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LUNG TUMOR PRODUCTION AND TISSUE METAL DISTRIBUTION AFTER EXPOSURE TO MANUAL METAL ARC–STAINLESS STEEL WELDING FUME IN A/J AND C57BL/6J MICE

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Stainless steel welding produces fumes that contain carcinogenic metals. Therefore, welders may be at risk for the development of lung cancer, but animal data are inadequate in this regard. Our main objective was to examine lung tumor production and histopathological alterations in lung-tumor-susceptible (A/J) and -resistant C57BL/6J (B6) mice exposed to manual metal arc-stainless steel (MMA-SS) welding fume. Male mice were exposed to vehicle or MMA-SS welding fume (20 mg/kg) by pharyngeal aspiration once per month for 4 mo. At 78 wk postexposure, gross tumor counts and histopathological changes were assessed and metal analysis was done on extrapulmonary tissue (aorta, heart, kidney, and liver). At 78 wk postexposure, gross lung tumor multiplicity and incidence were unremarkable in mice exposed to MMA-SS welding fume. Histopathology revealed that only the exposed A/J mice contained minimal amounts of MMA-SS welding fume in the lung and statistically increased lymphoid infiltrates and alveolar macrophages. A significant increase in tumor multiplicity in the A/J strain was observed at 78 wk. Metal analysis of extrapulmonary tissue showed that only the MMA-SS-exposed A/J mice had elevated levels of Cr, Cu, Mn, and Zn in kidney and Cr in liver. In conclusion, this study further supports that MMA-SS welding fume does not produce a significant tumorigenic response in an animal model, but may induce a chronic lung immune response. In addition, long-term extrapulmonary tissue alterations in metals in the susceptible A/J mouse suggest that the adverse effects of this fume might be cumulative.

Approximately 3 million workers perform welding as part of their work duties. Welding processes produce vaporized metals, derived primarily from the consumable electrode wire. These metals react with air and form the fume, which consists of a complex mixture of metal oxides. Depending on the welding process employed, the electrode coating, shielding gases, fluxes, base metal, and paint or surface coatings also may comprise the welding fume (Antonini, 2003). Shielded manual metal arc (MMA) welding is the most common type of welding used worldwide (Harris, 2002). MMA welding with a stainless steel (SS) electrode

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produces fumes that contain carcinogenic metals such as Cr and Ni; therefore, a primary health concern for welders is the development of lung cancer.

The harmful health effects of welding are well documented, and epidemiological evidence generally supports the hypothesis that exposure to welding fume increases lung cancer risk, but confounders such as asbestos exposure and smoking obscure these findings (Danielsen et al., 1998; Moulin, 1997; Sorensen et al., 2007; Sferlazza & Beckett, 1991). The International Agency for Research on Cancer (IARC) classifies welding fume as *possibly carcinogenic to humans* (Group 2B), based on limited evidence in humans and inadequate evidence in animals (IARC, 1990).

This study is a continuation of our ongoing research, which has used the lung-tumor-susceptible A/J mouse, a common animal model for lung carcinogenesis studies. Compared to the lung-tumor-resistant C57BL/6J (B6) strain, A/J mice exhibit high susceptibility to spontaneous and chemically induced lung tumors (Shimkin & Stoner, 1975). Further, the lung tumors in the A/J mouse display many similarities to human pulmonary adenocarcinomas, which makes them a relevant model for lung cancer research (Malkinson, 1998; Meuwissen & Berns, 2005). Our previous study characterized and compared the lung inflammatory response in both the A/J and B6 mouse strains and examined the tumorigenic potential of different welding fumes, including MMA-SS fume (Zeidler-Erdely et al., 2008). No evidence of MMA-SS-induced tumorigenesis with a repeated, low-dose (equivalent to a total of approximately 196 d of welding) exposure protocol was found (Zeidler-Erdely et al., 2008). However, Solano-Lopez et al. (2006) found persistent atypical hyperplastic bronchiolar and peribronchiolar changes in A/J mice exposed to higher doses of this fume. Here, as a continuation of previous investigations, A/J and B6 mice were given a dose that reflects approximately 4 yr of exposure to further elucidate the potential in vivo tumorigenic effects of this commonly used welding fume. As a second objective, extrapulmonary tissue was examined at 78 wk postexposure in both mouse strains for alterations in metal concentrations after the initial lung exposure. Because significant strain-dependent differences in the lung response to welding fume between A/J and B6 mice were observed, it was postulated that cellular metal processing and transport of welding fume-derived metals after pulmonary deposition and eventual translocation to other organs may also differ.

METHODS

Animals

Male A/J and B6 mice, 6–8 wk of age weighing 21–25 g, were purchased from Jackson Laboratories (Bar Harbor, ME) and housed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited, specific-pathogen-free, environmentally controlled facility. All mice were free of endogenous viral pathogens, parasites, mycoplasmas, *Helicobacter*, and CAR bacillus. Mice were individually housed in ventilated cages and provided HEPA-filtered air under a controlled light cycle (12-h light/dark) at a standard temperature (22–24°C) and 30–70% relative humidity. Animals were acclimated to the animal facility for a minimum of 2 wk and allowed access to a conventional diet (6% Irradiated NIH-31 Diet, Harlan Teklad, Madison, WI) and tap water ad libitum. All procedures were performed using protocols approved by the National Institute for Occupational Safety and Health Institutional Animal Care and Use Committee.

Welding Fume Collection and Characterization

The welding fume used in this study was provided by Lincoln Electric Co. (Cleveland, OH). The collection and characterization of the fume were previously described (Antonini et al., 1999). Briefly, the fume was generated in a cubical open-front fume chamber (volume = 1 m^3) by a skilled welder, using a

manual technique, and then collected on a sterile 0.2-μm filter. The sample was generated by manual metal arc welding using a flux-cored stainless steel electrode. After recovery of the fume from the filters, the filters and the total welding fume were analyzed for metal constituents. Due to the soluble nature of the MMA-SS welding fume, the insoluble and soluble fractions were extracted and also analyzed as described by Antonini et al. (1999). Seven different metals (Cr, Cu, Fe, Mn, Ni, Ti, and V) commonly found in welding fumes were measured using inductively coupled argon plasma-atomic emission spectroscopy (see Table 1) (Antonini et al., 1999). No detectable metals were found on the filters which indicated complete recovery of the MMA-SS welding fume. The count mean diameter was 0.92 μm for the MMA-SS sample, as determined by electron microscopy (Antonini et al., 1999).

Welding Fume Preparation

The total welding fume was weighed and suspended in sterile Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (PBS) in a 50-ml sterile conical tube. The fume samples were then vortexed and sonicated (Cole-Parmer ultrasonic model 08849-00). Prior to dosing, the samples were vortexed immediately before each mouse was exposed to ensure a consistent sample. The same total welding fume preparation was used to expose both strains of mice.

Mouse Pharyngeal Aspiration Exposure

Age- and weight-matched mice were exposed to MMA-SS or sterile Ca^{2+}- and Mg^{2+}-free PBS vehicle (sham) by pharyngeal aspiration as previously described (Rao et al., 2003). Briefly, each mouse was placed in a glass jar with a gauze pad moistened with isoflurane (Abbott Laboratories, North Chicago, IL) until slowed breathing was observed. The mouse was then suspended, by its top incisors, on a slanted board in a dorsal recumbent position. The tongue was extended with forceps and the solution was pipetted to the oropharynx. A 60-μl aspiration volume was used and shams were administered an equal volume of PBS. The tongue was held extended until the solution was aspirated into the lung and the mouse resumed a regular breathing pattern. When performed properly, this technique allows minimal sample loss to the digestive tract. The mouse was then returned to its cage to recover, typically 10–15 s. In this study, mice were exposed once per month for 4 mo to a bolus dose of test material in lieu of a single-bolus exposure. This regime achieved an accumulation of particles in the lung over time, which may be more representative of an occupational exposure. Mice were exposed 4 times (once per month) to 20 mg/kg of MMA-SS welding fume. The cumulative fume lung burden (approximately 1.6 mg) was derived from our previous pharyngeal aspiration experiment in the

TABLE 1. Characterization of MMA-SS Welding Fume by ICP-AES

Metal	Total fume sample weight % metals[a]	Soluble fraction (μg/sample)	Insoluble fraction (μg/sample)
Fe	41	0.845 (0.39%)	343 (53.7%)
Cr	29	191 (87%)	63.7 (9.97%)
Mn	17	25.7 (11.7%)	118 (18.4%)
Ni	3	1.43 (0.65%)	21.4 (3.35%)

Note. ICP-AES, inductively coupled argon plasma–atomic emission spectroscopy. For the soluble or insoluble fractions, numbers in parentheses indicate the percent of the metal measured relative to all metals analyzed in that fraction. MMA-SS, manual metal arc–stainless steel welding fume. Data presented were modified from Antonini et al., 1999.

[a]Relative to all metals analyzed. Only trace amounts of Cu, Ti, were found and V was undetectable.

A/J mouse and is equivalent to approximately 4 yr of exposure in a 75-kg welder working an 8-h shift (Solano-Lopez et al., 2006). Mice were euthanized 78 wk after the first exposure. All mice were weighed before the exposure period, at 39 wk, and at the 78-wk sacrifice. No strain- or treatment-related differences in body weight were found (data not shown).

Metal Analysis

Aorta, heart, kidney, and liver were excised, trimmed, and lyophilized. The freeze-dried tissue was weighed then acid digested. The amount of Al, Cr, Cu, Fe, Mn, Ni, Ti, and Zn present in the tissue was determined by ICP-AES at NIOSH-DART (Cincinnati, OH) according to NIOSH method 7300 modified to accommodate the sample matrix (NIOSH, 1994).

Gross Lung Tumor Counts and Histopathology

A/J and B6 mice were euthanized by carbon dioxide asphyxiation, weighed, then the abdomen was opened and the mice were exsanguinated via the vena cava. The whole lung was excised and gross tumor counts and size were recorded for each lung lobe. Apparent merged tumors, defined as a single tumor pattern in double-nodule form or an apparent collision of two different tumors, were counted as one because this was impossible to distinguish at necropsy. The lungs were inflated and fixed with 10% neutral buffered formalin for a minimum of 24 h. Each lung lobe (apical, azygos, cardiac, diaphragmatic, left) was separately embedded in paraffin and then a 5-μm standardized section was cut from each lung lobe. Slides were stained with hematoxylin and eosin and interpreted by a contracted board-certified veterinary pathologist in a blinded fashion for morphological changes and proliferative/neoplastic lesions. If abnormal changes were found, severity was scored as follows: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked, 5 = severe. The final severity score reflects the average of the five lung lobe scores. Proliferative/neoplastic changes were scored as P = preneoplastic epithelial proliferation, AP = adenoma arising within a proliferation, A = adenoma, CA = carcinoma arising within an adenoma, C = carcinoma, or MC = microcarcinoma according to Belinsky et al. (1992). Since examination of a single histological section per lung underestimates the total number of lesions per lung, the gross count at necropsy would be more representative of the response (Rehm & Ward, 1989). However, for completeness, both microscopic and gross exam were statistically evaluated in this study.

Statistical Comparisons and Analysis

All analyses were performed either using JMP version 5.0.1, or the SAS system for Windows version 9.1 (SAS Institute, Inc., Cary, NC). All post hoc comparisons were carried out using Fisher's least significant difference test. Metal concentrations was analyzed using analysis of variance, while histopathology was analyzed with the exact version of the Wilcoxon signed rank test using "Proc npar1way" in SAS. Gross tumor counts and histopathology counts from sections were analyzed similarly. Tumor incidence (presence or absence of tumors) was analyzed using a chi-squared test in SAS "Proc Freq,' while tumor multiplicity (number of tumors/lung) was analyzed using Poisson regression in SAS "Proc Genmod." All analyses on tumor data utilized only those animals surviving to 78 wk.

RESULTS

Extrapulmonary Tissue Metal Analysis After MMA-SS Welding Fume Exposure

Metal analysis was conducted on aorta, heart, kidney, and liver from A/J and B6 mice after MMA-SS exposure. At 78 wk, concentrations of Cr, Cu, Mn, and Zn were increased in the kidney of the exposed A/J strain only (Figure 1). In the liver, only Cr was significantly increased after MMA-SS welding fume exposure in A/J mice (Figure 2). No marked changes

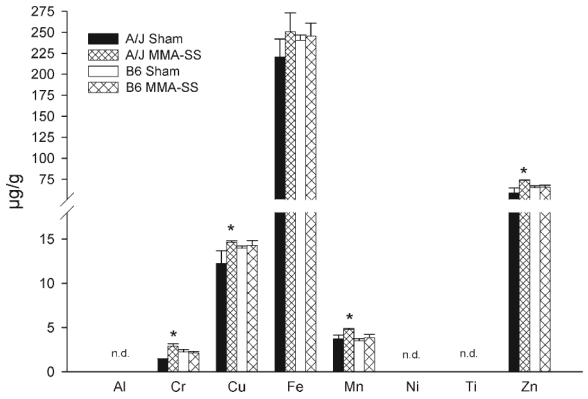

FIGURE 1. Metal deposition in the kidney of A/J and B6 mice 78 wk after 4 monthly exposures to MMA-SS welding fume (20 mg/kg). Asterisk indicates a significant increase versus corresponding sham ($p < .05$, $n > 4$); nd, below limit of detection.

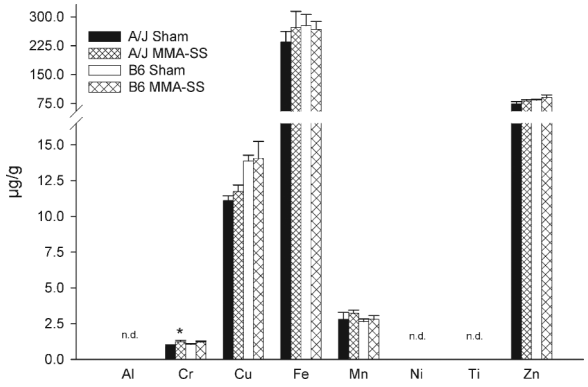

FIGURE 2. Metal deposition in the liver of A/J and B6 mice 78 wk after 4 monthly exposures to MMA-SS welding fume (20 mg/kg). Asterisk indicates a significant increase versus corresponding sham ($p < .05$, $n > 4$); nd, below limit of detection.

in metal concentrations in the aorta (data not shown) or heart (data not shown) were found in MMA-SS-exposed A/J mice compared to sham. In addition, there was no difference in metal levels between exposed B6 and sham groups in any tissue at 78 wk.

Gross Lung Tumor Findings at 78 wk After MMA-SS Welding Fume Exposure

At 78 wk postexposure, lung tumor multiplicity and incidence were unremarkable in mice exposed to MMA-SS welding fume (Table 2). Tumor incidence was 88 and 100% in the sham- and MMA-SS-exposed A/J groups, respectively. There were no significant differences in tumor size between sham- and welding fume-exposed groups. Nearly all tumors measured in both groups were <2 mm. As expected, the resistant B6 strain had no lung tumors upon gross examination at this time point.

Lung Histopathological Findings at 78 wk After MMA-SS Welding Fume Exposure

In addition to the gross lung tumor evaluation at necropsy, histopathological analysis was done to evaluate lung morphological changes (Table 3). There were no significant findings related to MMA-SS welding fume exposure found at 78 wk in the B6 strain. In the A/J strain, all exposed mice had minimal amounts of MMA-SS welding fume in the lung and increased lymphoid infiltrates and alveolar macrophages. Upon histopathologic examination, the preneoplastic/tumor multiplicity was significantly increased compared to sham. Of note, exclusion of preneoplastic lesions resulted in no significant difference between the groups. Incidence was not changed due to MMA-SS welding fume exposure (Table 3). Lesion types were as follows (total number indicated in parentheses): sham: P (3), AP (2), A (4), and MC (1); MMA-SS: P (10), AP (0), A (6), MC (2), CA (8).

DISCUSSION

This preliminary study examined the tumorigenic response in lung-tumor-susceptible (A/J) and -resistant (B6) mice exposed to MMA-SS welding fume after repeated, high-dose exposure by pharyngeal aspiration. The extrapulmonary fate of welding fume-derived metals was also investigated in these mouse strains. Overall, it was found that MMA-SS welding fume did not induce significant lung tumor multiplicity or incidence in A/J or, as expected, B6 mice. However, increased amounts of Cr, Cu, Mn, and Zn were observed in extrapulmonary tissue of susceptible A/J mice, but not resistant B6 mice, at 78 wk after exposure to MMA-SS fume.

TABLE 2. Gross Lung Tumor Findings for A/J and B6 Mice 78 wk Postexposure

	Tumor multiplicity[a]		Tumor incidence[b]	
Exposure	A/J	B6	A/J	B6
Sham	2.38 ± 0.42(8)	0 ± 0(6)	88% (7/8)	0% (0/6)
MMA-SS	3.00 ± 0.57(11)	0 ± 0(5)	100% (11/11)	0% (0/5)

Note. MMA-SS, manual metal arc-stainless steel welding fume.

[a]Average number of tumors per lung (±SE) and includes mice with no tumors. Numbers in parentheses indicate total animal number.

[b]Percentage of tumor-bearing mice out of the total. Numbers in parentheses indicate tumor-bearing/total animal number.

TABLE 3. Lung Histopathology for A/J and B6 Mice 78 wk Postexposure

Strain	Exposure	Alveolar macrophages	Lymphoid infiltrates[a]	Welding fume-laden cells	Tumor multiplicity[b]	Tumor incidence[c]
A/J	Sham	0.2 ± 0.1	0.25 ± 0.01	0.00 ± 0.00	1.25 ± 0.31 (8)	75% (6/8)
	MMA-SS	0.56 ± 0.14[d]	1.35 ± 0.16[d]	1.75 ± 0.11[d,e]	2.36 ± 0.39 (11)[d]	100% (11/11)
B6	Sham	0	0.20	0.00	0.00 (1)	0% (0/1)
	MMA-SS	0.2 ± 0.13	0.96 ± 0.25	0.92 ± 0.16	0.47 ± 0.25 (5)	20% (1/5)

Note. If abnormal changes were found, severity was scored as follows: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked, 5 = severe. The final severity score reflects the average of the five lung lobe scores. For MMA-SS-exposed B6 incidence, a single epithelial proliferative lesion was found and was likely age-related. All exposed and a selected sham ($n = 1$) from the B6 strain were evaluated to confirm the negative gross findings. MMA-SS, manual metal arc stainless steel welding fume. Data are mean ± SE.

[a]Perivascular/peribronchial associated lymphocytes, macrophages, and plasma cells.

[b]Average number of tumors/preneoplastic lesions per lung, includes mice with no lesions. Numbers in parentheses indicate total animal number.

[c]Percentage of tumor/preneoplasia-bearing mice out of the total. Numbers in parentheses indicate tumor-bearing/total animal number.

[d]Significantly increased compared to sham ($p < .05$).

[e]Significantly increased versus B6 ($p < .05$).

Stainless steel electrodes used during MMA welding generate fumes containing carcinogenic Cr and Ni. MMA welding fumes are highly soluble in water; therefore, the bioavailability of these carcinogenic metals may be increased compared to a more insoluble fume such as from gas metal arc (GMA) welding. Stainless steel fumes reportedly are toxic and mutagenic to mammalian cells (Hedenstedt et al., 1977; Maxild et al., 1978), produce DNA strand breaks in vitro and increased apoptosis in vivo (Antonini et al., 2005), and induce lung cell hyperplasia and atypia in mice (Solano-Lopez et al., 2006). Furthermore, previous data showed that MMA-SS welding fume generated free radicals and produced significant lung macrophage toxicity (Antonini et al., 1999; Taylor et al., 2003). Taken together, these studies provided an initiative to investigate the carcinogenic potential of this welding fume.

Nikitin et al. (2004) reported that A/J mice begin to develop spontaneous pulmonary tumors at 12–16 wk of age. Grossly observed background tumor frequency, as reported in the literature, can range from 31 to 40% between 43 and 53 wk of age and increase to 65% by approximately 66 wk (Witschi et al., 2004; Groch et al., 1997; Curtin et al., 2004). Microscopically, adenomas and proliferations are the most commonly observed pathologies of both spontaneous and chemically induced lung lesions in the A/J mouse (Gunning et al., 1991). In this study, a significant increase was found in proliferative lesion/tumor number in MMA-SS welding fume-exposed A/J mice. These effects, however, were not consistent

with gross observation or if the proliferative lesions were excluded from the dataset. Although, in most cases, both human and A/J mouse lung tumors originate from atypical hyperplastic foci in the lung periphery, one cannot conclude that MMA-SS welding fume at this dose is a carcinogen in this animal model based on histopathology alone (Belinsky et al., 1992; Westra, 2000; Westra et al., 1996; Foley et al., 1991). In fact, this result supports our previous study that showed no evidence of a carcinogenic effect with this welding fume (Zeidler-Erdely et al., 2008).

In this study, at a cumulative exposure dose of approximately 1.6 mg, significant amounts of MMA-SS welding fume were noted in the lung and an ongoing immune cell infiltration (i.e., lymphoid cells, macrophages) in susceptible A/J mice only. In a previous study, at a lower cumulative exposure dose (340 μg), significant amounts of GMA-SS, but not MMA-SS, welding fume were detected in the lungs of A/J mice at 78 wk postexposure. This was also accompanied by an ongoing immune response, characterized by a mild lymphoid infiltrate, in the lung (Zeidler-Erdely et al., 2008). This was attributed to the enhanced and prolonged acute inflammatory response that was observed in A/J compared to B6 mice (Zeidler-Erdely et al., 2008; Rondini et al., 2010). Perhaps the most unexpected finding here was the increased levels of welding fume-derived metals, Cr, Cu, and Mn, as well as Zn in extrapulmonary tissue of the A/J strain. This observation seems to directly correlate with the attenuated pulmonary clearance of MMA-SS welding fume in this mouse strain that was found in this study. Indeed, if both mouse strains equally cleared the particles from the lung, it could be a deficient extrapulmonary mechanism that contributed to the increased retention of metals. However, because the A/J strain had greater fume lung levels compared to the B6, the effect most likely is due to a specific lung clearance deficiency in this strain. Further of note was the increased Zn in the kidney. Zn has both antioxidant and anti-inflammatory properties and is essential to the functioning of the innate immune system (Prasad, 2008, 2009). Since MMA-SS welding fume has undetectable levels of this metal, the increase in kidney Zn may reflect a protective response since it has been documented that Cr exposure may produce renal injury (Wedeen & Qian, 1991). In fact, some studies support adverse, but transient, effects on the renal tubule system in welders (Bonde & Vittinghus, 1996).

In conclusion, this preliminary study further supports the hypothesis that welding fumes that contain primarily soluble carcinogenic metals, such as MMA-SS fume, do not appear to induce a significant tumorigenic response in an animal model. However, our findings do show that with high exposure levels, MMA-SS welding fume produces a chronic lung immune response in the A/J model similar to that observed with GMA-SS fume. Previously, MMA-SS welding fume was shown to suppress both local and systemic immune responses, which was likely associated with the bioavailability of soluble Cr and its interaction with local lung and systemic splenic cells (Antonini et al., 2004; Antonini & Roberts, 2007; Anderson et al., 2007). Thus, it is possible that higher, chronically administered levels, reflecting a lifetime of exposure, would allow the tumorigenic effects of MMA-SS welding fume to be realized. An interesting and novel finding of this study was the long-term extrapulmonary tissue alterations in Cr, Cu, Mn, and Zn levels in the susceptible A/J mouse but not the resistant B6 mouse. This could imply that, in a susceptible individual, toxic effects of this welding fume may continually occur long after intermittent exposures in the workplace.

REFERENCES

Anderson, S. E., Meade, B. J., Butterworth, L. F., and Munson, A. E. 2007. The humoral immune response of mice exposed to manual metal arc stainless steel-welding fumes. *J. Immunotoxicol.* 4:15–23.

Antonini, J. M. 2003. Health effects of welding. *Crit. Rev. Toxicol.* 33:61–103.

Antonini, J. M., Lawryk, N. J., Murthy, G. G., and Brain, J. D. 1999. Effect of welding fume

solubility on lung macrophage viability and function in vitro. *J. Toxicol. Environ. Health A* 58:343–363.

Antonini, J. M., Leonard, S. S., Roberts, J. R., Solano-Lopez, C., Young, S. H., Shi, X., and Taylor, M. D. 2005. Effect of stainless steel manual metal arc welding fume on free radical production, DNA damage, and apoptosis induction. *Mol. Cell. Biochem.* 279:17–23.

Antonini, J. M., and Roberts, J. R. 2007. Chromium in stainless steel welding fume suppresses lung defense responses against bacterial infection in rats. *J. Immunotoxicol.* 4:117–127.

Antonini, J. M., Taylor, M. D., Millecchia, L., Bebout, A. R., and Roberts, J. R. 2004. Suppression in lung defense responses after bacterial infection in rats pretreated with different welding fumes. *Toxicol. Appl. Pharmacol.* 200:206–218.

Belinsky, S. A., Devereux, T. R., Foley, J. F., Maronpot, R. R., and Anderson, M. W. 1992. Role of the alveolar type II cell in the development and progression of pulmonary tumors induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in the A/J mouse. *Cancer Res.* 52:3164–3173.

Bonde, J. P., and Vittinghus, E. 1996. Urinary excretion of proteins among metal welders. *Hum. Exp. Toxicol.* 15:1–4.

Curtin, G. M., Higuchi, M. A., Ayres, P. H., Swauger, J. E., and Mosberg, A. T. 2004. Lung tumorigenicity in A/J and rasH2 transgenic mice following mainstream tobacco smoke inhalation. *Toxicol. Sci.* 81:26–34.

Danielsen, T. E., Langard, S., and Andersen, A. 1998. Incidence of lung cancer among shipyard welders investigated for siderosis. *Int. J. Occup. Environ. Health* 4:85–88.

Foley, J. F., Anderson, M. W., Stoner, G. D., Gaul, B. W., Hardisty, J. F., and Maronpot, R. R. 1991. Proliferative lesions of the mouse lung: Progression studies in strain A mice. *Exp. Lung Res.* 17:157–168.

Groch, K. M., Khan, M. A., Brooks, A. L., and Saffer, J. D. 1997. Lung cancer response following inhaled radon in the A/J and C57BL/6J mouse. *Int. J. Radiat. Biol.* 71:301–308.

Gunning, W. T., Castonguay, A., Goldblatt, P. J., and Stoner, G. D. 1991. Strain A/J mouse lung adenoma growth patterns vary when induced by different carcinogens. *Toxicol. Pathol.* 19:168–175.

Harris, M. K. 2002. Welding processes. In *Welding health and safety: A field guide for the OEHS professional*, p. 184. Fairfax, VA: American Industrial Hygiene Association.

Hedenstedt, A., Jenssen, D., Lidestein, B. M., Ramel, C., Rannug, U., and Stern, R. M. 1977. Mutagenicity of fume particles from stainless steel welding. *Scand. J. Work Environ. Health* 3:203–211.

IARC. 1990. Chromium, nickel and welding. *IARC Monogr. Eval. Carcinogen. Risks Hum.* 49:1–648.

Malkinson, A. M. 1998. Molecular comparison of human and mouse pulmonary adenocarcinomas. *Exp. Lung Res.* 24:541–555.

Maxild, J., Andersen, M., and Kiel, P. 1978. Mutagenicity of fume particles from metal arc welding on stainless steel in the *Salmonella*/microsome test. *Mutat. Res.* 56:235–243.

Meuwissen, R., and Berns, A. 2005. Mouse models for human lung cancer. *Genes Dev.* 19:643–664.

Moulin, J. J. 1997. A meta-analysis of epidemiologic studies of lung cancer in welders. *Scand. J. Work Environ. Health* 23:104–113.

National Institute for Occupational Science and Health. 1994. *NIOSH manual of analytical methods*. Washington, DC: NIOSH.

Nikitin, A. Y., Alcaraz, A., Anver, M. R., Bronson, R. T., Cardiff, R. D., Dixon, D., Fraire, A. E., Gabrielson, E. W., Gunning, W. T., Haines, D. C., Kaufman, M. H., Linnoila, R. I., Maronpot, R. R., Rabson, A. S., Reddick, R. L., Rehm, S., Rozengurt, N., Schuller, H. M., Shmidt, E. N., Travis, W. D., Ward, J. M., and Jacks, T. 2004. Classification of proliferative pulmonary lesions of the mouse: Recommendations of the mouse models of human cancers consortium. *Cancer Res.* 64:2307–2316.

Prasad, A. S. 2008. Clinical, immunological, anti-inflammatory and antioxidant roles of zinc. *Exp. Gerontol.* 43:370–377.

Prasad, A. S. 2009. Zinc: Role in immunity, oxidative stress and chronic inflammation. *Curr. Opin. Clin. Nutr. Metab. Care* 12: 646–652.

Rao, G. V., Tinkle, S., Weissman, D. N., Antonini, J. M., Kashon, M. L., Salmen, R., Battelli, L. A., Willard, P. A., Hoover, M. D., and Hubbs, A. F. 2003. Efficacy of a technique for exposing the mouse lung to particles aspirated from the pharynx. *J. Toxicol. Environ. Health A.* 66:1441–1452.

Rehm, S., and Ward, J. M. 1989. Quantitative analysis of alveolar type II cell tumors in mice by whole lung serial and step sections. *Toxicol. Pathol.* 17:737–742.

Rondini, E. A., Walters, D. M., and Bauer, A. K. 2010. Vanadium pentoxide induces pulmonary inflammation and tumor promotion in a strain-dependent manner. *Part. Fibre Toxicol.* 7:9.

Sferlazza, S. J., and Beckett, W. S. 1991. The respiratory health of welders. *Am. Rev. Respir. Dis.* 143:1134–1148.

Shimkin, M. B., and Stoner, G. D. 1975. Lung tumors in mice: Application to carcinogenesis bioassay. *Adv. Cancer Res.* 21:1–58.

Solano-Lopez, C., Zeidler-Erdely, P. C., Hubbs, A. F., Reynolds, S. H., Roberts, J. R., Taylor, M. D., Young, S. H., Castranova, V., and Antonini, J. M. 2006. Welding fume exposure and associated inflammatory and hyperplastic changes in the lungs of tumor susceptible A/J mice. *Toxicol. Pathol.* 34: 364–372.

Sorensen, A. R., Thulstrup, A. M., Hansen, J., Ramlau-Hansen, C. H., Meersohn, A., Skytthe, A., and Bonde, J. P. 2007. Risk of lung cancer according to mild steel and stainless steel welding. *Scand. J. Work Environ. Health* 33:379–386.

Taylor, M. D., Roberts, J. R., Leonard, S. S., Shi, X., and Antonini, J. M. 2003. Effects of welding fumes of differing composition and solubility on free radical production and acute lung injury and inflammation in rats. *Toxicol. Sci.* 75:181–191.

Wedeen, R. P., and Qian, L. F. 1991. Chromium-induced kidney disease. *Environ. Health Perspect.* 92:71–74.

Westra, W. H. 2000. Early glandular neoplasia of the lung. *Respir. Res.* 1:163–169.

Westra, W. H., Baas, I. O., Hruban, R. H., Askin, F. B., Wilson, K., Offerhaus, G. J., and Slebos, R. J. 1996. K-ras oncogene activation in atypical alveolar hyperplasias of the human lung. *Cancer Res.* 56:2224–2228.

Witschi, H., Espiritu, I., Uyeminami, D., Suffia, M., and Pinkerton, K. E. 2004. Lung tumor response in strain a mice exposed to tobacco smoke: Some dose-effect relationships. *Inhal. Toxicol.* 16:27–32.

Zeidler-Erdely, P. C., Kashon, M. L., Battelli, L. A., Young, S. H., Erdely, A., Roberts, J. R., Reynolds, S. H., and Antonini, J. M. 2008. Pulmonary inflammation and tumor induction in lung tumor susceptible A/J and resistant C57BL/6J mice exposed to welding fume. *Part. Fibre Toxicol.* 5:12.