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## Cardiovascular effects in rats after intratracheal instillation of metal welding particles

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### Abstract

Studies have indicated that pulmonary exposure to welding fumes can induce a series of adverse effects in the respiratory system, including infection, bronchitis, siderosis and decreased pulmonary function. Recent clinical and epidemiological studies have found that pulmonary exposure to welding fumes is also associated with a higher incidence of cardiovascular events. However, there is insufficient evidence to confirm a direct effect of welding fumes on the cardiovascular system. The present study investigated the effects of pulmonary exposure to welding fumes on the heart and the vascular system in rats. Two chemically distinct welding fumes generated from manual metal arc-hard surfacing (MMA-HS) and gas metal arc-mild steel (GMA-MS) welding were tested. Three groups of rats were instilled intratracheally with MMA-HS (2 mg/rat), GMA-MS (2 mg/rat) or saline as control once a week for seven weeks. On days 1 and 7 after the last treatment, basal cardiovascular function and the cardiovascular response to increasing doses of adrenoreceptor agonists were assessed. MMA-HS treatment reduced the basal levels of left ventricle end-systolic pressure and  $dP/dt_{max}$  at 1 day post-treatment, and decreased  $dP/dt_{min}$  in response to isoproterenol (ISO) at 7 days post-treatment. Unlike MMA-HS, GMA-MS only affected left ventricular end-diastolic pressure in response to ISO at 7 days post-treatment. Treatment with MMA-HS or GMA-MS did not alter heart rate and blood pressure. Our findings suggest that exposure to different welding fumes can induce different adverse effects on the cardiovascular system, and that cardiac contractility may be a sensitive indicator of cardiovascular dysfunction.

### Keywords

Cardiovascular function; pulmonary exposure; welding fume

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### Declaration of interest

The opinions expressed in this manuscript are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health, Center for Disease Control and Prevention.

## Introduction

Welding fumes are generated during welding operations and usually consist of a wide range of chemical species of metals. Every year, large numbers of workers are exposed to welding fumes due to various welding operations in occupational settings. According to the Occupational Outlook Handbook, edition 2010–2011, published by the US Bureau of Labor Statistics, there were about 450 000 Americans employed as welders, cutters and welding machine operators in 2009. However, by including the workers involved in welding from other professions, such as assemblers, machinists and steel metal workers, the number of workers being exposed to welding fumes can reach above two million. The adverse health effects of welding fumes are complicated and difficult to predict due to the variety of metal components used in welding rods for different welding purposes and the routes of exposure. Pulmonary exposure to welding fumes has been reported to result in inflammation, lung injury and even cancer in the lung in animal models (Antonini et al., 2003; Scharrer et al., 2007; Sorensen et al., 2007). However, the respiratory system is not the only system that is affected by inhalation of welding fume, the brain and immune systems can also be impaired (Antonini et al., 2012; Kenangil et al., 2006; Nakata et al., 2006; Sriram et al., 2010). More recently, animal studies demonstrated that pulmonary exposure to welding fumes can induce expression of stress response genes in the aorta and heart (Erdely et al., 2011b), and increase the lesion area of atherosclerotic plaques (Erdely et al., 2011a). In humans, exposure to welding fumes can cause a systemic cytokine response, inflammation, oxidative stress and alteration in the autonomic nervous function (Cavallari et al., 2008; du Plessis et al., 2010; Luo et al., 2009). All these biological changes observed in human and animal studies have been reported as potential pathogenic factors in the development of cardiovascular dysfunction. Evidence accumulated from epidemiological studies also suggests a strong link between inhalation of welding fumes and the increased incidence of cardiovascular events such as cardiac arrhythmia, myocardial ischemia and atherosclerosis (Cavallari et al., 2007; Chinn et al., 1990). A study conducted in a group of more than 10 000 male metal workers found that exposure to welding processed particles significantly increased the risk of cardiovascular disease, including acute myocardial infarction and angina pectoris (Ibfelt et al., 2010).

In the present study, we investigated the effects of intratracheal instillation to welding fumes collected from manual metal arc-hard surfacing (MMA-HS) or gas metal arc-mild steel (GMA-MS) welding process on cardiovascular function in rats. These two types of welding fumes are very different in their metal composition and solubility. MMA-HS welding fume consists mainly of manganese (Mn; 50.9%) and chromium (Cr 8.46%), and has higher water solubility compared with GMA-MS, whereas GMA-MS welding fume contains mainly Fe (72.2%) and less Mn (21.7%) compared with MMA-HS (Table 1) (Antonini et al., 2010). Both types of welding fumes have been confirmed to cause adverse lung effects in an animal model, with MMA-HS fumes inducing more severe inflammatory response and injury in the lung than GMA-MS fumes due to the differing metal components. It has also been confirmed that inhalation of MMA-HS or GMA-MS welding fumes can alter mitochondrial function and reduce the synthesis of certain neurotransmitters that may contribute to the early onset of Parkinsonism in humans (Sriram et al., 2010), and depress the total number of

circulating T-lymphocytes (Antonini et al., 2012). These observations suggest that inhalation of MMA-HS or GMA-MS can induce multiple organ or system dysfunction. However, the possible short- and long-term pathogenic effects of inhalation of these welding fumes on the cardiovascular system have not been well studied. Thus, research is necessary to investigate the comparative impacts of the two different types of welding fumes on the cardiovascular system after pulmonary exposure.

## Methods and materials

### Animals

Male Sprague-Dawley rats [H1a:(SD) CVF] from Hilltop Lab Animals (Scottsdale, PA), 6–7 weeks of age and free of viral pathogens, parasites, mycoplasmas, *Helicobacter* and CAR bacillus were used for all experiments. The rats were housed in cages ventilated with HEPA-filtered air under controlled temperature and humidity conditions and a 12-h light/12-h dark cycle. Food (Teklad 7913) and tap water were provided *ad libitum*. The rats were allowed to acclimate to the facilities for one week before exposure was performed. The animal facilities are specific pathogen-free and accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All experimental procedures were approved by the Animal Care and Use Committee of the National Institute for Occupational Safety and Health and conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Research Council.

### Welding fume collection and characterization

Bulk samples of different welding fumes were collected by Lincoln Electric Co. (Cleveland, OH). The fumes were generated in a cubical open front fume chamber (volume = 1 m<sup>3</sup>) by a skilled welder using a manual or semi-automatic technique appropriate to the electrode and collected on 0.2 µm Nuclepore filters (Nuclepore Co., Pleasanton, CA). The fume samples were generated using two different processes: (1) gas metal arc welding using a mild steel E70S-3 electrode (GMA-MS; L-50 carbon steel electrode, Lincoln Electric Co., Cleveland, OH) and (2) manual metal arc welding using a flux-covered stainless steel hardsurfacing electrode (MMA-HS; Wearshield 15CrMn, Lincoln Electric Co., Cleveland, OH) that contains elevated amounts of Mn compared with standard welding electrodes/rods/wires.

Welding fume samples (GMA-MS and MMA-HS) were suspended in distilled water, pH 7.4 and sonicated for 1 min with a Sonifier 450 Cell Disruptor (Branson Ultrasonics, Danbury, CT). The two particle suspensions (total samples) were incubated for 24 h at 37°C, and the samples were centrifuged at 12 000 g for 30 min. The supernatant of the sample (soluble fraction) was recovered and filtered with a 0.22 µm filter (Millipore Corp., Bedford, MA). The pellet (insoluble fraction) was resuspended in water. The sample suspensions (total, soluble and insoluble fractions) were digested, and the metals analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES) by the Division of Applied Research and Technology (DART, Cincinnati, OH) according to the NIOSH method 7300 (NIOSH, 1994). The metals that were measured in the particle suspensions included Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, La, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Sb, Se, Sr, Te, Ti, Tl, V, Zn and Zr (Antonini et al., 2010, 2011; NIOSH, 1994).

## Welding fume treatment

Welding fume collection and preparation were described in a previous study (Antonini et al., 2012). Briefly, welding fume samples (GMA-MS and MMA-HS) were suspended in distilled water, pH 7.4 and sonicated for 1 min with a Sonifier 450 Cell Disrupter (Branson Ultrasonics Corp., Danbury, CT) prior to use. Rats were lightly anesthetized by an intraperitoneal injection of 0.6 ml of a 1% solution of sodium methohexital (Brevital; Eli Lilly, Indianapolis, IN) and instilled intratracheally once a week for 7 weeks with 2 mg/rat of the suspended MMA-HS or GMA-MS welding fumes in 300  $\mu$ l of sterile phosphate-buffered saline (PBS). These suspensions were vortexed immediately prior to instillation to assure a homogenous suspension. Vehicle control animals were instilled intratracheally with 300  $\mu$ l of sterile PBS. The intratracheal instillation dose of 2 mg/rat was chosen based on results from previous welding fume studies at NIOSH (Antonini et al., 2010; Popstojanov et al., 2014).

The intratracheal instillation method of treatment is a widely used procedure to deliver materials into lungs of laboratory animals and was chosen for this pilot study to initially examine if welding particles that have accumulated in the lungs can induce cardiac effects. A welding fume generation and inhalation exposure system has been developed by our group (Antonini et al., 2006), and based on the findings of the current intratracheal instillation investigation, the inhalation study has been initiated. Although not as physiologic as an inhalation exposure, intratracheal instillation of particles has some advantages, such as simplicity, relative low cost and the delivery of a well-defined dose of particles (Driscoll et al., 2000). Intratracheal instillation is also useful for assessing effects of bulk-collected particles and particles of limited quantities.

To estimate how the intratracheal instillation particle dose used in the study correlated with a ‘real world’ worker exposure to welding fumes, the total cumulative exposure over time was calculated. The calculations made here do not account for particle clearance, but provides an estimate of the plausible welder exposure concentrations that our exposure paradigm mimics. The daily lung burden of a welder was estimated, assuming 8 h of continuous welding, a worker minute ventilation of 20 000 ml/min, a particle deposition efficiency in the alveolar region of 15% for fumes having a mass medium aerodynamic diameter of 200 nm and a fume concentration of 5 mg/m<sup>3</sup> (previous Threshold Limit Values for 8-h day for welding fume). The following calculations were made:

$$\begin{aligned} & \text{Fume concentration} \times \text{minute volume} \times \text{exposure duration} \\ & \quad \times \text{deposition efficiency} = \text{Daily deposited dose} \\ & 5 \text{ mg/m}^3 \times (20\,000 \text{ ml/min} \times 10^{-6} \text{ m}^3/\text{ml}) \times (8 \text{ h} \times 60 \text{ min/h}) \\ & \quad \times 0.15 = 7.2 \text{ mg deposited per day} \end{aligned}$$

Morphometric analysis indicates that the alveolar surface area in the human is 102 m<sup>2</sup>, while the value for the rat is 0.4 m<sup>2</sup> (Stone, 1992). Therefore, the alveolar surface area for a human is 255 times greater than the rat. In the current study, the rat lung burden is 14 mg, that is, 2 mg  $\times$  7 days. Thus, on an alveolar surface basis, the equivalent lung burden in a welder would be 3570 mg. At a deposited dose of 7.2 mg/day, this lung burden would be achieved

in 496 day or approximately 2 working years, assuming 250 work days/year at the TLV. However, field studies indicated that welding fume levels often exceed the TLV of 5 mg/m<sup>3</sup>, being as high as 13 mg/m<sup>3</sup> in confined work spaces (Harris et al., 2005; Korczynski, 2000; Susi et al., 2000). In such cases, for example, boiler workers, a lung burden equivalent to the current rat exposure could be achieved in 0.76 years. It is true that the dose rate in these welders would be far less than that for the rats in the current study. However, the data from the present study do suggest that a cardiovascular effect is possible and are being used to justify that further inhalation studies with welding fume are warranted. Such studies are in progress.

### ***In vivo* hemodynamic measurements**

At 1 and 7 days after the last weekly intratracheal instillation, rats (6–8/group) were anesthetized with inhaled 3% isoflurane mixed with oxygen at a flow rate of 2 l/min. A temperature-regulated heating pad was used to maintain the normal body temperature of the rat. Using an aseptic technique, a custom catheter made according to the method described by Khanna et al. (2007) was inserted into the left ventricle through the carotid artery. The correct position of the catheter tip in the left ventricle was confirmed by the waveform of left ventricular pressure visualized on a computer monitor. Cardiac function was assessed by measuring left ventricular end-systolic pressure (LVESP), left ventricular end-diastolic pressure (LVEDP), the maximum rate of increase in left ventricular pressure ( $dP/dt_{max}$ ) and the minimum rate of decrease in left ventricular pressure ( $dP/dt_{min}$ ). To study vascular function *in vivo*, mean blood pressure (MBP), systolic blood pressure (SBP) and diastolic blood pressure (DBP) were determined by using a fluid-filled arterial catheter placed in the femoral artery and connected to a pressure transducer coupled to a computerized cardiovascular continuous monitoring system, a PowerLab/4SP analog-to-digital converter (AD Instruments, Colorado Springs, CO). The heart rate (HR) was recorded by the same monitoring system at a sampling rate of 1000 Hz. Increasing doses of isoproterenol (ISO) or norepinephrine (NE) (Sigma-Aldrich, St. Louis, MO) were administered through a catheter (polyurethane, 3 French size) pre-placed in the jugular vein. Cardiopulmonary responses and spinal reflexes were checked to determine the proper depth of anesthesia. Each rat was euthanized with a carbon dioxide overdose at the end of the experiment.

### **Statistical analysis**

Data were compared using analysis of variance, followed by pairwise comparisons between the control and treated groups using a Student's *t*-test. All data were analyzed using JMP software (Version 9.0, Cary, NC) and differences were considered statistically significant at  $p < 0.05$ . The values in the figures are expressed as the mean  $\pm$  SD.

## **Results**

### **Body weight**

The effect of welding fumes on the body weight was determined at 1 and 7 days after the last treatment. The body weight was not affected by the treatment of GMA-MS welding fumes either at 1 or 7 days post-treatment; however, the body weight was slightly but significantly reduced in the group treated with MMA-HS welding fumes at 7 days post-

treatment (Figure 1). There was no difference in the body weights for all experimental groups at the beginning of treatment with the welding fumes.

### Effects of welding fumes on basal cardiovascular function

The basal level of cardiovascular function was assessed by a computerized cardiovascular continuous monitoring system at 1 and 7 days after last treatment. Repeated intratracheal instillation of MMA-HS for seven weeks did not alter basal HR, LVEDP and  $dP/dt_{\min}$ ; however, LVESP and  $dP/dt_{\max}$  were decreased significantly at one day post-treatment, but the decrease in LVESP and  $dP/dt_{\max}$  returned to control at seven days after the last treatment. Unlike MMA-HS, intratracheal instillation of GMA-MS did not change basal cardiovascular parameters at either days 1 or 7 after last treatment (Table 2). An transient decrease in LVESP and  $dP/dt$ , occurred in 1 day not 7 days post-treatment, indicated that intratracheal instillation of MMA-HS can temporally depress cardiac pumping function. A continuous decline in cardiac pumping function could eventually result in chronic heart failure. In the meantime, it is not clear whether longer welding fume exposure will cause more severe and persistent impact on cardiac contractile function.

### Effects of welding fumes on cardiac function in response to $\beta$ -adrenoceptor stimuli

It is possible that the evidence of cardiac dysfunction of the rats exposed to welding fumes may not be detectable under unstressed conditions due to the compensatory mechanism of cardiovascular system at the early stage of the disease *in vivo*. Subtle changes at the early stage of the disease in rats exposed to welding fumes may be uncovered only under stressful conditions. Therefore, cardiac function after intratracheal instillation of welding fumes was further evaluated by administration of the  $\beta$ -adrenoceptor agonist, ISO. The left ventricular function was measured as above. Administration of ISO induced a dose-dependent increase in HR as expected in welding fume-treated and control groups (Figure 2). No statistical difference in HR responses in response to ISO was found between the welding fume-treated and control groups. GMA-MS seemed to slightly attenuate the HR in response to ISO at one day post-treatment, but the effect did not reach a significant level (Figure 2A).

The contractile function of the left ventricle in response to  $\beta$ -adrenoceptor stimuli after welding fume exposure was studied by measuring both LVESP and LV  $dP/dt_{\max}$ , the two main parameters that reflect the systolic function of the left ventricle. Although our data indicated that pulmonary exposure to MMA-HS altered the basal level of LVESP and LV  $dP/dt_{\max}$  at 1 day post-treatment, exposure to MMA-HS did not change responsiveness to ISO at days 1 or 7 post-treatment (Figures 3 and 4). Intratracheal instillation of GMA-MS did not affect the contractile function of left ventricle in response to ISO compared with the control group (Figures 3 and 4).

The diastolic function of the heart in response to  $\beta$ -adrenoceptor stimuli after welding fume exposure was evaluated by measuring LV  $dP/dt_{\min}$ . Our data indicated that LV diastolic function  $dP/dt_{\min}$  in response to ISO was slightly impaired in rats treated with MMA-HS at day 1 post-treatment and reached a significant level at days 7 post-treatment compared with the control group (Figure 5). GMA-MS did not have any effect on LV  $dP/dt_{\min}$  in response to ISO at either days 1 or 7 post-treatment (Figure 5). In addition, we also found that the left

ventricle filling pressure indicated by LVEDP in response to lower doses of ISO was increased by GMA-MS at day 1 post-treatment and returned to the normal level at days 7 post-treatment (Figure 6). In the current study, the mechanism of impaired diastolic function  $dP/dt_{\min}$  in response to  $\beta$ -adrenoceptor stimuli after MMA-HS treatment is not known. However, it could be due to the intrinsic changes of myocyte function, which may include alterations in calcium handling, myofilament function and the sensitivities of the receptors (Engelhardt et al., 2001; Wilson et al., 2014).

### Effects of welding fumes on blood pressure in response to $\alpha$ -adrenoceptor stimuli

The effects of pulmonary exposure to welding fumes on vascular function were also studied by measuring mean, systolic and DBP responses to an  $\alpha$ -adrenoceptor agonist, NE. Intratracheal instillation of either MMA-HS or GMA-MS for seven weeks did not alter the basal MBP compared with the control group (Table 2). Additionally, the MBP response to NE was not affected by pulmonary exposure to two types of welding fume (Figure 7). We also found that both systolic and DBPs were not affected by welding fume exposure either at basal level or in response to NE (data are not shown).

### Discussion and conclusion

In the present study, we treated rats with different welding fumes (2 mg/rat) once a week for seven weeks; this amount of welding fume exposure is equivalent to two working years of a welder exposure of 5 mg/m<sup>3</sup> for 8 h/day (Antonini et al., 2012; Popstojanov et al., 2014). Our results indicated that intratracheal instillation of welding fumes can alter cardiac function either at basal level or in response to  $\beta$ -adrenoceptor stimuli, but did not affect blood pressure (Figure 7). The effects of welding fume on cardiac function were induced mainly by MMA-HS. MMA-HS decreased the basal levels of LVESP and LV  $dP/dt_{\max}$  at 1 day post-treatment (Table 2). Exposure to MMA-HS also significantly depressed diastolic function of the heart in response to  $\beta$ -adrenoceptor stimulation at 7-days post-treatment, as evidenced by reducing LV  $dP/dt_{\min}$  (Figure 5). GMA-MS increased LVEDP responses to  $\beta$ -adrenoceptor stimulation at 1 day post-treatment but not at 7 days post-treatment (Figure 6). We also noticed that the ratio of heart weight to body weight was increased in the group treated with MMA-HS at 7 days post-treatment (Table 2). The increase in heart weight to body weight ratio usually indicates a cardiac muscle mass increase, which is often found in cardiac remodeling following heart failure (Hu et al., 2013). However, in our study, this increased heart weight to body weight ratio was more likely due to the reduced body weight by MMA-HS exposure since the heart weight was not significantly different comparing MMA-HS-treated group and the control group (Table 2).

Our study indicated that both types of welding fume can alter cardiac function with MMA-HS showing the greater effect. However, in regard to the effect of welding fume on the blood pressure, both types of welding fume seem to have no effect on the basal vascular function nor on this vascular reactivity to  $\alpha$ -adrenoceptor agonist stimulation (Figure 7). However, the absence of a blood pressure effect by the two welding fumes in this study could be related to exposure time or dose. The results from our study do suggest, however, that measures of cardiac contractility are more sensitive to welding fumes than blood pressure following pulmonary welding fume exposure.

Intratracheal instillation of either MMA-HS or GMA-MS can change cardiac function as shown in our study; however, our data further indicated that MMA-HS exhibited more impact on the heart function than GMA-MS did at the same exposure dose. In addition, MMA-HS also reduced body weight. In a precursor study to this current one using repeated intratracheal instillation of rats to MMA-HS or GMA-MS, repeated MMA-HS induced greater pulmonary injury and inflammation than GMA-MS (Antonini et al., 2010). These differences in pulmonary and cardiac reaction induced by two types of welding fume can be explained apparently by the composition and water solubility of these two types of welding fume. MMA-HS contains more Mn and less Fe than GMA-MS. Additionally, MMA-HS also contains Cr and has higher water solubility than GMA-MS (Antonini et al., 2012). A review of welder and animal studies confirmed that pulmonary exposure to the welding fume with a higher percentage of Mn and higher water solubility caused great pulmonary reactions (Antonini et al., 2003). The current study indicates that not only does the response vary in the pulmonary system due to exposure to the different welding fumes, but also the sensitivity of cardiovascular changes varies.

From the results of this study, it appears that intratracheal instillation of welding fumes could be a risk factor which increases the incidence of cardiovascular events. Unfortunately at this time, the mechanism by which welding fume exposure alters cardiac function is unclear and requires further investigation. There are several potential possibilities that may contribute to the alterations in cardiac function by welding fume exposure observed in our study. First, it has been demonstrated that intratracheal instillation of either MMA-HS or GMA-MS induces significantly lung inflammation or injury, which results in the release of large amount of chemokines and cytokines in the lung (Antonini et al., 2010; Leonard et al., 2010). These small signaling proteins, including MCP-1, MIP-2, TNF $\alpha$  and Il-6, may enter into the circulatory system and induce adverse cardiac effects (Farina et al., 2013). In addition, a study on shipyard welders suggests that pulmonary exposure to welding fumes can induce a systemic oxidative stress response (Han et al., 2005). The heart has been proven to be particularly prone to oxidative stress due to its continuous contractile activity, high metabolic demand and loss of ability to regenerate in adulthood. Moreover, antioxidants and antioxidant enzyme activities of superoxide dismutase, glutathione peroxidase, glutathione and  $\alpha$ -tocopherol are found to be lower in the heart of experimental animals than in other organs. Therefore, it is very likely that the heart may be affected or damaged by welding fume-induced systemic oxidative stress. Second, it has been confirmed that some components, for instance, iron and chromium, of welding fumes can enter into the systemic circulation and deposit in organs such as liver, spleen and kidney after they are inhaled into the lung. Mn, the major component in MMA-HS, was found to deposit in the heart after exposure to MMA-HS (Antonini et al., 2010). Therefore, it is possible that once the components of welding fume circulate in the blood and deposit in the heart, they may induce direct toxicity to the heart. Whether deposited Mn in the heart could cause toxicity and affect cardiac function as observed in this study are not clear and warrant further investigation. Third, we and other investigators have shown that pulmonary inhalation of small particles like nanoparticles can change the activity of the autonomic nerves that regulate cardiovascular function (Kan et al., 2014; Nurkiewicz et al., 2011). The size of

primary welding particles is less than 100 nm; therefore, they may behave like nanoparticles after being inhaled (Antonini et al., 2011; Brand et al., 2013).

In this study, alteration in cardiac diastolic function indicated by reduced-LV  $dP/dt_{\min}$  and increased LVEDP in response to adrenoceptor stimuli suggest that welding fumes can affect adrenoceptor signaling pathways. We reported previously that pulmonary exposure to nanoparticles alters adrenoceptor signaling activity through a neuron-regulated pathway by activating sensory receptor in the lung and increasing of substance P synthesis in nodose ganglia (Kan et al., 2014). In order to determine whether welding fume-induced cardiac dysfunction was regulated through a sensory receptor/neuronal pathway, we examined substance P synthesis in nodose ganglia after welding fume treatment, and we did not find any changes in substance P synthesis in nodose ganglia (data are not shown). This result could suggest that the welding fume-induced cardiac dysfunction observed in our study is less likely through a lung sensory receptor/ neuron-regulated mechanism. Considering the findings that pulmonary exposure to welding fumes induces lung inflammation and systemic oxidative stress, and the fact that inhalation of welding fumes is able to cause toxicity in the remote organs other than lungs, welding fume-induced alterations in cardiac function may be regulated through inflammation and oxidative stress pathway, or through a direct local toxicity mechanism due to welding fume translocation. However, the sensory receptor/ neuronal mechanism cannot be discounted, since the timing of assays for substance P may have missed a response due to the exposure lasted over a seven-week period.

In conclusion, our *in vivo* observations suggest that pulmonary exposure to welding fumes causes toxicity and damage not only in the lung, but also in the cardiovascular system. The study also suggests that the effects of welding fume on the cardiovascular system are dependent on the characteristics of different types of welding fumes and that the heart may be sensitive to the development of dysfunction. In the present study, since all the assessments were made in rats under anesthesia which impacts cardiovascular function, further study to assess cardiovascular function in conscious, freely moving rats is warranted.

## Acknowledgments

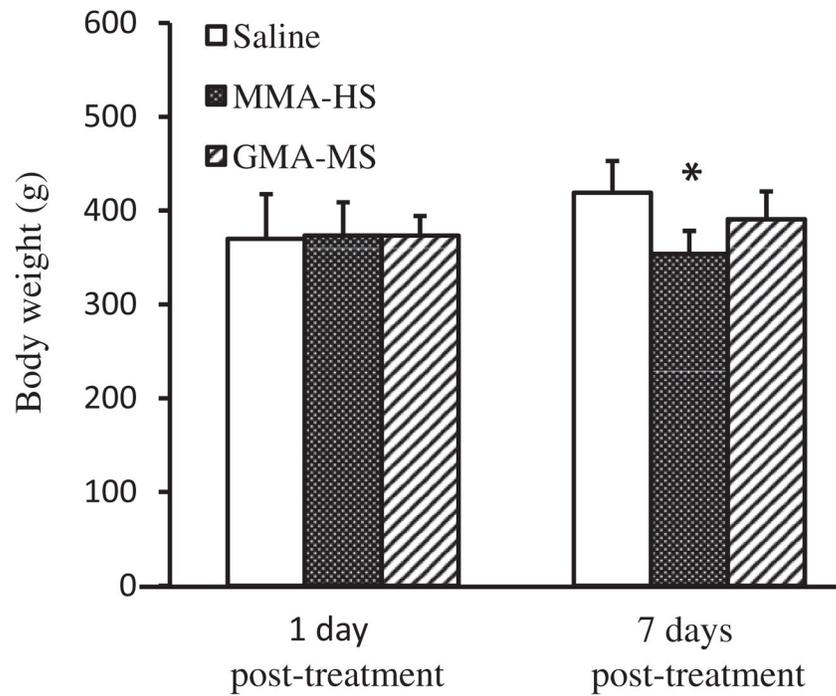
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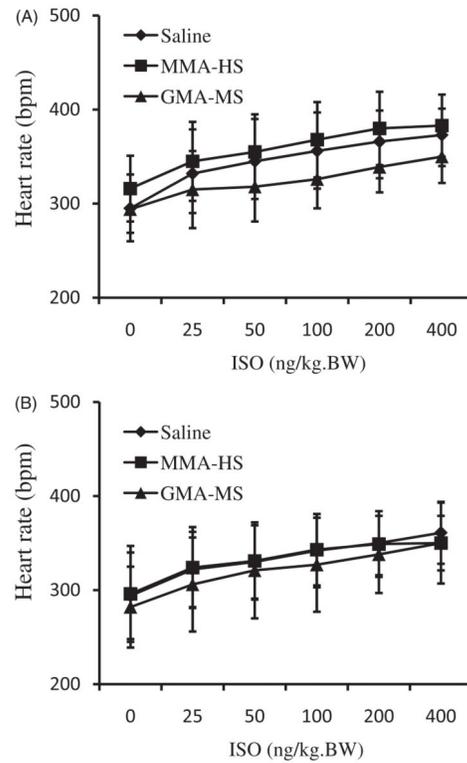
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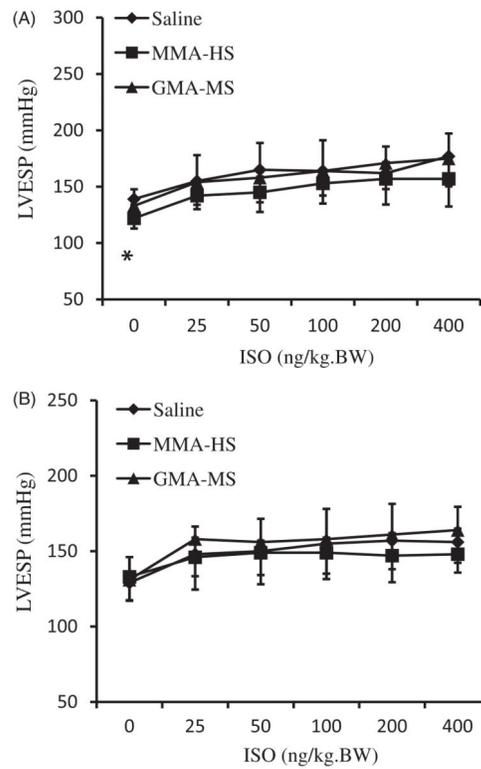


**Figure 1.**

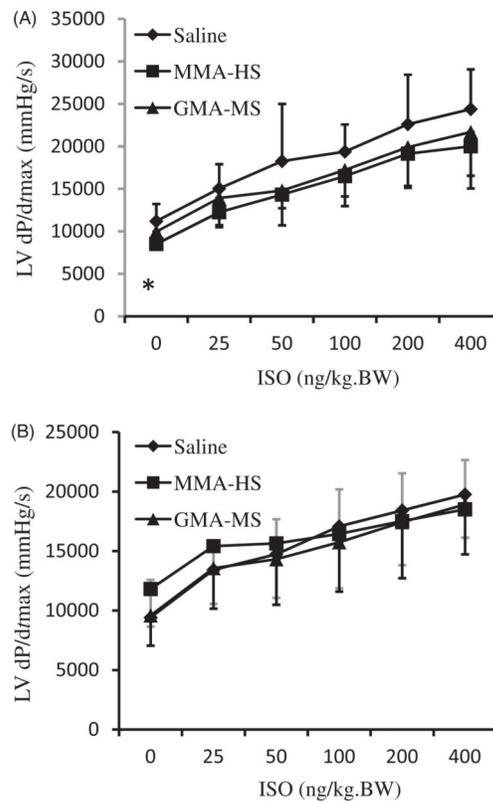
Body weights at 1 and 7 days after the last intratracheal instillation of MMA-HS or GMA-MS. The initial body weights (in grams) right before the treatment of welding fumes were  $228 \pm 6.5$ ,  $232 \pm 5.5$ ,  $235 \pm 11$  (control versus MMA-HS versus GMA-MS, respectively) for 1 day post-treatment study, and were  $233 \pm 9.4$ ,  $237 \pm 6.9$ ,  $244 \pm 8.2$  (control versus MMA-HS versus GMA-MS, respectively) for 7 days post-treatment study. Each value represents the mean  $\pm$  SD of 6–8 rats; \* $p < 0.05$  compared with the control group.



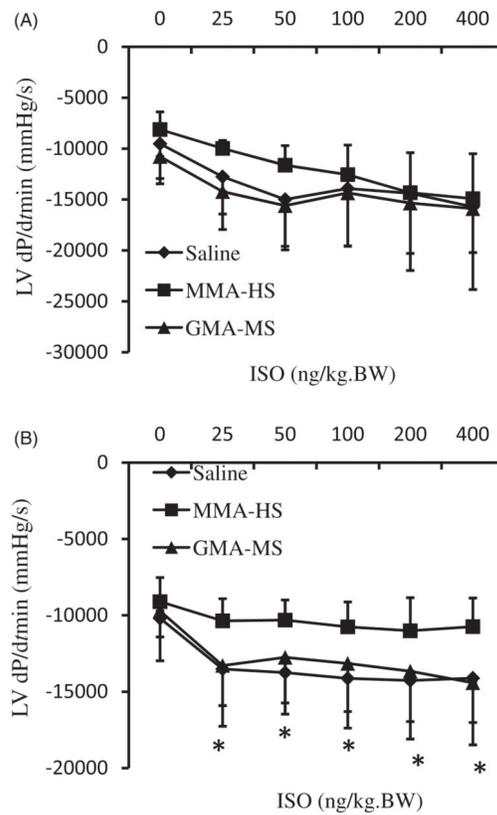
**Figure 2.** Effects of intratracheal instillation of welding fumes on HR. (A) The dose-response curve of HR in response to ISO at 1 day post-treatment. (B) The dose-response curve of HR in response to ISO at 7 days post-treatment. Each value represents the mean  $\pm$  SD of 6 rats;  $p > 0.05$  compared with the control group.



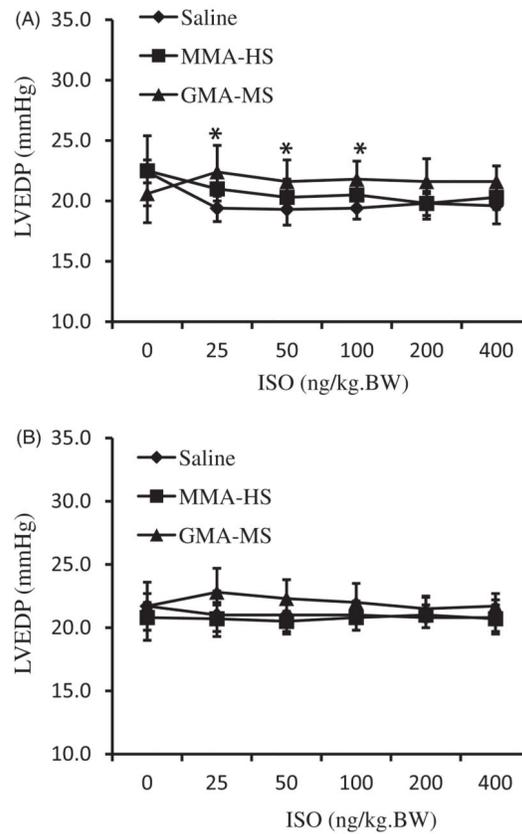
**Figure 3.** Effects of intratracheal instillation of welding fumes on LVESP. (A) The dose-response curve of LVESP in response to ISO at 1 day post-treatment. (B) The dose-response curve of LVESP in response to ISO at 7 days post-treatment. Each value represents the mean  $\pm$  SD of six rats; \* $p < 0.05$  compared with the control group.



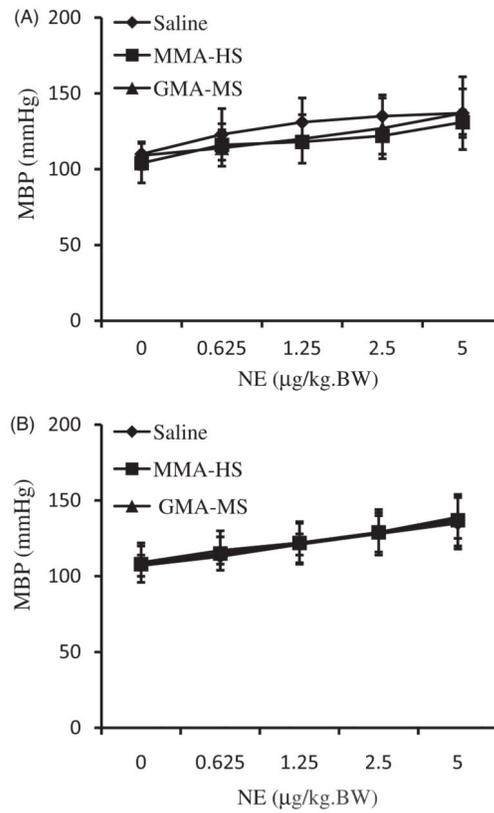
**Figure 4.** Effects of intratracheal instillation of welding fumes on contractile function of the heart. The contractile function of the heart was assessed by measuring the maximum rate of increase in left ventricular pressure ( $dP/dt_{max}$ ). (A) The dose-response curve of LV  $dP/dt_{max}$  in response to ISO at 1 day post-treatment. (B) The dose-response curve of LV  $dP/dt_{max}$  in response to ISO at 7 days post-treatment. Each value represents the mean  $\pm$  SD of six rats; \* $p < 0.05$  compared with the control group.



**Figure 5.** Effects of intratracheal instillation of welding fumes on diastolic function of the heart. Diastolic function of the heart was assessed by measuring the minimum rate of decrease in left ventricular pressure (LV dP/dt<sub>min</sub>). (A) The dose-response curve of LV dP/dt<sub>min</sub> in response to ISO at 1 day post-treatment. (B) The dose-response curve of LV dP/dt<sub>min</sub> in response to ISO at 7 days post-treatment. Each value represents the mean ± SD of six rats; \**p*<0.05 compared with the control group.



**Figure 6.** Effects of intratracheal instillation of welding fumes on LVEDP. (A) The dose-response curve of LVEDP in response to ISO at 1 day post-treatment. (B) The dose-response curve of LVEDP in response to ISO at 7 days post-treatment. Each value represents the mean  $\pm$  SD of six rats; \* $p < 0.05$  compared with the control group.



**Figure 7.** Effects of intratracheal instillation of welding fumes on MBP. (A) The dose-response curve of MBP in response to NE at 1 day post-treatment. (B) The dose-response curve of MBP in response to NE at 7 days post-treatment. Each value represents the mean  $\pm$  SD of six rats;  $p > 0.05$  compared with the control group.

**Table 1**

Metal composition and solubility properties of welding fume samples.

| <b>Fume samples</b> | <b>Weight % total metals</b>  | <b>Soluble/ insoluble ratio</b> | <b>% Soluble/total metals<sup>a</sup></b>                |
|---------------------|---|---------------------------------|--|
| <b>GMA-MS</b>       | <b>Fe 72.2</b><br><b>Mn 21.7</b>  | <b>0.0139</b>                   | <b>1.35 (Fe 0.482, Mn 47.3, K 42.9)</b>                  |
| <b>MMA-HS</b>       | <b>Fe 19.3</b><br><b>Mn 50.9</b><br><b>Cr 8.46</b><br><b>K 12.1</b><br><b>Na 6.73</b><br><b>Ni 0.09</b> | <b>0.2182</b>                   | <b>19.4 (Fe 1.39, Mn 1.43, Cr 15.1, K 56.0, Na 25.4)</b> |

Metal composition was determined in particles suspended in saline by inductively coupled plasma atomic emission spectroscopy (ICP-AES) as adapted from Antonini et al. (2010).

<sup>a</sup>Relative to all metals analyzed.

Table 2

Baseline hemodynamic parameters and indices of cardiovascular function.

| Parameter                        | 1 Day post-exposure |              |                | 7 Days post-exposure |              |               |
|----------------------------------|---------------------|--------------|----------------|----------------------|--------------|---------------|
|                                  | Saline              | MMA-HS       | GMA-MS         | Saline               | MMA-HS       | GMA-MS        |
| HW (g)                           | 1.18 ± 0.10         | 1.21 ± 0.14  | 1.20 ± 0.11    | 1.35 ± 0.10          | 1.31 ± 0.05  | 1.35 ± 0.11   |
| HW/BW (mg/g)                     | 3.2 ± 0.4           | 3.24 ± 0.5   | 3.34 ± 0.3     | 3.24 ± 0.2           | 3.72 ± 0.3*  | 3.46 ± 0.2    |
| HR (bpm)                         | 295 ± 35            | 316 ± 35     | 294 ± 25       | 293 ± 45             | 296 ± 21     | 282 ± 43.9    |
| LVESP (mmHg)                     | 138 ± 8.8           | 121 ± 9.1*   | 133 ± 11.6     | 129 ± 11             | 133 ± 15     | 131 ± 13.9    |
| LVEDP (mmHg)                     | 22 ± 2.2            | 23 ± 3       | 20.6 ± 1.5     | 22 ± 3               | 21 ± 1       | 21.7 ± 1.9    |
| LV dP/dt <sub>max</sub> (mmHg/s) | 11 193 ± 2030       | 8715 ± 500*  | 9961 ± 1668    | 9468 ± 2122          | 11 805 ± 265 | 69 585 ± 2532 |
| LV dP/dt <sub>min</sub> (mmHg/s) | -9523 ± 2418        | -8121 ± 1728 | -10 771 ± 2672 | -10 192 ± 1223       | -9104 ± 1581 | -9717 ± 3257  |
| MBP (mmHg)                       | 110 ± 6             | 104 ± 13     | 109 ± 8.6      | 107 ± 7              | 108 ± 12     | 110 ± 13.6    |
| SBP (mmHg)                       | 132 ± 11            | 125 ± 17     | 130 ± 9.7      | 122 ± 10             | 128 ± 12     | 130 ± 20.5    |
| DBP (mmHg)                       | 93 ± 10             | 87 ± 11      | 91 ± 7.4       | 87 ± 8               | 90 ± 10      | 92 ± 10.2     |

HW, heart weight; HW/BW, heart weight to body weight ratio; HR, heart rate; LVESP, left ventricular end-systolic pressure; LVEDP, left ventricular end-diastolic pressure; LV dP/dt<sub>max</sub>, the maximum rate of increase in left ventricular pressure, LV dP/dt<sub>min</sub>, the minimum rate of decrease in left ventricular pressure; MBP, mean blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure.

\*  $p < 0.05$  versus control (saline).