Arch Environ Contam Toxicol 53, 513–518 (2007)
DOI 10.1007/s00244-007-0030-1

Production of Ozone and Reactive Oxygen Species After Welding

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Received: 12 March 2007 / Accepted: 6 May 2007

Abstract Many toxic substances including heavy metals, ozone, carbon monoxide, carbon dioxide, and nitrogen oxides are generated during welding. Ozone (O_3) is a strong oxidant that generates reactive oxygen species (ROS) in tissue, and ambient ROS exposure associated with particles has been determined to cause DNA damage. Ozone is produced within 30 seconds during welding. However, the length of time that O_3 remains in the air after welding is completed (post-welding) is unknown. The current study aimed to assess the distributions of ambient ROS and O_3 before the start of welding (pre-welding), during welding, and after welding. The highest O_3 levels, equal to 195 parts per billion (ppb), appeared during welding. Ozone levels gradually decreased to 60 ppb 10 minutes after the welding was completed. The highest ROS level was found in samples taken during welding, followed by samples taken after the welding was completed. The lowest ROS level was found in samples taken before the welding had started. Ozone and ROS levels were poorly correlated, but a similar trend was found for O_3 and ROS levels in particles (μM/mg). Although particles were not generated after welding, ROS and O_3 still persisted for more than 10 minutes. Meanwhile, because O_3 continues after welding, how long the occupational protective system should be used depends on the welding materials and the methods used. In addition, the relationship between metal fumes and ROS generation during the welding process should be further investigated.

Keywords O_3 · Particle · ROS · Ultraviolet light · Welding

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Introduction

The relation between welding and many occupational health effects, including respiratory illness (Antonini et al. 1998), neurotoxicity (Antonini et al. 2006; Bowler et al. 2003), and genotoxicity (Yu et al. 2004), is well known. A study also has indicated that breathing frequency increases and tidal volume decreases within several minutes when the rat exposed to welding fumes (Saito et al. 2000). However, the phenomenon disappears after repeated exposures.

Welding joins pieces of metal or sheet metals by producing very high heat. A filler metal from a consumable electrode wire is continuously fed into the weld. Many toxic substances including heavy metals, toxic gases (ozone, carbon monoxide, carbon dioxide, and nitrogen oxides) are generated during welding (Antonini 2003). Ozone (O_3) is produced in a photochemical reaction induced by ultraviolet light with atmospheric oxygen gas during the welding process.

Findings have shown that O_3 alters pulmonary morphology, physiology, and biochemistry, and it also is a proven cause of asthma in school children (Cheng et al. 2003; Fauroux et al. 2000; Grievink et al. 1998). Furthermore, O_3 is a strong oxidant that generates reactive oxygen species (ROS) in tissue, and even causes DNA damage (Cheng et al. 2003). Oxidative stress may be involved in the genetic changes associated with the initiation, promotion, and progress of carcinogenesis (Dreher and Junod 1996), and it has

been implicated in the pathogenesis of certain diseases, including lung, skin, and breast cancers (Matsui et al. 1999; Rosenkransz 1993; Sozzi et al. 1991; Yeh et al. 2005).

The rate of O_3 production depends on the wavelengths and the intensity of ultraviolet light generated during welding, which in turn is affected by the material being welded and the type of electrode used (Pattee et al. 1973). Ozone has been evaluated during the welding process (Dennis et al. 1997), but there is no published comprehensive analysis of O_3 production after welding has been completed.

In addition to O_3, welding has other hazardous by-products, for example, the fumes of metals including cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), and others. Findings have shown that exposure to some heavy metals, including Cd, Mn, Cr, and Fe, is related to the generation of ROS (Pourahmad et al. 2003; Ranieri et al. 2005; Wang et al. 2004). Antonini et al. (1998) reported that freshly generated stainless steel welding fumes induced greater lung inflammation than ''aged'' fumes. They interpreted this as indicating a higher concentration of ROS on fresh fume surfaces.

A later study by Churg (2003) also suggested that the combination of particles and O_3, cigarette smoke, or reagent hydrogen peroxide augments the inflammatory response to particles, increases cell proliferation, and liberates higher levels of chemo-attractant mediators. Therefore, complex mixtures of pollutants produced as welding by-products are important in terms of industrial hygiene. The current study aimed to assess the ambient exposure of the welding operator to ROS and O_3, and to assess the distribution of ROS and O_3 before the start of welding, during welding, and after welding in an effort to determine what protective equipment should be used during welding.

Many types of welding including manual shielded metal arc welding (also known as ''stick welding''), gas metal arc welding, flux-cored arc welding, gas tungsten arc welding, and others provide a powerful manufacturing tool for the high-quality joining of metal components. Shielded metal arc welding uses an electric arc between a flux-covered metal electrode (the ''stick'') and the metal objects to be joined (the base metal and the weld metal). Heat from the arc melts the flux, forming gas and slag that shield the arc and the molten weld pool. In this study, the ambient sampling was taken during shielded metal arc welding.

Materials and Methods

Welding and Collecting Ambient Samples

In this study, the ambient sampling was taken during manual metal arc welding. The base metal was low carbon steel, and the weld stick (Chinese National Standard, CNS-E308L-16) was stainless steel, which contains nickel, chromium, molybdenum, iron, and the like. The welding was done using 50 V in a laboratory, but without natural ultraviolet light. The welding was performed by an operator using a respiratory mask and safety glasses to prevent exposure to the occupational hazards.

Inhalable dust sampler (IOM) inhalable dust samples were taken from the breath zone of the welding operator and the O_3 detector located 0.3 to 0.4 m from the arc welding. The laboratory space was 64.49 m^3, and the window was open (open face, 2.23 m^2) during the welding process. The average wind velocity of the open face for window was 0.22 m/second, and the ventilation rate was 29.65 m^3/minute.

Air samples were taken during welding using IOM inhalable dust samplers with 0.4-μm pores of polycarbonate membrane filters (Nucleopore, Inc., Cabin John, MD, USA) in polystyrene cassettes with a diameter of 25 mm. A personal air pump sampler with a flow rate of 2.0 l/minute was used for this sampling. The sampling time was 30 minutes per sample. To minimize the effect of time delay, the samples were analyzed immediately after the sampling was completed. Five samples were taken separately before welding, during welding, and then 30 and 60 minutes after welding. Three experiments were performed, and 60 samples finally were analyzed in this study.

O_3 Measuring Equipment

To assess how long O_3 persisted, we used an indoor air quality monitor (AirBoxx; KD Engineering Inc., Blaine, WA, USA), a real-time measurement instrument, to detect the variation of O_3 levels before welding (for 27 minutes), during welding (65 minutes), and in two periods after welding (10 and 65 minutes). Indoor air quality monitoring is electrochemical. The response time for O_3 detection was 150 seconds to 90% of the final value. The measuring range was 0 to 2 parts per million (ppm), and the resolution was 0.01 ppm. The O_3 concentration was recorded 27 minutes before the welding started, for 65 minutes during the welding, and 75 minutes after the welding had been completed. The data were recorded while O_3 levels varied.

Measuring ROS

Reactive oxygen species activity was measured as an equivalent to hydrogen peroxide, which was used for calibration of the assay. The method was modified from that of the previous study (Hung and Wang 2001; Venkatachari et al. 2005). We used 0.5 ml of 1 m*M* 2',7'-dichlorofluorescin diacetate ($DCFH_2$-DA) (Sigma Chemical Co., St. Louis, MO, USA) to generate dichlorofluorescin ($DCFH_2$)

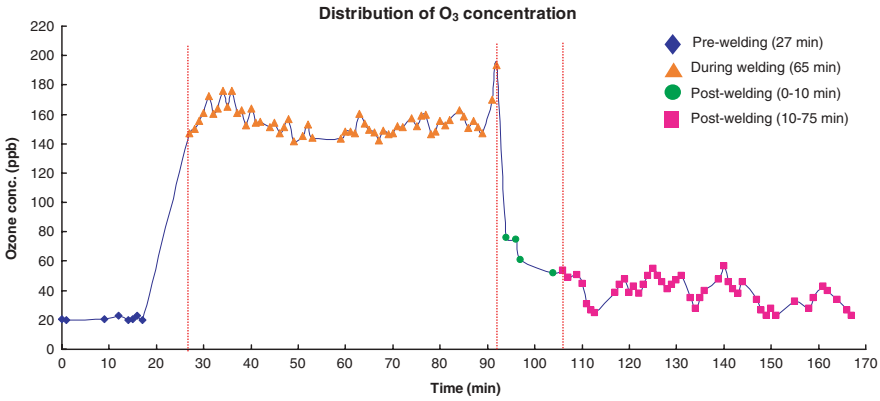

Fig. 1 Distribution of ozone (O_3) levels before, during, and after welding

Fig. 2 Average ozone (O_3) levels before, during, and after welding. *p value < 0.05, Kruskal–Wallis test comparing O_3 (ppb) levels among pre-, during-, post-welding-1, and post-welding-2. †p value < 0.05, Wilcoxon rank sum test comparing O_3 (ppb) levels between pre- and during-welding. ‡p value < 0.05, Wilcoxon rank sum test comparing O_3 (ppb) levels between pre- and post-welding-1. #p value < 0.05, Wilcoxon rank sum test comparing O_3 (ppb) levels between pre- and post-welding-2. The bar chart shows mean and standard error

in ethanol mixed with 2 ml of 0.01 *M* NaOH. The hydrolyzate was allowed to stand at room temperature for 30 minutes, after which it was neutralized by adding 10 ml of sodium phosphate buffer (pH 7.2). The solution was kept on ice in the dark until it was used. The working solution, 1 μM $DCFH_2$ in sodium phosphate buffer that contained 2.2 units of hydrogen-peroxide oxidoreductase (HRP) (Sigma Chemical) per ml of reagent, was freshly prepared before the analysis (Hung and Wang 2001).

The collected particle samples were weighed immediately. Subsequently, 1 μM of $DCFH_2$-HRP was added, 3 ml to each sample and filter blank. After ultrasonic shaking for 10 minutes to extract the ROS from particulates, the solution was incubated for 15 minutes at 37°C. After the incubation, 0.1-ml aliquots were placed in 96-well plates, and the fluorescence intensity of fluorescent dichlorofluorescin in each sample was measured using a fluorescence spectrophotometer (Fluoroskan Ascent FL; Thermo Electron Corp., Waltham, MA, USA) at an excitation wavelength of 485 nm and an emission wavelength of 530 nm. The hydrogen peroxide concentrations were 0.25, 1.0, 2.0, 3.0, and 4.0×10^{-7} *M* in standard solutions.

Statistical Analysis

Commercial software (JMP 5.0; SAS Institute, Cary, NC, USA, and Statistica 6.0 StatSoft, Inc., Tulsa, OK, USA) was used for data management and statistical analysis. Data are shown as mean ± standard deviation. The differences between pre-welding, welding, and post-welding ROS levels in particulates were determined using the Kruskal–Wallis and Wilcoxon rank sum tests. In addition, Spearman correlation was used to test the correlation between ROS and O_3 levels. Statistical significance was set at a p value less than 0.05.

Results

Distribution of O_3 Levels Before, During, and After Welding

The pre-welding O_3 levels were equal to 20 parts per billion (ppb). They rose to a maximum level of 195 ppb (range, 20–195 ppb) 5 minutes after the welding started, (Fig. 1)., then decreased gradually to 80 ppb 3 minutes after welding, and finally fell to 50 ppb 10 minutes after welding. The average O_3 levels were 20.9 ppb before welding, 155.1 ppb during welding, 65.8 ppb 10 minutes after welding, and 39.2 ppb 65 minutes after welding (Fig. 2). The O_3 levels during and after welding were significantly higher than the pre-welding levels ($P < 0.05$).

Distribution of Particle Levels Before, During, and After Welding

The particle levels in the samples taken during welding were significantly higher ($p < 0.05$; average, 8,097 $\mu g/m^3$)

than the pre-welding (405.8 $\mu g/m^3$) and post-welding (289.3 $\mu g/m^3$) levels (Fig. 3). The pre- and post-welding levels were not significantly different.

Distribution of ROS Levels Before, During, and After Welding

The highest ROS levels were found in the samples taken during welding (average, 5.19 $\mu M/m^3$). The second highest levels were found after welding (average, 1.43 $\mu M/m^3$), and the lowest levels were found before welding (average, 0.58 $\mu M/m^3$) (Fig. 4) The ROS levels during welding were significantly higher than the pre- and post-welding levels ($p < 0.05$).

Correlation of ROS and Particle Levels

A significant correlation between ROS and particle levels was found only for post-welding ($r = 0.764$; $p = 0.016$) (Table 1).

O_3 and ROS Levels

The average O_3 level increased when welding started, then decreased gradually until 10 minutes after welding. The trend was consistent when O_3 levels were compared with ROS levels in particles (μM/mg). The ROS levels ($\mu M/m^3$), however, were not correlated with the O_3 levels (Fig. 5).

Discussion

O_3 Levels

Ozone is a well-known oxidant pollutant that causes DNA strand breaks, alters pulmonary function and bronchial responsiveness (Bornholdt et al. 2002), and causes membrane oxidation or mutations *in vivo* (Ballinger et al. 2005). Among the gases produced during welding, O_3 is formed not only by thermal and chemical reactions, but also by the reaction of a specific wavelength band of ultraviolet light on oxygen molecules (Lyttle 1997).

The O_3 level in the current study was approximately 140 to195 ppb during welding, which is higher than the 0.1-ppm legal permissible exposure limit set by the Occupational Safety and Health Administration (OSHA) for an average 8-hour work shift. Compared with other indoor environments, the average O_3 concentration in the welding process was extremely higher (mean, 3.37 ± 7.7 ppb) than in the homes of asthmatic children in the inner city of Baltimore (Breysse et al. 2005). It also was higher than the O_3 concentration in the museums of Poland (range, 3.2–8.5

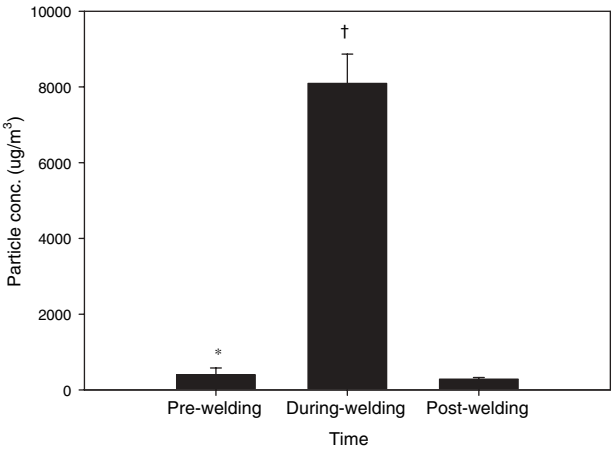

Fig. 3 Average particle levels ($\mu g/m^3$) before, during, and after welding. *p value < 0.05 by Kruskal–Wallis test comparing particle concentrations ($\mu g/m^3$) among pre-, during-, and post-welding. †p value < 0.05 by Wilcoxon rank sum test comparing particle concentrations ($\mu g/m^3$) between pre- and during-welding. The bar chart shows mean and standard error

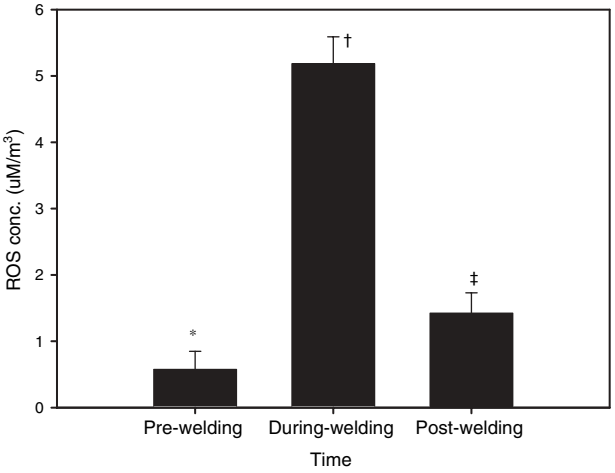

Fig. 4 Average reactive oxygen species (ROS) concentrations ($\mu M/m^3$) before, during, and after welding. *p value < 0.05 by Kruskal–Wallis test comparing ROS concentrations ($\mu M/m^3$) among pre-, during-, and post-welding. †p value < 0.05 by Wilcoxon rank sum test comparing ROS concentrations ($\mu M/m^3$) between pre- and during-welding. ‡p value < 0.05 by Wilcoxon rank sum test comparing ROS concentrations ($\mu M/m^3$) between pre- and post-welding. The bar chart shows mean and standard error

Table 1 Correlation coefficients between reactive oxygen species (ROS) concentration ($\mu M/m^3$) and particle concentration ($\mu g/m^3$) at different welding times

Time	Correlation coefficient	p Value
Before welding	–0.173	0.842
During welding	0.056	0.413
After welding	0.794	0.016[a]

[a] $p < 0.05$ (Spearman correlation)

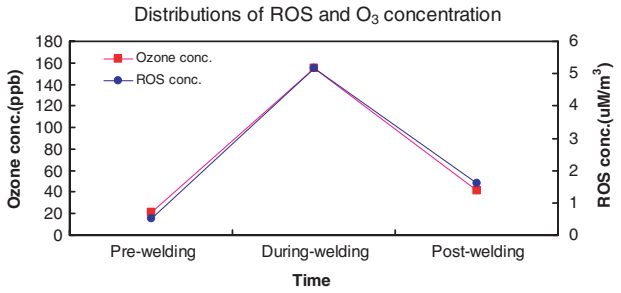

Fig. 5 Distributions of reactive oxygen species (ROS) and O_3 levels before, during, and after welding

ppb) (Salmon et al. 2000). Therefore, O_3 is a significant pollutant in the welding environment.

In this study, O_3 was produced during welding within 30 seconds (Fig. 1). However, it is not known how long O_3 persists. The current study indicated that the O_3 level was still high 10 minutes after welding. These data suggest that welding workers should protect themselves for more than 10 minutes after welding to avoid O_3 exposure. Other studies report that welding voltage and materials, shield gas (Scharffetter-Kochanek et al. 1997), and ultraviolet light (Antonini 2003) influence factors to affect O_3 levels in the welding process. Therefore, O_3 production under different welding conditions and the ultraviolet light produced during the welding process should be investigated further.

Particle and ROS Levels

Shibata et al. (2000) reported that the fume concentration near the welder's breathing zone was affected by welding currents, hood position, and flow rates in a small enclosed workspace. Antonini et al. (1998) also reported that freshly generated stainless steel welding fumes induced greater lung inflammation in rats than did aged fumes, suggesting that this might be due to a higher concentration of ROS on fresh fume surfaces.

The current study showed that the particle mass (mean, 3,919.7 $\mu g/m^3$) and ROS levels (mean, 2.97 $\mu M/m^3$) sampled during the welding process substantially exceeded those reported by Hung and Wang (2001) for vehicle exhausts (mass concentration of ultrafine to coarse particles, 116.9–14.2 $\mu g/m^3$; ROS level,: 0.006–0.592 nM/m^3) as well as those from our previous report on foundry plants (mean, 1,202 $\mu g/m^3$ and 8.1 $\mu M/m^3$, respectively) (unpublished data). According to Hung and Wang (2001), the level of ROS is high, especially in particles 0.18 to 1 μm in size, perhaps because the surface area of the particles in the photochemical reaction is high. Therefore, particle size also may influence ROS distribution during the welding process.

A previous investigation (Dick et al. 2003) showed that ultrafine particles may cause adverse effects via oxidative stress, and that further oxidative stress may be induced by occupational or environmental particles in susceptible individuals, such as those with chronic obstructive pulmonary disease (COPD) or asthma, who already exhibit preexisting oxidative sensitivity. The experimental and epidemiologic data also indicated that airborne particulate matter with an aerodynamic diameter smaller than 2.5 μm is easily deposited in the human lung and has a severe impact on health, although particles of other sizes also can be toxic (Squadrito et al. 2001; Tao et al. 2003). Therefore, the distribution of ROS levels in particles of various sizes in welding fumes needs to be elucidated for an assessment of their adverse health effects on welding workers. In addition, the relationship between metal fumes and ROS generation during the welding process should be investigated further.

O_3 and ROS Levels

The ROS levels ($\mu M/m^3$) were not correlated with the O_3 levels. However, a similar trend was found for O_3 concentrations and ROS levels in the particles (μM/mg). Hung and Wang (2001) found reasonable correlations between ROS ($\mu M/m^3$) and O_3 levels, especially for small particles. Therefore, a non-size-sensitive determination of the particle mass generally underestimates the contribution of small particles. Because ROS concentration tends to be higher in smaller than in larger particles (Hung and Wang 2001), this could be one reason for the lack of correlation. In addition, Churg (2003) reported that combinations of occupational or environmental particles, exogenous O_3, and cigarette smoke could generate ROS. Although the particles are not generated after welding, the persistence of ROS may be attributable to O_3 in the ambient air.

Implications

Welding fumes contain particles, ROS, O_3, and heavy metals. Therefore, protective equipment should be provided to prevent welders from exposure to these health hazards. In addition, because O_3 still existed after welding in this study, how long the occupational protective system should be applied depends on the welding material and the welding methods used.

Acknowledgment The authors thank the National Science Council of the Republic of China, Taiwan, for supporting this research financially.

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