water bath. To this solution, 2 μl of gel loading buffer (50 mM EDTA, 2.5% sodium dodecyl sulfate (SDS), 0.1% bromophenol blue) was added, and then electrophoresis was performed in 0.7% agarose at 1–2 V/cm in 40 mM tris acetate buffer containing 2 mM EDTA (pH 8.0). Gels were stained in vistra green nucleic acid stain (5 μl/ml) for 30 min and photographed under UV light using a Stratagene Eagle Eye II (Stratagene Inc., La Jolla, CA 92037, USA).

2.9. Statistics

Data were expressed as mean ± standard error of the mean (S.E.M.) ($n = 3$) for each group. One-way ANOVA test was performed using SigmaStat statistical software (Jandel Scientific, San Rafael, CA, USA) to compare the responses between treatments. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Particle size distribution

Table 1 shows the mass-based distribution of particle sizes collected by the 12 MOUDI samplers. The wildland fire smoke shows a bell-shaped distribution with the highest %masses, 14.5, 11.3 and 13 being found on filters 4, 5 and 6, respectively. These sizes range from a mean of 4.2–1.34 μm. The ultrafine (filters 9, 10, 11 and 12) particles have the lowest overall mass, making up 20.2%

Table 1
Particle mass-based size distribution

Filter number	Mean diameter (μm)	Mass concentration (% of total)
1	24	6.3
2	13.4	7
3	7.4	8.3
4	4.2	14.5
5	2.4	11.3
6	1.34	13
7	0.74	10
8	0.42	9.5
9	0.24	7.8
10	0.134	5.7
11	0.074	3.7
12	0.042	3

of the total mass collected, while the fine (filters 5, 6, 7 and 8) particles have the highest overall mass (43.8%) of the overall mass collected. Our toxicological studies showed that the ultrafine particle generated the highest amount of ROS associated damage in assays preformed. The complete %distribution can be seen in Table 1.

3.2. Stable free radicals

Fig. 1 shows a typical ESR spectrum recorded from a wildland smoke filter sample. The spectrum shape and magnetic field location were assigned to carbon-centered radicals ($R^{\bullet}$). Carbon-centered radials are relatively stable free radicals which, depending on environmental conditions, can have a half-life of up to several days. The carbon radical spectra were not observed in the aqueous samples with DMPO added. Control PVC filters were tested and showed no radical formation. The spectra strength was found to vary with particle size and composition as reported in Figs. 3 and 4.

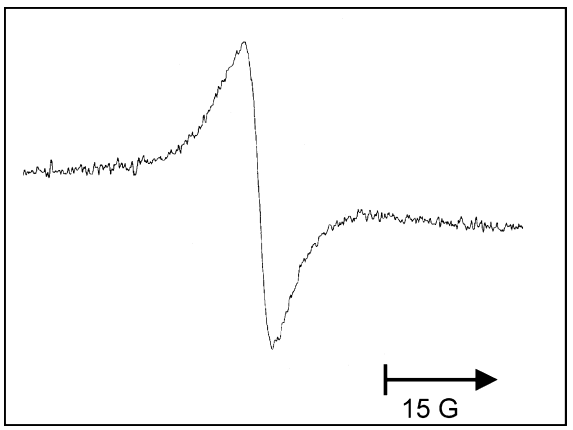

Fig. 1. A typical ESR spectrum of the carbon radical recorded from a dry filter measurement. No change in spectral intensity was observed over a 3-day period. ESR settings were center field, 3490 G; scan width, 100 G; time constant, 0.40 s; modulation amplitude, 1 G; receiver gain, 6.32×10^4; frequency, 9.828 GHz; power, 100 mW.

3.3. Radicals generated from H_2O_2 reaction

Fig. 2 shows an example of the spectra generated from wildland fire smoke exposed filters when treated with H_2O_2, to create a Fenton-like reaction, in the presence of the radical spin trap DMPO. Filters were treated in order to measure their potential to generate reactive radical species when inhaled. The spectrum shown in Fig. 2 demonstrates the 1:2:2:1 quartet splitting of $a_N = a_H = 14.9$ as indicated by the magnetic field legend. Using these splitting constants, this 1:2:2:1 quartet can be assigned to the DMPO/$^{\bullet}$OH adduct, showing $^{\bullet}$OH radical generation. To further verify the involvement of transition metals in the formation of the $^{\bullet}$OH radical, a chelator, deferoxamine, was added at various concentrations. Concentrations of 0.1, 0.5, 1.0 and 2.0 mM deferoxamine showed decreasing $^{\bullet}$OH peaks as the concentration of the chelator increased (data not shown).

3.4. Radical characteristics

Fig. 3 displays the relationship between radical peak height per unit milligram and aerodynamic diameter of the particles. The results show that the carbon radical was stronger in the larger particles per unit mass, while the $^{\bullet}$OH radical was stronger per unit mass in the smaller particles. This could indicate that the larger particles

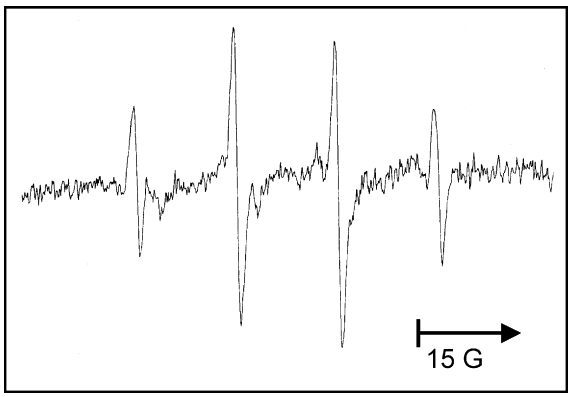

Fig. 2. The generation of short-lived $^{\bullet}$OH radicals by wildland smoke upon reaction with H_2O_2. ESR spectrum recorded 3 min after reaction was initiated in PBS (pH 7.4) containing H_2O_2 [1 mM] and DMPO [100 mM] vortexed for 1 min with the filter. ESR settings were center field, 3490 G; scan width, 100 G; time constant, 0.40 s; modulation amplitude, 1 G; receiver gain, 6.32×10^4; frequency, 9.828 GHz; power, 100 mW.

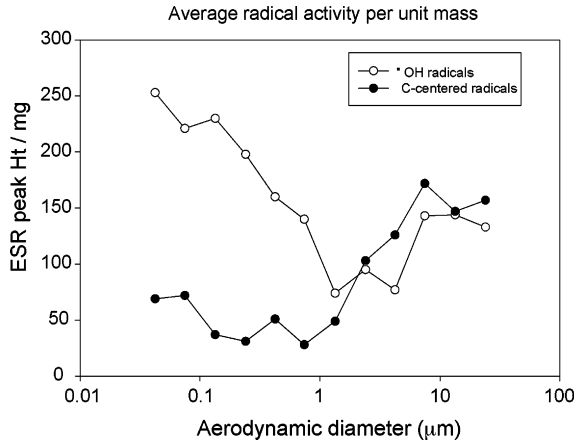

Fig. 3. Free radical activity per unit particle mass. Radical strength of •OH was greatest per unit mass in the smaller particle sizes, while carbon radicals were stronger per unit mass in the coarser particles.

were produced at a lower temperature and generated particles with less complete combustion in which the carbon radicals stayed intact. The smaller particles may have been produced at higher temperatures and lower oxygen states, which may have dissipated the carbon radical by forming cross-links and produced more potentially reactive hydroxyl radical precursors (Halliwell and Gutteridge, 2000a).

Fig. 4 demonstrates the relationship between radical peak height per unit particle surface area (μm^2) and median aerodynamic diameter of each particle size fraction. The surface area relationship was based on the assumption that all particles were spherical in shape with unit density. The results indicate that the ESR peaks are surface area-dependent for the ultrafine and fine sizes,

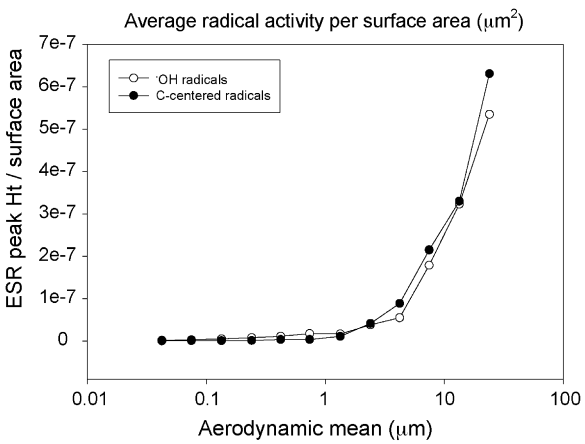

Fig. 4. Free radical activity per unit particle surface area. Both •OH and carbon radicals showed good surface area correlation for ultrafine and fine sizes however, underestimation of true particle surface area or particle bounce may have influenced the coarse particle results.

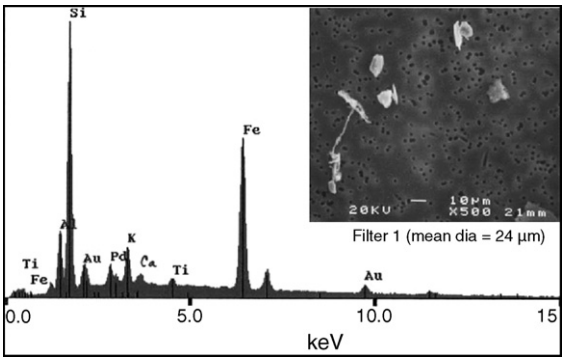

Fig. 5. Elemental particle profile and electron micrograph of particles on first filter stage (median particle diameter 24 μm). Particle profile shows high amounts of Si and Fe, which could be found in soil residue taken up in the burning process.

i.e., over these particle sizes, equivalent surface area produced equivalent radicals. The coarse particles departure from this correlation may be partly due to an underestimate of the coarse particle surface area (i.e., true particle shape may provide substantially larger surface area than calculated from the assumption of spherical particle shape), or party due to particle bounce of large particles, an artifact of the required long sampling time.

3.5. Electron microscopy

Figs. 5 and 6 show representative samples from two of the filter sizes. Fig. 5 shows the first, and largest particle size, filter stage (24 μm) and a typical particle elemental profile and electron micrograph. The elemental profile shows high amounts of both silica (Si) and

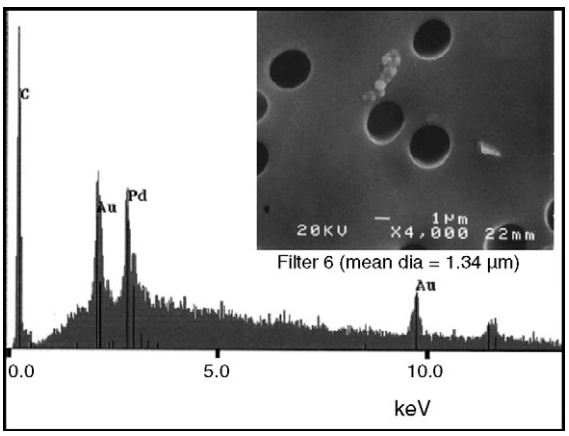

Fig. 6. Elemental particle profile and electron micrograph of particles on sixth filter stage (median particle diameter 1.34 μm). Smaller particles show a different profile than the larger fraction containing carbon, which could be the result of combustion. The clumping of the smaller particles can also be observed.

iron (Fe), these reactive elements may have been part of the combusted wood fiber or taken up from soil during the burning process. Both Si and Fe have been shown to be capable of reactive oxygen species generation. Si acts through the splitting of Si–O bonds generating •Si and Si–O• radicals on the fracture surfaces (Castranova et al., 1995). Fe generates ROS through its transition metal characteristics and the Fenton reaction with H_2O_2 (Halliwell and Gutteridge, 2000b). Fig. 6 shows filter stage (1.34 μm); the elemental profile for these smaller particles shows no measurable Si or Fe. It can also be noted that while the particles are small, they show clumping which may further affect their surface area related characteristics.

3.6. H_2O_2

It has been shown in the previous section that H_2O_2 was involved in the •OH radical generation from wildfire smoke particles via Fenton-like reaction. H_2O_2 generation was measured from RAW 264.7 cells exposed to wildfire smoke suspended in PBS. As shown in Fig. 7, this mixture generated H_2O_2 as determined by an assay, which measures the oxidation of ferrous ions (Fe^{2+}) to ferric ions (Fe^{3+}) by hydrogen peroxide under acidic conditions. Exposure of cells to wildfire smoke resulted in a significant increase in H_2O_2 production in the ultrafine (0.042–0.24 μm) and the fine (0.42–2.4 μm) sizes groups, demonstrating activation of ROS systems within the cells. However, the coarse (4.2–24 μm) size group was not significantly different from the control in cellular H_2O_2 production. Therefore, on an equivalent mass basis, small particles were more potent stimulants of H_2O_2 production by monocytes than large particles.

3.7. Lipid peroxidation

Fig. 8 shows the generation of malondialdehyde (MDA) after exposure of RAW 264.7 cells to wildfire smoke suspensions. The results show that the ultrafine (0.042–0.24 μm) and the fine (0.42–2.4 μm) sized groups induced a significant increase in MDA, from lipid peroxidation, compared to the clean filter groups. The coarse (4.2–24 μm) sized group showed a small increase in lipid peroxidation over the clean filter group, which was not significant.

3.8. DNA damage

Fig. 9 shows λ Hind III digested DNA after exposure to wildfire smoke suspensions and H_2O_2. Lane 1 shows DNA alone with sharp, undamaged DNA bands. Lane 2 contains DNA + H_2O_2 [1 mM] + clean filter suspension, which also shows undamaged focused bands. However, in lane 3, which contains DNA + H_2O_2 [1 mM] + ultrafine (0.042–0.24 μm) wildfire filter suspension, DNA damage is apparent in the form of smeared bands. The band smearing is caused by DNA being cut into randomly sized pieces and blurring the gel when stained instead of staying in sharp focused bands. DNA damage is also seen in lane 4 which contains

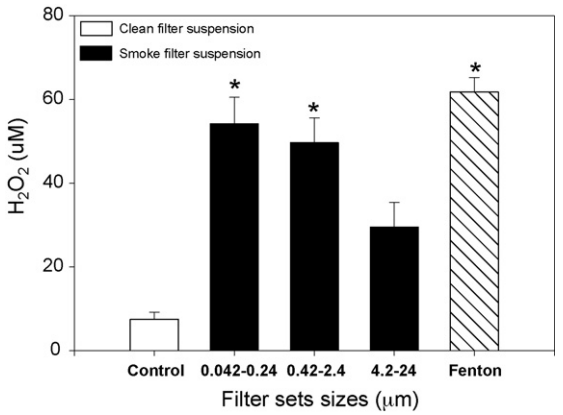

Fig. 7. H_2O_2 production in 5×10^6/ml RAW 264.7 cells stimulated by wildland fire smoke. Cells were incubated with wildfire smoke [100 μg/ml], for 30 min in a 37 °C incubator. H_2O_2 production in incubation mixtures of filter size groups, both control and smoke exposed and a Fenton reaction positive control were measured. Data presented are means of ±S.D. for four sets of experiments. Asterisks (*) indicate a significant increase in H_2O_2 production compared to control ($P < 0.05$).

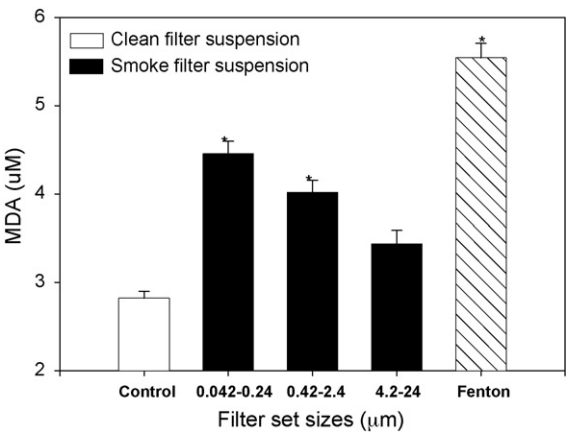

Fig. 8. Wildland fire smoke-induced lipid peroxidation. Exposure mixture contained 100 μl wildland smoke sample [100 μg/ml], and 5×10^7 RAW 264.7 cells. Fenton reaction-induced lipid peroxidation exposure mixture contained 1.0 mM $FeSO_4$, 0.1 mM H_2O_2 and 5×10^7 RAW 264.7 cells. Data presented are means of ±S.D. for four sets of experiments. Asterisks (*) indicate a significant increase in lipid peroxidation compared to control ($P < 0.05$).

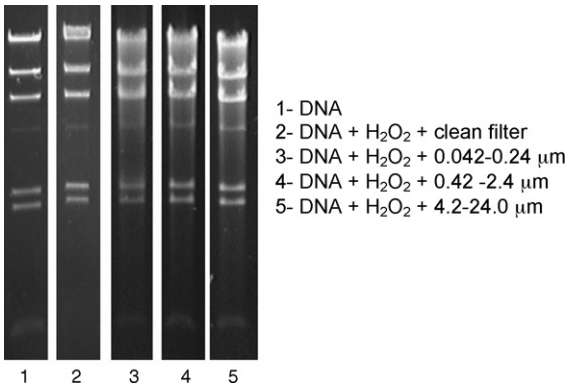

Fig. 9. DNA strand breaks induced by wildfire smoke. Lane 1, λ Hind III digested DNA alone; lane 2, DNA + H_2O_2 [1 mM] + clean control filter suspension; lane 3, DNA + H_2O_2 [1 mM] + 0.042–0.24 μm wildfire filter suspension [100 μg/ml]; lane 4, DNA + H_2O_2 [1 mM] + 0.42–2.4 μm wildfire filter suspension [100 μg/ml]; lane 5, DNA + H_2O_2 [1 mM] + 4.2–24.0 μm wildfire filter suspension [100 μg/ml].

DNA + H_2O_2 [1 mM] + fine (0.42–2.4 μm) filter suspension and in lane 5 which contains DNA + H_2O_2 [1 mM] + coarse (4.2–24.0 μm) filter suspension. The results suggest generation of radicals from the reaction of the wildfire smoke suspension with H_2O_2 and the resulting DNA damage. Sodium formate, an $^{\bullet}OH$ radical scavenger, and deferoxamine, a metal chelator, inhibited DNA damage caused by wildfire smoke plus H_2O_2, demonstrating that a transition metal reaction with the H_2O_2 was involved in the $^{\bullet}OH$ generated DNA damage (data not shown).

4. Discussion

The present study was undertaken to investigate possible radical generation in wildfire smoke particles and to determine if their effects were size and surface area-dependent. Our results determined that wildfire smoke contains both carbon radicals and precursors, which are able to react and generate hydroxyl radicals ($^{\bullet}OH$) from a Fenton-like reaction with H_2O_2 as well as ROS generation after exposure to cells. Electron spin resonance (ESR) analysis showed that carbon radicals were found in all filter samples; however, a higher concentration per milligram was found in the coarse (4.2–24 μm) particles leading us to believe that the larger particles were made up mostly of ash, not fully combusted wood particles, and some soil particles swept up during the process of burning. The decreasing amount of carbon radicals measured in the fine (0.42–2.4 μm) and the ultrafine (0.042–0.24 μm) sizes demonstrate that these groups were made up of particles further along in the pyrolysis process and due to oxygen depletion as well as temperature the carbon radicals had cross-linked to form covalent bonds (Halliwell and Gutteridge, 2000b). Carbon radicals are relatively stable and unlikely to do much damage in biological systems (Jankovic et al., 1993). ESR was also used to determine the $^{\bullet}OH$ generating potential of the particles. All wildfire filter sizes showed generation of $^{\bullet}OH$ upon reaction with H_2O_2. Hydroxyl radical generation was decreased when deferoxamine, a metal chelator, was added to the reaction, indicating that a transition metal or metals were at least partly responsible for the $^{\bullet}OH$ generation (Halliwell, 1989). The reaction with H_2O_2 was significant because inhaled particles are likely to undergo phagocytosis once deposited in the lung (Patierno and Landolph, 1989). In this process, alveolar macrophages and other cellular constituents generate H_2O_2 as part of the respiratory burst (Halliwell and Gutteridge, 2000c; Forman and Torres, 2002; Liu et al., 1997). The H_2O_2 generated in response to the inhaled particles can react with transition metals and other precursors present in the smoke, which by a Fenton-like reaction can generate $^{\bullet}OH$ (Halliwell and Gutteridge, 2000a).

Table 1 showed the distribution of particle mass-based size. This distribution shows that the equivalency of mass among the three groups: ultrafine (20.2%), fine (43.8%) and coarse (36.1%) was good and would be a good model as to the bioavailability in humans. The three basic size groups of the particles tested deposit into different regions of the lung. Coarse particles are mostly deposited in the nasal area with some penetrating into the conducting airways. Fine particles are deposited in all three regions, but the majority are found in alveolar region of the lung. Ultrafine particles penetrate into the conducting tracheo-bronchial zone, deep into the pulmonary zones and alveoli, where they can gain access to the pulmonary interstitium (Castranova et al., 1991; Pietropaoli et al., 2004; Chalupa et al., 2004).

Our results indicated that there were more ROS per milligram in the smaller particles. Specifically, ultrafine particles showed the highest production per unit milligram, which is of interest because these same particles are able to penetrate deep into the pulmonary system and into the alveolar region. This increase in radical production corresponds to the greater surface area exposed to allow reaction with the H_2O_2. In an organism, this same increase in surface area would allow greater interaction with macrophages and other defense systems, many of which would react with the particle contents and generate ROS. Measurement of radicals showed surface area dependence in the ultrafine and fine sizes; however, the coarse particles demonstrated a possible experimental

artifact that could be related either to the assumption that particle surface area was equivalent to that of round smooth spheres, or to particle bounce due to the long sampling time. By the same token (similar to the collection of coarse particles on the lower stage as a result of particle bounce), the dry tar-like fine particles could be collected on the higher stage of the impactor as well. The SEM micrographs indicate that depending on the humidity of the environment, tar-like fine particles could coagulate into aggregate particles while still in the airborne state. As a result, these aggregate particles would be collected on the higher stages of the impactor and considered as coarse particles. As a result, this phenomenon also promotes the higher ratio for the coarse particles as indicated in our results.

The results indicate that wildfire smoke is heterogeneous and complex. Particle surface area may be a critical factor in radical production characteristics of wildfire smoke.

On an equivalent mass basis, the ultrafine and fine sizes also showed significant increase in H_2O_2 generation from RAW cells, which indicate that these particles are more reactive, generate a stronger respiratory burst in cells, and therefore, may have more $^{\bullet}OH$ generation potential. The strong respiratory burst acts synergistically with the surface area and chemical composition of the particles to provide more reactive surfaces. The ultrafine and fine particles also showed a significant increase in lipid peroxidation in exposed RAW cells again indicating their potential for damage to cells and organisms. Lipid peroxidation results in a release of lipid-derived radicals ($R^{\bullet}$, $RO^{\bullet}$ and $ROO^{\bullet}$) (Shi et al., 1988) These radicals can have a cascade effect and lead to further reactions in the cell membrane and release catalytically active iron (Vladimirov, 1986), which will further increase the generation of ROS. Lipid peroxidation and its products have been found to cause DNA damage (Vaca et al., 1988) and may function as tumor initiators (Comporti, 1985).

The significant increase seen in H_2O_2 production and lipid peroxidation by exposure of cells to both ultrafine and fine wildfire smoke suspensions correlates well with the results measured from $^{\bullet}OH$ generation by these same particles.

The data further demonstrate that the $^{\bullet}OH$ radicals generated from the reaction between the wildfire smoke and H_2O_2 caused DNA strand breaks. All size groups were observed to cause DNA damage with no significant difference seen between them. It should be noted that $^{\bullet}OH$ radicals generated in the reaction of H_2O_2 with certain metal ions, such as nickel (Inoue and Kawanishi, 1989), copper or zinc containing SOD (Yim et al., 1990), exhibit very little reactivity. For example, the $^{\bullet}OH$ radicals produced by these systems cannot be scavenged by ethanol or formate. The reason for this non-reactivity is believed to be that $^{\bullet}OH$ radicals are generated within the domain of certain macromolecules, and hence are not "free" to exhibit significant reactivity (Inoue and Kawanishi, 1989; Yim et al., 1990). However, the results obtained from the present study show that $^{\bullet}OH$ radicals generated from materials found in wildfire smoke have the potential to cause DNA damage. Furthermore, H_2O_2, and $^{\bullet}OH$ may cause other cellular damage via mechanism typically associated with reactions initiated by reactive oxygen species, for example, dG hydroxylation and protein–DNA cross-links. They may also cause activation of nuclear transcription factors, such as NF-κB, over-expression of certain oncogenes and induction of p53 mutation (Chen et al., 2000; Shi et al., 1998).

In conclusion, the results of the present study demonstrate that wildfire smoke particles have different chemical characteristics over different size-ranges and can generate ROS, which are affected by both surface area and these different characteristics. The particles can cause cellular respiratory burst, increased generation of radical precursors, such as H_2O_2 and can also cause lipid peroxidation. These same radicals are also responsible for DNA damage. The most reactive particles were observed to be the ultrafine group, which can penetrate furthest into the lung of an exposed firefighter. Future studies involving the *in vivo* exposure of wildfire smoke are planned to further study the impact the free radical formation can have in lipid peroxidation and DNA damage. Therefore, our results suggest that wildfire smoke may cause acute lung injury. Since particle size and surface area of the smoke exposure are significant factors in radical generation and particle deposition, particle size should be considered when developing protective strategies.

Acknowledgements

We would like to thank the Alpine Interagency Hotshot crew, the National Interagency Fire Center and Steve Hart's Type 1 Incident Management Team for allowing us to collect data at the Boundary Fire, Fox, AK.

References

Betchley, C., Koenig, J.Q., van Belle, G., 1997. Pulmonary function and respiratory symptoms in forest firefighters. Am. J. Ind. Med. 31, 503–509.

Bizovi, K.E., Leikin, J.D., 1995. Smoke inhalation among firefighters. Occup. Med. 10 (4), 721–733.

Brambilla, G., Martelli, A., Marinari, U.M., 1989. Is lipid peroxidation associated with DNA damage? Mutat. Res. 214, 123–127.

Brown, M., Desai, M., Traber, L.D., Herndon, D.N., Traber, D.L., 1988. Dimethylsulfoxide with heprin in the treatment of smoke inhalation injury. J. Burn Care Rehabil. 9, 22–25.

Buettner, G.R., 1987. ESR parameters of spin adducts. Free Radic. Biol. Med. 3, 259–303.

Bergstrom, C.E., Eklund, A., Skold, M., Tornling, G., 1997. Bronchoalveolar lavage findings in firefighters. Am. J. Ind. Med. 32, 332–336.

Burgess, J.L., Nanson, C.J., Bolstad-Johnson, D.M., Gerkin, R.D., Hysong, T.A., Lantz, R.C., Sherrill, D.L., Crutchfield, C.D., Quan, S.F., Bernard, A.M., Witten, M.L., 2001. Adverse respiratory effects following overhaul in firefighters. J. Occup. Environ. Med. 43, 467–473.

Castranova, V., Dalal, N.S., Vallyathan, V., 1995. Role of surface free radicals in the pathogenicity of silica. In: Castranova, V., Vallyathan, V., Wallace, W.E. (Eds.), Silica and Silica-induced Lung Diseases. CRC Press, New York, pp. 91–94.

Castranova, V., Kang, J.H., Moore, M.D., Pailes, W.H., Frazer, D.G., Schwegler-Berry, D., 1991. Inhibition of stimulant-induced activation of phagocytic cells with tetrandrine. J. Leukoc. Biol. 50 (4), 412–422.

Chalupa, D.C., Morrow, P.E., Oberdörster, G., Utell, M.J., Frampton, M.W., 2004. Ultrafine particle deposition in subjects with asthma. Environ. Health Perspect. 112 (8), 879–882.

Chen, F., Ding, M., Lu, Y., Leonard, S.S., Vallyathan, V., Castranova, V., Shi, X., 2000. Participation of MAP kinase p38 and IkB kinase in chromium(VI)-induced NF-κB and AP-1 activation. J. Environ. Pathol. Toxicol. Oncol. 19, 231–238.

Chia, K.S., Jeyaratnam, J., Chan, T.B., Lim, T.K., 1990. Airway responsivness of firefighters after smoke exposure. Br. J. Ind. Med. 47 (8), 524–527.

Comporti, M., 1985. Biology of disease: lipid peroxidation and cellular damage in toxic liver injury. Lab. Invest. 53, 599–623.

Daniel, L.N., Mao, Y., Saffiotti, U., 1993. Oxidative DNA damage by crystalline silica. Free Rad. Biol. Med. 14, 463–472.

Forman, H.J., Torres, M., 2002. Reactive oxygen species and cell signaling: respiratory burst in macrophage signaling. Am. J. Res. Crit. Care Med. 166, S4–S8.

Gonzalez, P.K., Zhuang, J., Doctrow, S.R., Malfroy, B., Benson, P.F., Menconi, N.J., 1996. Role of oxidant stress in the adult respiratory distress syndrome. Shock 6 (Suppl. 1), S23–S26.

Goodglick, L.A., Kane, A.B., 1986. Role of reactive oxygen metabolites in crocidolite asbestos toxicity mouse macrophages. Cancer Res. 46, 5558–5566.

Halliwell, B., 1989. Protection against tissue damage in vivo by desferrioxamine: what is its mechanism of action? Free Radic. Biol. Med. 7, 645–651.

Halliwell, B., Gutteridge, J.M.C., 2000a. The chemistry of free radicals and related 'reactive species'. In: Free Radicals in Biology and Medicine. Oxford Science Publications, New York, pp. 38–39.

Halliwell, B., Gutteridge, J.M.C., 2000b. The chemistry of free radicals and related 'reactive species'. In: Free Radicals in Biology and Medicine. Oxford Science Publications, New York, pp. 52–54.

Halliwell, B., Gutteridge, J.M.C., 2000c. Reactive species as useful biomolecules. In: Free Radicals in Biology and Medicine. Oxford Science Publications, New York, pp. 448–467.

Hiura, T.S., Kaszubowski, M.P., Li, N., Nel, A.E., 1999. Chemicals in diesel exhaust particles generate reactive oxygen radicals and induce apoptosis in macrophages. J. Immunol. 163, 5582–5591.

Inoue, S., Kawanishi, S., 1989. ESR evidence for superoxide, hydroxyl and singlet oxygen produced from hydrogen peroxide and nickel(II) complex of glycylglycyl-L-histidine. Biochem. Biophys. Res. Commun. 159, 445–451.

Jalava, P.I., Raimo, O., Halinen, A.I., Penttinen, P., Pennanen, A.S., Sillanpaa, M., Sandell, E., Hillamo, R., Hirvonen, M.R., 2006. In vitro inflammation and cytotoxic effects of size-segregated particulate samples collected during long-range transport of wildfire smoke to Helsinki. Toxicol. Appl. Pharmacol. 215, 341–353.

Janzen, E.G., Blackburn, B.J., 1968. Detection and identification of short-lived free radicals by an electron spin resonance trapping technique. J. Am. Chem. Soc. 90, 5909–5910.

Jankovic, J., Jones, W., Burkhart, J., 1991. Environmental study of firefighters. Ann. Occup. Hyg. 35, 581–602.

Jankovic, J., Jones, W., Castranova, V., Dalal, N., 1993. Measurement of short-lived reactive species and long-lived free radicals in air samples from structural fires. Appl. Occup. Environ. Hyg. 8 (7), 650–654.

Jarjour, N.N., Calhoun, W.J., 1994. Enhanced production of oxygen radicals in asthma. J. Lab. Clin. Med. 123, 131–136.

Kamp, D.W., Graceffa, P., Pryor, W.A., Weitzman, S.A., 1992. The role of free radicals in asbestos-induced diseases. Free Radic. Biol. Med. 12, 293–315.

Kimura, R., Traber, L.D., Herndon, D.N., Neuhous, G.D., Traber, D.L., 1988. Treatment of smoke-induced pulmonary injury with nebulized dimethylsulfoxide. Circ. Shock 25, 333–341.

Lachoki, T.M., Nuggahalli, S.K., Church, D.E., Pryor, W.A., Scherer, K.V., 1989. The smoke produced from the oxidative pyrolysis of perfluoropolymers: an ESR spin-trapping study. Chem. Res. Toxicol. 2, 174–180.

Large, A.A., Owens, G.R., Hoffman, L.A., 1990. The short-term effect of smoke exposure on the pulmonary function of firefighters. Chest 97, 806–809.

Lees, P.S.J., 1995. Combustion products and other firefighter exposures. Occup. Med. 10 (4), 691–706.

Leigh, J.P., 1995. Causes of Death in the Workplace. Greenwood Publishing Group, Inc., Westport, CT.

Leonard, S.S., Wang, S., Shi, X., Jordan, B.S., Castranova, V., Dubick, M.A., 2000. Wood smoke particles generate free radicals and cause lipid peroxidation, DNA damage, NF-κB activation and TNF-α release in macrophages. Toxicology 150, 147–157.

Leonard, S.S., Roberts, J.R., Antonini, J.M., Castranova, V., Shi, X., 2004a. $PbCrO_4$ mediates cellular responses via reactive oxygen species. Mol. Cell. Biochem. 255 (1/2), 171–179.

Leonard, S.S., Bower, J.J., Shi, X., 2004b. Metal-induced toxicity, carcinogenesis, mechanisms and cellular responses. Mol. Cell. Biochem. 255 (1/2), 3–10.

Li, N., Sioutas, C., Cho, A., Schmitz, D., Misra, C., Sempf, J., Wang, M., Oberley, T., Froines, J., Nel, A., 2003. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. Environ. Health Perspect. 111 (4), 455–460.

Liu, L., Elwing, H., Karlsson, A., Nimeri, G., Dahlgren, C., 1997. Surface-related triggering of the neutrophil respiratory burst. Characterization of the response induced by IgG adsorbed to hydrophilic and hydrophobic glass surfaces. Clin. Exp. Immunol. 109, 204–210.

Liu, D., Tager, I.B., Balmes, J.R., 1992. The effect of smoke inhalation on lung function and airway responsiveness in wildland firefighters. Am. Rev. Respir. Dis. 146, 1469–1473.

Lowry, W.T., Peterson, J., Petty, C.S., Badgett, J.L., 1985. Free radical production from controlled low-energy fires: toxicity considerations. J. Forensic Sci. 1, 73–85.

MacFarland, H.N., 1968. The pyrolysis of plastics—problems in defining their toxicity. Am. Ind. Hyg. Assoc. J. 29, 7–9.

Materna, B.L., Jones, J.R., Sutton, P.M., Rothman, N., Harrison, R.J., 1992. Occupational exposures in California wildland fire fighting. Am. Ind. Hyg. Assoc. J. 53 (1), 69–76.

McCord, J.M., Gao, B., Leff, J., Flores, S.C., 1994. Neutrophil-generated free radicals: possible mechanisms of injury in adult respiratory distress syndrome. Environ. Health Perspect. 102 (Suppl. 10), 57–60.

Mott, J.A., Mannino, D.M., Alverson, C.J., Kiyu, A., Hashim, J., Lee, T., Falter, K., Redd, S.C., 2005. Cardiorespiratory hospitalizations associated with smoke exposure during the 1997 Southeast Asian forest fires. Int. J. Hyg. Environ. Health 208, 75–85.

Musk, A.W., Peters, J.M., Bernstein, L., Rubin, C., Monroe, C.B., 1982. Pulmonary function in firefighters: acute changes in ventilatory capacity and their correlates. Br. J. Ind. Med. 36 (1), 29–34.

Patierno, S.R., Landolph, J.R., 1989. Soluble vs. insoluble hexavalent chromate. Relationship of mutation to in vitro transformation and particle uptake. Biol. Trace Elem. Res. 21, 469–474.

Pietropaoli, A.P., Frampton, M.W., Hyde, R.W., Morrow, P.E., Oberdörster, G., Cox, C., Speers, D.M., Frasier, L.M., Chalupa, D.C., Huang, L.S., Utell, M.J., 2004. Pulmonary function, diffusing capacity, and inflammation in healthy and asthmatic subjects exposed to ultrafine particles. Inhal. Toxicol. 16 (Suppl. 1), 59–72.

Pryor, W.A., Nuggehalli, S.K., Scherer, K.V., Church, D.F., 1990. An electron spin resonance study of the particles produced in the pyrolysis of perfluoro polymers. Chem. Res. Toxicol. 3, 2–7.

Rothman, N., Ford, D.P., Baser, M.E., 1991. Pulmonary function and respiratory symptoms in wildland firefighters. J. Occup. Med. 33, 1163–1167.

Scannell, C.H., Balmes, J.R., 1995. Pulmonary effects of firefighting. Occup. Med. 10 (4), 789–801.

Serra, A., Mocci, F., Randaccio, F.S., 1996. Pulmonary function in Sardinian firefighters. Am. J. Ind. Med. 30, 78–82.

Shi, X., Chiu, A., Chen, C.T., Halliwell, B., Castranova, V., Vallyathan, V., 1998. Reduction of chromium(VI) and its relationship to carcinogenesis. J. Toxicol. Environ. Health 2, 101–118.

Shi, X., Dalal, N.S., Vallyathan, V., 1988. ESR evidence for the hydroxyl radical formation in aqueous suspension of quartz particles and its possible significance to lipid peroxidation in silicosis. J. Toxicol. Environ. Health 25, 237–245.

Shi, X., Mao, Y., Knapton, A.D., Ding, M., Rojanasakul, Y., Gannett, P.M., Dalal, N.S., Liu, K., 1994. Reaction of Cr(VI) with ascorbate and hydrogen peroxide generates hydroxyl radicals and causes DNA damage: role of Cr(IV)-mediated Fenton-like reaction. Carcinogenesis 15, 2475–2478.

Sutton, P.M., Castorina, J., Harrison, R.J., 1990. Carbon monoxide exposure in wildland firefighters. Cal. Dept. Health Sci., FI-87-008.

Traber, D.L., Herndon, D.N., Tarber, L.D., 1989. Free radical scavengers in the cardiopulmonary response to endotoxin. Progr. Clin. Biol. Res. 308, 885–890.

Tuomala, M.H., Hirvonen, M.R., Savolainen, K.M., 1992. Production of inositol phosphates and reactive oxygen metabolites in quartz-dust-stimulated human polymorphonuclear leukocytes. FEBS Lett. 296, 57–60.

Tuve, R.L., 1976. Principles of fire protection chemistry. NFPA, 125–135.

US Bureau of Labor Statistics, 2003. Occupational Injuries and Illnesses in the US by Industry. International Association of Firefighters, Washington, DC, 1995–1996.

Ward, D.E., Rothman, N., Strickland, P., 1989. The Effects of Fire Smoke on Firefighters: A Comprehensive Study Plan. United States Department of Agriculture Forest Service Intermountain Research Station, Missoula, MT.

Vaca, C.E., Wilhelm, J., Harms-Ringdahl, M., 1988. Interaction of lipid peroxidation products with DNA. Rev. Mutat. Res. 195, 137–149.

Vallyathan, V., Castranova, V., Pack, D., Leonard, S., Shumaker, J., Hubbs, A.F., Shoemaker, D.A., Ramsey, D.M., Pretty, J.R., Malaurin, J.L., 1995. Freshly fractured quartz inhalation leads to enhanced lung injury and inflammation: potential role of free radicals. Am. J. Respir. Crit. Care Med. 152, 1003–1009.

Vladimirov, Y.A., 1986. In: Johnson Jr., J.E., Walford, R., Harman, D., Miquel, J. (Eds.), Free Radicals, Aging and Degenerative Diseases. Liss, New York, pp. 141–195.

Yim, M.B., Chock, P.B., Stadtman, E.R., 1990. Copper, zinc superoxide dismutase catalyzes hydroxyl radical production from hydrogen peroxide. Proc. Natl. Acad. Sci. U.S.A. 87, 5006–5010.

Yamaguchi, K.T., Stewart, R.J., Wang, H.M., Hudson, S.E., Vierra, M., Akhtar, A., Hoffman, C., George, D., 1992. Measurement of free radicals from smoke inhalation and oxygen exposure by spin trapping and ESR spectroscopy. Free Radic. Res. Commun. 16 (3), 167–174.

Zhang, Y., Fong, C.C., Wong, M.S., Tzang, C.H., Lai, W.P., Fong, W.F., Sui, S.F., Yang, M., 2005. Molecular mechanisms of survival and apoptosis in RAW 264.7 macrophages under oxidative stress. Apoptosis 10 (3), 545–556.