Effect of Process Parameters Upon the Dopamine and Lipid Peroxidation Activity of Selected MIG Welding Fumes as a Marker of Potential Neurotoxicity

N. J. HUDSON†, A. T. EVANS‡*, C. K. YEUNG‡ and P. J. HEWITT†

†*Dept of Environmental Science, University of Bradford, Bradford BD7 1DP, UK*; ‡*School of Pharmacy, University of Bradford, Bradford BD7 1DP, UK*

There is growing concern over the neurotoxic effects of chronic occupational exposure to metal fume produced by welding. Elevated iron and manganese levels in the brain have been linked to an increase in lipid peroxidation, dopamine depletion and predisposition to the development of a Parkinson's type condition in advanced cases. Chemical and toxicological analysis of selected welding fumes, generated by model processes, were used in order to evaluate their potential to release solutes that promote oxidation of dopamine and peroxidation of brain lipids in cell free assays. This study compared the effect of shield gas, electrode type and voltage/current upon the dopamine and brain lipid peroxidation potential of selected welding fume, obtained from metal inert gas (MIG) welding systems. Overall, fume extracts were found to enhance dopamine oxidation and inhibit lipid peroxidation. Significant differences were also found in the oxidising potential of fume generated under differing process conditions; it may therefore be possible to determine the potential neurotoxicity of fumes using this system. © 2001 British Occupational Hygiene Society. Published by Elsevier Science Ltd. All rights reserved

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INTRODUCTION

The widespread use of welding in the steel processing industry raises a number of occupational health issues concerning both the immediate and long-term health risks posed to workers employed within this sector. Welders as a group are exposed to a number of occupational health hazards, which are intrinsic to the welding process. One particular aspect of concern is directed to the long-term occupational health implications due to chronic exposure to metal fumes generated during the processing of steel alloys (Tanala, 1996; Castner and Null, 1998). The potential occupational health risks posed by chronic welding fume inhalation have been reported to include the development of specific neuropathological disorders, characterised by psychotic changes, choreoform type symp-toms and a Parkinson's like condition in advanced cases. This syndrome, termed manganism, has been attributed to chronic exposure to soluble compounds of manganese, and possibly iron, in fume generated by industrial metal processes such as welding, and has been reported in a number of case studies conducted upon workers employed within the metal processing industry. In one recent study, a 48 year old welder was shown to have symptoms representative of the idiopathic Parkinsonism associated with long-term exposure to metal fume that contains manganese (Kim *et al.*, 1999). Concentrations of manganese in blood and urine samples, taken after two months of cessation of exposure, were 3.26 mg/dl and 3.57 mg/l. respectively. Other studies have also demonstrated early symptoms of manganese poisoning in welders. The symptoms were characterised by behavioural changes and reduction in short-term memory, symptoms characteristic of organic brain damage due to chronic inhalation of fumes generated by the processing of steel alloys, using welding consumables that contain manganese (Barrington *et al.*, 1998). The

results of this study reported cases of manganese dementia in workers employed in the welding and processing of alloys containing high levels of manganese.

Fume generated by MIG welding of mild steel alloys, mainly consists of mixed metal oxides, 'spinels', where the metal cation is typically present in the divalent or trivalent oxidation state. The composition of the welding fume is determined by the electrode type, the metal alloy being welded and the process conditions employed i.e. shield gas mixture and the voltage/current setting. Flux components, present in cored electrodes such as Tubrod 15.00, are composed of compounds of elements such as silicon, sodium, potassium and fluorine etc., also contribute to the overall fume composition and may therefore also influence the dopamine and brain lipid oxidation potential of the fume.

The electrode composition, voltage/current and shield gas employed determine the composition of the fume and hence its physico-chemical properties (Voitkevich, 1986). These are important factors in determining the ability of fume particles to enter the respiratory system and the subsequent dissolution rate of fume leachates into lung alveoli and hence the blood plasma level of the fume components. This influences their ability to affect dopaminergic systems, and possibly levels of other neurotransmitters present within the central nervous system (CNS). Manganese is transported in the blood primarily in the divalent oxidation state but is, however, able to cross the blood–brain barrier, via specific carriers, in both the divalent and trivalent oxidation states (Aschner *et al.*, 1999). Accumulation of manganese within the CNS occurs mainly in the astrocytes, due to binding with the astrocyte-specific enzyme glutamine synthase. The potential neurotoxicity of the fume may therefore be highly dependent on selected process parameters.

Both manganese and iron have been reported to be synergistic in causing the direct oxidation of dopamine to melanin, due to the production of hydrogen peroxide via an iron catalysed Fenton type reaction and the subsequent release of hydroxy-free radicals within dopamine containing cells in the brain striatum. This may result in the depletion of dopamine within the substantia nigra and may therefore be implicated in the mode of pathogenicity associated with the neurological disorders attributed to the long-term inhalation of metal fume containing soluble compounds of manganese and iron (Barrington *et al.*, 1998). It has also been suggested that a manganese induced neuronal necrosis may occur, mediated by an oxidative cascade within the dopinergic brain regions, by the synergism between manganese and dopamine. This cascade may cause the oxidation of DNA, proteins and brain lipids, resulting in mithochondrial and cellular damage (Brenneman *et al.*, 1999). Paradoxically, a recent study has suggested that low levels of manganese may even be neuroprotective, due to the binding of manganese to specific sites in the fourth iron site of aconitase, thus resulting in the suppression of energy and iron metabolism within the brain. (Sziraki *et al.*, 1998; Zheng *et al.*, 1998).

In this study we provide a comparison between the oxidative potential of MIG fume solutes and soluble compounds of iron, manganese, nickel and chromium upon dopamine and brain lipids. The results also compare the dopamine and brain lipid oxidation potential of metal fumes produced by MIG welding, systems, enabling the effect of the electrode type, voltage/current and shield gas composition upon the dopamine oxidation potential of the metal fumes generated to be compared.

MATERIALS AND METHODS

Generation and sampling of welding fume

Fume samples were generated by metal inert gas (MIG) welding of mild steel plates (0.300×0.300×0.01 m) using industrial solid steel and flux cored electrodes, in a conical welding chamber, with a rotating table upon which the workpiece was situated. Three replicate fume samples were collected onto 0.15 m diameter glass fibre filters using an extraction pump to draw air and fume through the chamber.

The welding rig consisted of a BOC SMR 500 constant voltage rectifier with a BOC TF 2.0S feed unit. The welding gun was a BOC MG5/MXA503 air-cooled device fitted with a 0.025 m tapered gas nozzle.

Process parameters

Nominal voltage:	30 and 40 V at positive plate polarity
Stand-off distance from the welding gun nozzle to the surface of the workpiece (mild steel metal sheet):	0.020 m
Shield gas mixture:	(*a*) CO$_2$ (98% CO$_2$/2% O$_2$) (*b*) Argoshield 5 (95% Ar/5% CO$_2$)
Flow rate:	15 l. min^{-1}
Weld time:	10 s
Torch position:	0.134 m from the centre of rotation
Torch angle:	90° to the direction of travel
Electrodes:	Bostrand BW1 (solid steel electrode) and Tubrod 15.00 (flux-cored electrode)
Wire diameter:	0.0012 m

Elemental analysis of welding fume

25 mg of welding fume was fused with $KHSO_4$ (AR Grade) for 60 min at 650°C and digested in 50 ml of 2.5 mol dm^{-3} HNO_3. Following dilution in double distilled water, the iron and manganese content was determined by emission, using a Philips PU 7450 ICP/AES. The wavelengths used for analysis of iron and manganese were 259.9 nm (Fe) and 257.1 nm (Mn) respectively.

Fume sample preparation for toxicological assay

Fume samples were suspended in Dulbeco's modified Eagle's medium, without phenol red, at a concentration of 10 mg ml^{-1}. The suspensions were shaken at 37°C for 2 h and centrifuged at 10 000*g* for 15 min. The supernatant was removed for analysis.

Analysis of the dopamine oxidation potential of welding fume samples

Dopamine was added to 200 µl aliquots of each supernatant sample at a final concentration of 10 mg ml^{-1} and the oxidation rate measured as an increase in absorbance at 550 nm over 10 min.

Analysis of the lipid peroxidation potential of welding fume samples

100 µl aliquots of fume supernatant extracts were mixed with equal volumes of fresh mouse brain homogenate, prepared in 4 volumes of Dulbeco's modified Eagle's medium. The mixture was then incubated at 37°C with shaking for 1 h; 1 ml of 0.375% thiobarbituric acid and 15% trichloroacetic acid in 0.25 M HCl was added to the mixture and boiled for 15 min. After removal of the precipitate by centrifugation at 1000*g* for 10 min, the absorbance of 200 µl aliquots was measured at 550 nm. Both the dopamine and lipid peroxidation assays were performed in triplicate.

RESULTS

Effect of process parameters upon dopamine oxidation potential of welding fume solutes

The results obtained from toxicological assays upon welding fume solutes demonstrated a significantly higher dopamine oxidation rate compared to controls (mean control 0.0899); test range 0.131–0.223 (+0.001–0.056), $P<0.05$ Anova and Bonferroni *t*-test (Fig. 1). Elemental analysis of the welding fumes generated in this study showed iron and manganese to be the main elements present with smaller amounts of chromium and nickel also contributing to the fume (Table 1). Dopamine oxidation assays conducted upon soluble compounds of these elements showed a significantly higher dopamine oxidation potential for Fe^{2+} and Mn^{2+} compared with Cr^{6+} and Ni^{2+}, (Fig. 2). N.B. the dichromate salt was used in this study due to the ability of hexavalent chromium to cross cell membranes, unlike divalent chromium, which is not readily taken up by cells.

Metal fume generated by welding using the solid steel electrode (BW1) generally showed a lower dopamine oxidation rate, per shield gas type, compared to the flux cored electrode (15.00) (Fig. 1). Elevated dopamine oxidation rates were also found in metal fumes produced using high levels of CO_2 (98% CO_2/2% O_2) in the shield gas compared to shield gas mixtures consisting mainly of Ar (95%Ar/5%CO_2) per electrode type. The potential difference and current across the welding arc was also found to influence the dopamine oxidation potential of the metal fume. At the 30 V setting, the fume produced was found to have an increased dopamine oxidation potential compared to that generated by using a 40 V setting per electrode type (Fig. 1). As the current across the arc increased, the dopamine oxidation potential of the metal fume was found to generally decrease for both the solid steel and flux cored electrode.

Effect of process parameters upon brain lipid peroxidation rates of welding fume solutes

The welding fume solutes studied, generally demonstrated a lower rate of brain lipid peroxidation compared to control assays (Fig. 3). *In vitro* toxicological studies showed the brain lipid peroxidation potential to be higher for metal fume solutes generated by welding using the solid steel electrode (BW1) and the CO_2 shield gas than fume produced using an Ar shield gas mixture at both voltage settings. Welding using the flux cored electrode (15.00) did, however, show an elevated rate of brain lipid peroxidation, per voltage setting, using the 95% Ar/5% CO_2 shield gas mixture.

The current across the welding arc was found to influence the brain lipid peroxidation potential of the welding fume. An increase in current was shown to coincide with a decrease in the rate of brain lipid oxidation potential of the welding fume per electrode and shield gas, with fume generated at 30 V settings being generally higher than that of fume produced at 40 V.

Elemental analysis of welding fume and effect upon dopamine and brain lipid peroxidation

Process parameters, were shown to influence the ratio of iron and manganese in the fume generated by the welding systems studied and the rate of dopamine and brain lipid peroxidation. As the ratio of iron to manganese in the welding fume increased, the dopamine oxidation potential of the welding fume was found to decrease accordingly, per electrode type (Fig. 1). Fume produced by welding using the flux cored electrode (15.00), at a 30 V setting and CO_2 shield gas, contained iron and manganese in the ratio 8:1. This fume did, however, show a higher dopamine oxidation rate compared with fume containing lower ratios of these elements for the same electrode.
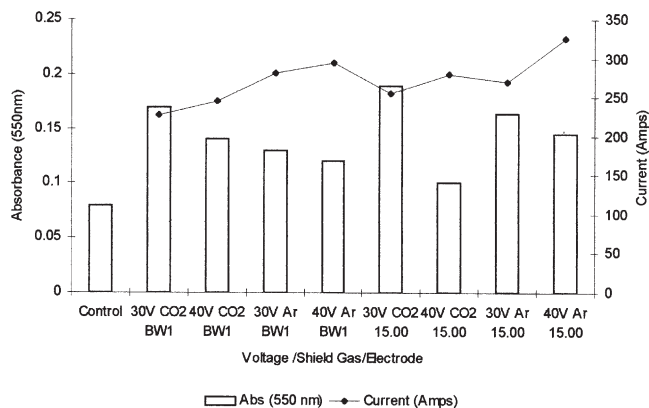
Fig. 1. Comparison between dopamine oxidation levels of welding fume solutes with current using solid and flux cored electrodes.

Table 1. Comparison between iron and manganese levels in welding fume with process parameters

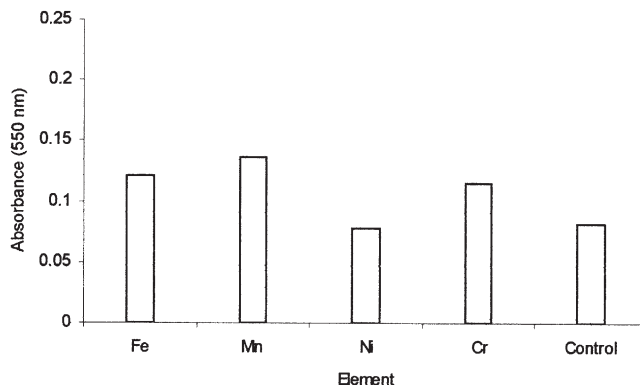| Electrode	Shield gas	Voltage (V)	Current (A)	Resistance (Ω)	% Fe	% Mn	Ratio Fe:Mn
BW1	CO_2	30	228	0.132	52.1	12.0	4:1
BW1	CO_2	39	245	0.159	55.8	6.1	9:1
BW1	Ar5	31	281	0.110	54.9	4.7	12:1
BW1	Ar5	40	290	0.138	59.1	6.6	12:1
15.00	CO_2	31	255	0.122	50.3	6.5	8:1
15.00	CO_2	40	280	0.143	49.5	5.3	9:1
15.00	Ar5	28	270	0.111	33.3	9.5	4:1
15.00	Ar5	39	325	0.123	45.0	8.9	5:1

Fig. 2. Comparison between dopamine oxidation levels of soluble compounds of metals found in welding fume.

The results obtained from the brain lipid peroxidation assays demonstrated a similar trend to the dopamine oxidation analysis in the relationship between the iron to manganese ratio and the oxidative potential of the fume. The fume produced by welding using the solid steel electrode (BW1) at 30 V and an Ar shield gas, produced fume with a 12:1 iron to manganese ratio. This also resulted in a higher rate of brain lipid peroxidation compared to fume with lower ratios of these elements for the same electrode type.

DISCUSSION

Analysis of welding fume showed that iron and manganese were the main elemental components generated by the welding systems employed in this study. Chromium and nickel were also found to be present, but in substantially lower levels. Toxicological assays conducted upon equimolar concentrations of soluble compounds of Fe^{2+} and Mn^{2+}, demonstrated significantly higher dopamine oxidation rates compared to
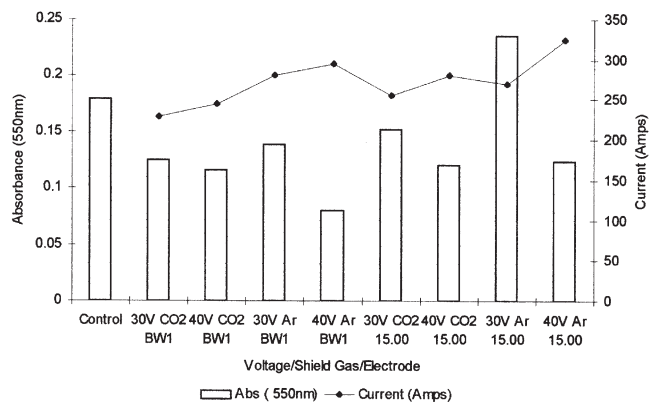
Fig. 3. Comparison between brain lipid peroxidation levels of welding fume solutes with current using solid and flux cored electrodes.

soluble metal compounds of Ni^{2+} and Cr^{6+}. This strongly suggests that iron and manganese are the main causative agents in the oxidation process of dopamine to melanin observed in the toxicological studies conducted upon actual welding fume solutes. The mode of dopamine oxidation by welding fume solutes may be due to the ability of Fe^{2+} to react with H_2O_2 and promote the release of cytotoxic hydroxy-free radicals, via a Fenton reaction, and Mn^{2+} to act as a catalyst in the oxidation process of dopamine to melanin (Linert *et al.*, 1996). The rate of dopamine oxidation was, however found to be generally lower for individual metal compounds compared to actual welding fume extracts. This also suggests that both the oxidation state and phase composition of the welding fume are important factors in determining its dopamine oxidation potential and hence potential neurotoxicity. These results further indicate that the mechanism of dopamine oxidation is too selective to be due to iron alone, and that a synergistic or additive relationship may therefore exist between iron and manganese in the oxidative process of dopamine and brain lipids due to exposure to welding fume solutes. These results are in agreement with the findings of Zheng *et al.* (1998), strongly indicating that manganese is a main causative agent in the dopamine oxidation process.

In vitro toxicological assays conducted upon homogenates of rodent brain tissue exposed to metal fume extracts, generally demonstrated a lower rate of brain lipid peroxidation compared to controls. This precludes brain lipid peroxidation as being an underlying mechanism in the mode of neuropathogenicity associated with chronic exposure to metal fumes containing iron and manganese.

Toxicological assays conducted upon metal fume solutes generated by welding using the flux cored electrode, generally showed a higher dopamine oxidation rate compared to that produced by welding using the solid steel electrode per voltage setting and shield gas mixture. These results suggest that flux components contribute to the rate of dopamine oxidation and may result in the evolution of metal fume with an elevated potential neurotoxicity. Other process parameters were also demonstrated to affect the rate of dopamine oxidation. A higher dopamine oxidation rate was found in fume samples produced using the 30 V setting compared with that produced at 40 V per electrode type and shield gas mixture. This coincides with an increase in the ratio of iron to manganese, suggesting that the voltage setting is an important factor in determining the potential neurotoxicity of welding fume and also that ratio of iron to manganese is an important factor in determining the dopamine oxidation rate.

The shield gas composition was shown to influence the dopamine oxidation potential of the welding fumes studied. Welding using a 98% CO_2/2% O_2 mixture resulted in the evolution of fume with a higher dopamine oxidation potential compared to the 95% Ar/5% CO_2 mixture per electrode type and voltage setting. This may be attributed to the ability of carbon dioxide to act as an oxidising medium at the temperatures present across the welding arc and result in production of fume which is composed of higher oxidation state transition elements, thus increasing the dopamine oxidation potential of the fume. The presence of 2% O_2 in the CO_2 shield gas may also contribute to the elevated oxidising potential observed upon dopamine. This effect may be attenuated in fume with high ratios of iron to manganese, where lower dopamine oxidation rates were generally observed per electrode type and voltage setting.

The current and resistance across the welding arc were also shown to influence the dopamine oxidation potential of the fume. High resistance values were demonstrated to result in the evolution of fume with

a reduced dopamine oxidation potential per voltage setting/electrode type, with the carbon dioxide shield gas mixture demonstrating the highest level. This may be due to the higher temperatures produced during welding with this shield gas mixture compared to the argon mixture.

CONCLUSIONS

This study has demonstrated the ability of welding fume solutes to cause the oxidation of dopamine to melanin and consequently that the metal fumes produced by MIG welding may represent a serious occupational health hazard due to the potential neurotoxicity of the metal fumes generated. Modern health and safety protocol recommends that the inherent health hazards associated with an industrial process be reduced at source. Due to the complex series of interactions which occur between the different process parameters employed during MIG welding, an holistic approach to hazard reduction must be applied, in order that the potential hazards are assessed in terms of the system as a whole and not as individual components within the system. It is also imperative to ensure that the modifications made to the process do not solve one problem but create others, which may present greater inherent occupational health hazards than those they replaced.

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