3. CANCER IN EXPERIMENTAL ANIMALS

A previous *IARC Monographs* Working Group concluded in 1989 that there was *inadequate evidence* for the carcinogenicity of welding fumes in experimental animals (IARC, 1990).

3.1 Mouse

See Table 3.1

3.1.1 Inhalation

Groups of age- and weight-matched male A/J mice (age, 5 weeks) were exposed by whole-body inhalation to gas metal arc stainless steel (GMA-SS) welding fumes at 40 mg/m^3 of filtered air for 3 hours per day for 6 (n = 45 per group) or 10 (n = 55 per group) days (Zeidler-Erdely et al., 2011a). The automated system for the generation of welding fumes consisted of a welding power source, an automated, programmable six-axis robotic arm, a water-cooled arc welding torch, a wire feeder, and an automatic welding torch cleaner. For the initial studies on characterization of fumes, GMA welding was performed using a SS electrode. Welding was performed on A36 carbon steel plates. A shielding gas combination of 95% argon (Ar) and 5% carbon dioxide (CO_2) was used during welding. The resulting aerosol was carried into a whole-body animal exposure chamber through a flexible tube. Particle concentrations within the exposure chamber were continuously monitored. Mice inhaled welding fumes composed of iron (57 percentage by weight or wt%), chromium (20.2 wt%), manganese (13.8 wt%), nickel (8.8 wt%), and copper (0.2 wt%), with trace amounts of silicon, aluminium, and vanadium. The particle diameters ranged from ultrafine (0.01–0.10 μm) to coarse (1.0–10 μm), with most particles in the fine size range (0.10–1.0 μm). Gas generation, including carbon monoxide (CO) and ozone (O_3), was continuously monitored. In the exposure chamber, carbon monoxide and ozone concentrations were not significantly higher than background levels (Antonini et al., 2006; Erdely et al., 2011). The 6- and 10-day inhalation regimes were estimated to be equivalent to 30 and 50 days of exposure, respectively, in a 75 kg person working an 8-hour shift using the previous threshold limit value time-weighted average of 5 mg/m^3 for welding fumes (Zeidler-Erdely et al., 2011a). The deposited human dose was calculated as: fume concentration (5 mg/m^3) multiplied by minimum volume (20 L/min × 10^{-3} m^3/L), exposure duration (8 hours per day × 60 minutes per hour), and alveolar deposition efficiency (0.16). The deposited human dose at these conditions is 7.7 mg/day. The proportional equivalent deposition in mice, assuming a mouse body weight of 20 g, is 7.7 mg/day multiplied by 20 g divided by 75 kg, which equals 2.05 μg/day. To simulate an exposure period of approximately 50 days, measured deposition in the study was 11 μg/day for 10 days of inhalation exposure (Erdely et al., 2011). The effect of welding fumes on grossly observed lung tumour multiplicity (average number of tumours per lung) and incidence

Table 3.1 Studies of carcinogenicity in experimental animals exposed to welding fumes

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Results	Significance	Comments
Full carcinogenicity Mouse, A/J (M) 5 wk 78 wk Zeidler-Erdely et al. (2011a)	Inhalation (whole-body) GMA-SS welding fumes (see Comments) Air Air for 6 d, GMA-SS for 6 d (40 mg/m^3 for 3 h/d), air for 10 d, GMA-SS for 10 d (40 mg/m^3 for 3 h/d) 45, 45, 55, 55 33, 37, 43, 42	Lung: tumours (gross lesions) Tumour incidence 6 d: air, 24/33; GMA-SS, 19/37 10 d: air, 33/43; GMA-SS, 26/42 Tumour multiplicity: 6 d: air, 1.36 ± 0.21; GMA-SS, 0.84 ± 0.16 10 d: air, 0.93 ± 0.11; GMA-SS, 0.86 ± 0.14 Total tumours: 6 d: air, 45; GMA-SS, 31 10 d: air, 40; GMA-SS, 36	 NS NS NS	Principal limitations: short duration of exposure; histopathological examination of the lung only; use of a low dose; lung histopathology only on selected animals Metals (wt%): Fe (57), Cr (20.2), Mn (13.8), Ni (8.8), Cu (0.2); trace amounts of Si, Al, and V Mass median aerodynamic diameter: 0.255 µm with SD of 1.352
Full carcinogenicity Mouse, A/J (M) 5 wk 48 wk, 78 wk Zeidler-Erdely et al. (2008)	Oropharyngeal aspiration GMA-MS and GMA-SS welding fumes; MMA-SS welding fumes (see Comments) PBS 85 µg 4 × (once every 3 d) in 25 µL PBS Sham control (25 µL/aspiration), GMA-MS (340 µg), GMA-SS 340 µg), MMA-SS (340 µg) evaluated after 48 wk and 78 wk 25, 25, 25, 25, 25, 25, 25, 25 21, 24, 20, 24, 19, 20, 16, 20	Lung, alveolar/bronchiolar: tumours (gross lesions) Tumour incidence: 48 wk: sham control, 7/21; GMA-MS, 8/24; GMA-SS, 8/20; MMA-SS, 5/24 78 wk: sham control, 10/19; GMA-MS, 13/20; GMA-SS, 13/16; MMA-SS, 16/20 Tumour multiplicity: 48 wk: sham control, 0.38 ± 0.13; GMA-MS, 0.42 ± 0.14; GMA-SS, 0.45 ± 0.14; MMA-SS, 0.25 ± 0.11 78 wk: sham control, 1.00 ± 0.35; GMA-MS, 1.00 ± 0.22; GMA-SS, 1.75 ± 0.32; MMA-SS, 1.55 ± 0.34 Total tumours: 48 wk: sham control, 8; GMA-MS, 10; GMA-SS, 9; MMA-SS, 6 78 wk: sham control, 19; GMA-MS, 20; GMA-SS, 28; MMA-SS, 31	 NS NS NS	Principal strengths: well-described and -conducted study Principal limitations: only one dose; histopathological examination of the lung only; small numbers of animals; non-physiological route of exposure Metals (wt%): GMA-MS, Fe (85), Mn (14); GMA-SS, Fe (53), Mn (23), Cr (19), Ni (5) MMA-SS: Fe (41), Cr (29), Mn (17), Ni (3) Soluble/insoluble ratio: GMA-MS, 0.020; GMA-SS, 0.006; MMA-SS, 0.345 (soluble metals: Cr, 87%; Mn, 11%) Count mean diameters: GMA-MS, 1.22 µm; GMA-SS 1.38 µm; MMA-SS 0.92 µm

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Results	Significance	Comments
Full carcinogenicity Mouse, A/J (M) 8–10 wk 78 wk Zeidler-Erdely et al. (2011b)	Oropharyngeal aspiration MMA-SS welding fumes (see Comments) PBS 60 µL (sham control), 20 mg/kg bw 1 ×/mo for 4 mo NR 8, 11	Lung, alveolar/bronchiolar: preneoplastic epithelial proliferations, adenoma, carcinomas, or microcarcinomas (combined)		Principal strengths: well-described and -conducted study Principal limitations: only one dose; small number of animals; lung histopathology only; non-physiological route of exposure Metals (wt%): Fe (41), Cr (29), Mn (17), and Ni (3); trace amounts of Cu and Ti Soluble fraction (%): Cr (87), Fe (0.39), Mn (11.7), Ni (0.65) Insoluble fraction (%): Cr (9.97), Fe (53.7), Mn (18.4), Ni (3.35)
		Tumour incidence: sham control(grossly observed), 7/8; sham control (histopathology), 6/8; MMA-SS (grossly observed), 11/11; MMA-SS (histopathology), 11/11	NS	
		Tumour multiplicity: sham control (grossly observed), 2.38 ± 0.42; sham control (histopathology), 1.25 ± 0.31; MMA-SS (grossly observed), 3.00 ± 0.57; MMA-SS (histopathology), 2.36 ± 0.39*	*$P < 0.05$	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Results	Significance	Comments
Initiation–promotion (tested as promoter) Mouse, A/J (M) 6–7 wk 30 wk Zeidler-Erdely et al. (2013)	Oropharyngeal aspiration GMA-SS welding fumes (see Comments) PBS CO/sham, 3-MC/sham, CO/GMA-SS 340 µg, 3-MC/GMA-SS 340 µg, CO/GMA-SS 680 µg, 3-MC/GMA-SS 680 µg 3-MC: 1× intraperitoneal injection; GMA-SS (at 1 wk after initiation with 3-MC) 1×/wk for 5 wk 30, 30, 28, 28, 30, 30 28, 26, 26, 26, 28, 29	Lung: "tumours" (gross lesions)		Principal strengths: multiple-dose study; adequate number of tumours produced; well-described and -conducted study Principal limitations: lung histopathology only Metals (wt%): GMA-SS, Fe (57), Cr (20.2), Mn (13.8), Ni (8.8), and Cu (0.2); trace amounts of Si, Al, and V Chromium (VI) levels were 2929 pm (µg/g) Sham exposure with PBS Tumour incidence reported as average percentage for CO groups combined and 3-MC groups combined Histopathology: 3-MC/GMA-SS low, 4 adenocarcinomas were observed (2 were in 1 mouse); 3-MC/GMA-SS high, 6 adenocarcinomas and 1 carcinoma were observed ($P < 0.01$ vs 1 carcinoma in 3-MC/sham group)
		Tumour incidence: CO/sham, 25.8 ± 6.4%; 3-MC/sham and 3-MC/GMA-SS, > 93%; 3-MC/sham, > 93%; CO/GMA-SS, 25.8 ± 6.4%; 3-MC/GMA-SS, > 93%; CO/GMA-SS, 25.8 ± 6.4%; 3-MC/GMA-SS, > 93%	NS	
		Tumour multiplicity: CO/sham, 0.21 ± 0.09; 3-MC/sham, 4.77 ± 0.7; CO/GMA-SS low, 0.42 ± 0.11; 3-MC/GMA-SS low, 12.1 ± 1.5*; CO/GMA-SS high, 0.21 ± 0.08; 3-MC/GMA-SS high, 14.0 ± 1.8*	*$P < 0.0001$ vs 3-MC/sham	
		Total tumours: CO/sham, 6; 3-MC/sham, 124; CO/GMA-SS low, 11; 3-MC/GMA-SS low, 314*; CO/GMA-SS high, 6; 3-MC/GMA-SS high, 405*	*$P < 0.004$ vs 3-MC/sham	
		Lung, alveolar/bronchiolar: preneoplastic epithelial proliferation, adenoma, adenocarcinoma or carcinoma (combined)		
		Tumour multiplicity (histopathology): CO/sham, NR; 3-MC/sham, 2.15 ± 0.32; CO/GMA-SS low, NR; 3-MC /GMA-SS low, 5.85 ± 0.76*; CO/GMA-SS high, NR; 3-MC/GMA-SS high, 6.00 ± 0.87*	*$P < 0.0001$	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Results	Significance	Comments
Initiation–promotion (tested as promoter) Mouse, A/J (M) 5–6 wk 30 wk Falcone et al. (2017)	Inhalation (whole-body) GMA-SS welding fumes (see Comments) Filtered air CO/air, 3-MC/air, CO/GMA-SS 360 µg, 3-MC/GMA-SS 360 µg 40 mg/m³ for 4 h/d, 4 d/wk, for 9 wk 30, 30, 30, 30 28, 29, 29, 28	Lung, alveolar/bronchiolar: bronchiolo-alveolar hyperplasia and adenoma		Principal strengths: complete histopathology; well-described and -conducted study Principal limitations: only one dose group; lung histopathology only Metals (wt%): Fe (57), Cr (20.2), Mn (13.8), Ni (8.8), and Cu (0.2); trace amounts of Si, Al, and V Chromium (VI) levels: 2929 ppm (µg/g) Mass median aerodynamic diameter: 0.350 µm Lung metals analysis showed an increase of ~10.1 µg of total GMA-SS welding fume deposited in the lungs from a single 4-h exposure day Initiation with 3-MC: single intraperitoneal injection of 10 µg/g bw 1 wk before inhalation exposure
		Tumour incidence (grossly observed): CO/air, 29%; 3-MC/air, > 96%; CO/GMA-SS, 38%; 3-MC/GMA-SS, > 96%	NS	
		Tumour multiplicity (grossly observed): CO/air, 0.32 ± 0.10; 3-MC/air, 7.93 ± 0.82; CO/GMA-SS, 0.45 ± 0.13; 3-MC/GMA-SS, 16.11 ± 1.18*	*$P < 0.0001$ vs 3-MC/air	
		Total tumours: Grossly observed: CO/air, 9; 3-MC/air, 230; CO/GMA-SS, 13; 3-MC/GMA-SS, 451* Histopathology: CO/air, 5; 3-MC/air, 90; CO/GMA-SS, 2; 3-MC/GMA-SS, 153**	*$P < 0.009$ and **$P < 0.05$ vs 3-MC/air	
Full carcinogenicity Hamster, Syrian golden (M) 6 wk 100 wk Reuzel et al. (1986)	Intratracheal instillation MIG-SS welding fumes; MMA-SS welding fumes (see Comments) Saline MMA-SS 0.5 mg, MMA-SS 2.0 mg, MIG-SS 2.0 mg, saline 0.2 mL 1×/wk for 56 wk, reduced to 1×/wk every 4 wk (after wk 26) in MMA-SS high-dose group due to increased morbidity/mortality 35, 35, 35, 35 NR	Lung: malignant tumours		Principal strengths: multiple-dose study Principal limitations: lack of detailed histopathology; no survival data; limited methodology; no statistics reported; historical controls were undocumented Metals (wt%): MIG-SS, Cr (0.4), Ni (2.4); MMA-SS, Cr (5), and Ni (0.4) Historical control incidence for lung tumours: 0/429 males
		Tumour incidence: 1/35, 1/35, 0/35, 0/35		
		Total tumours: 1, 1, 0, 0	NS	

3-MC, 3-methylcholanthrene; Al, aluminium; bw, body weight; CO, corn oil; Cr, chromium; Cu, copper; d, day(s); Fe, iron; GMA, gas metal arc; h, hour(s); M, male; MIG, metal inert gas; Mn, manganese; MMA, manual metal arc; Mo, month(s); MS, mild steel; Ni, nickel; NR, not reported; NS, not significant; PBS, phosphate buffered saline; ppm, parts per million; SD, standard deviation; Si, silicon; SS, stainless steel; Ti, titanium; V, vanadium; vs, versus; wk, week(s); wt%, percentage by weight

(percentage of tumour-bearing mice out of the total number of mice) was evaluated 78 weeks after exposure; survival was greater than 73% for all groups. Lung tumour multiplicity or incidence was not significantly different between the groups exposed to air (6 days, 1.36 ± 0.21, 73%; 10 days, 0.93 ± 0.11, 77%) and to GMA-SS welding fumes (6 days, 0.84 ± 0.16, 51%; 10 days, 0.86 ± 0.14, 62%). Average tumour size was approximately 3 mm and no significant differences between the groups were found. Histopathological analysis of selected lungs (air, n = 10; GMA-SS, n = 28) showed no significant changes related to the 6-day or 10-day exposures, except for the presence of a minimal amount of welding fumes in the latter only (Zeidler-Erdely et al., 2011a). [This was a subchronic exposure study with an extended observation period. The Working Group noted the short-term duration of exposure of this inhalation study and also the low dose used.]

3.1.2 Oropharyngeal aspiration

Groups of 25 age- and weight-matched male A/J mice (age, 5 weeks) were exposed to 85 µg of gas metal arc mild steel (GMA-MS), GMA-SS, or manual metal arc stainless steel (MMA-SS) welding fumes, or 25 µL of Ca^{+2}- and Mg^{+2}-free phosphate-buffered saline vehicle (sham control) by oropharyngeal aspiration, once every 3 days for 4 exposures. The welding fumes were generated in a cubical open-front fume chamber by a skilled welder using a manual or automatic technique appropriate for the electrode, and then collected on a sterile 0.2 µm filter. The samples were generated by three welding processes: GMA (with Ar and CO_2 shielding gases) using a MS electrode; GMA using a SS electrode; and MMA using a flux-cored SS electrode. The cumulative dose of welding fumes, 340 µg, was reported to be equivalent to approximately 196 days of exposure in a 75-kg human working an 8-hour shift using a calculation similar to that described in Section 3.1.1. The effects of the different welding fumes on grossly observed lung tumour multiplicity (average number of tumours per lung) and incidence (percentage of tumour-bearing mice out of the total number of mice) were evaluated 48 and 78 weeks after exposure. Survival was greater than 91% 48 weeks after exposure for all groups. Survival was 80% for the sham control, GMA-MS, and MMA-SS groups and 73% for the GMA-SS group 78 weeks after exposure. No significant increases in grossly observed lung tumour multiplicity or incidence were found for the groups exposed to welding fumes compared with the sham control groups (sham control, 0.38 ± 0.13, 33%; GMA-MS, 0.42 ± 0.14, 33%; GMA-SS, 0.45 ± 0.14, 40%; MMA-SS, 0.25 ± 0.11, 21%) 48 weeks after exposure. A similar result was reported (sham control, 1.00 ± 0.35, 53%; GMA-MS, 1.00 ± 0.22, 65%; GMA-SS, 1.75 ± 0.32, 81%; MMA-SS, 1.55 ± 0.34, 80%) 78 weeks after exposure. Histopathological analysis of the lungs at 48 weeks showed that the group exposed to GMA-SS welding fumes had a significant ($P < 0.05$) increase in the incidence of preneoplastic or neoplastic lesions (combined) of the lung (65%) compared with the group exposed to GMA-MS welding fumes (33%), but not compared with sham controls (50%). The difference in lesion incidence between the groups exposed to MMA-SS (33%) and GMA-SS (65%) welding fumes was not significant. Lung lesion types were preneoplastic epithelial proliferations and adenomas. No significant differences were found among the groups 78 weeks after exposure, but the group exposed to GMA-SS welding fumes had the highest multiplicity and incidence (sham control, 1.47 ± 0.33, 68%; GMA-MS, 1.40 ± 0.32, 75%; GMA-SS, 1.94 ± 0.38, 88%; MMA-SS, 1.85 ± 0.46, 75%). Lesion types 78 weeks after exposure were similar to those at 48 weeks, with carcinomas arising in adenomas, carcinomas, and microcarcinomas also present, but less common. A significant increase ($P < 0.05$) in lymphoid infiltrates

was also found in the group exposed to GMA-SS welding fumes (sham control, 1.53 ± 0.29; GMA-MS, 0.78 ± 0.24; GMA-SS, 2.53 ± 0.36; MMA-SS, 1.70 ± 0.27) (Zeidler-Erdely et al., 2008). [The Working Group noted the non-physiological route of exposure.]

Male A/J and C57BL/6J mice (age, 8–10 weeks) were exposed to MMA-SS welding fumes at a dose of 20 mg/kg body weight (bw) or 60 µL of Ca^{+2}- and Mg^{+2}-free phosphate-buffered saline vehicle (sham control) by oropharyngeal aspiration (Zeidler-Erdely et al., 2011b) once per month for 4 months. The MMA-SS welding fumes were generated by a skilled welder and collected onto sterile filters as described in the paragraph above (Zeidler-Erdely et al., 2008). The cumulative dose of welding fumes, 1.6 mg, was estimated to be equivalent to approximately 4 years of exposure in a 75-kg human working an 8-hour shift using a calculation similar to that described in Section 3.1.1. The lung-tumour-resistant strain C57BL/6J served as a negative control. The effect of MMA-SS welding fumes on grossly observed lung tumour multiplicity (average tumour number per lung) and incidence (percentage of tumour-bearing mice of total number of mice) was evaluated 78 weeks after the first exposure. No significant difference in grossly observed tumour multiplicity or incidence was found between the groups of A/J mice: sham control (n = 8), 2.38 ± 0.42, 88%; and MMA-SS (n = 11), 3.00 ± 0.57, 100%. The C57BL/6J groups (sham control, n = 6; MMA-SS, n = 5) had no grossly observed tumours 78 weeks after exposure. Histopathological analysis of the A/J mice lungs showed that exposure to MMA-SS welding fumes significantly ($P < 0.05$) increased the multiplicity of preneoplastic or neoplastic lesions (combined) compared with sham controls (2.36 ± 0.39 vs 1.25 ± 0.31). Incidence was 75% and 100% for the sham control and group exposed to MMA-SS welding fumes, respectively, and the difference was not statistically different. Exclusion of the preneoplastic lesions from the histopathology data resulted in no significant difference in multiplicity between the A/J sham control group and the group exposed to MMA-SS welding fumes. Lung lesion types (total number in parentheses) were preneoplastic epithelial proliferations (sham control, 3; MMA-SS, 10), adenomas arising within a proliferation (sham control, 2; MMA-SS, 0), adenomas (sham control, 4; MMA-SS, 6), microcarcinomas (sham control, 1; MMA-SS, 2), and carcinomas arising within an adenoma (sham control, 0; MMA-SS, 8) (Zeidler-Erdely et al., 2011b). [The Working Group noted the high number of carcinomas found in the group exposed to MMA-SS welding fumes; however, the authors did not report the incidence of individual tumour type in each group so no additional conclusions could be made by the Working Group in this regard. Group sizes at the start of the study were not provided by the authors. The non-physiological route of exposure was also noted by the Working Group.]

3.1.3 Initiation–promotion studies

The effect of GMA-SS welding fumes as a lung tumour promoter was evaluated in a two-stage initiation–promotion model of lung tumorigenesis. Groups of 28 or 30 age- and weight-matched male A/J mice (age, 6–7 weeks) were given the chemical initiator 3-methylcholanthrene (3-MC; 10 µg/g bw) dissolved in corn oil or corn oil alone (vehicle) by intraperitoneal injection (Zeidler-Erdely et al., 2013). One week after initiation, mice were exposed to GMA-SS welding fumes (340 or 680 µg per exposure) or 50 µL of Ca^{+2}- and Mg^{+2}-free phosphate-buffered saline vehicle (sham control) by oropharyngeal aspiration once per week for 5 weeks. The welding aerosols were generated by the automated system described in Section 3.1.1. For the oropharyngeal exposure, the GMA-SS welding fumes from the weld area were collected onto sterile filters for use in the experimental protocol. The cumulative doses of welding fumes, 1700 and 3400 µg, were estimated

to be equivalent to approximately 450 days (1.84 working years) and 900 days (3.68 working years) of exposure, respectively, in a 75-kg human working an 8-hour shift using a calculation similar to that described in Section 3.1.1. Grossly observed lung tumour multiplicity (average number of tumours per lung) and incidence (percentage of tumour-bearing mice out of total number of mice) were determined 30 weeks after initiation. Survival was approximately 93% for all groups. Both groups exposed to GMA-SS welding fumes (low, 1700 μg; high, 3400 μg) initiated with 3-MC had significantly increased lung tumour multiplicity based on gross observations (low, 12.1 ± 1.5; high, 14.0 ± 1.8) compared with 3-MC/sham (4.77 ± 0.7; $P < 0.0001$). Similar results for total tumour number were also found across all five individual lung lobes (left:apical:cardiac:diaphragmatic:azygos): corn oil/sham, 1:1:2:2:0; corn oil/GMA-SS low, 4:3:2:1:1; corn oil/GMA-SS high, 4:1:0:1:0; 3-MC/sham, 52:12:13:39:8; 3-MC/GMA-SS low, 119:46:40:81:28 ($P < 0.004$, increase for all five lobes compared with 3-MC/sham); 3-MC/GMA-SS high, 132:64:66:106:37 ($P < 0.004$, increase for all five lobes compared with 3-MC/sham). Tumour multiplicity across the groups given corn oil was similar (sham, 0.21 ± 0.09; low, 0.42 ± 0.11; high, 0.21 ± 0.08). There were no significant differences in tumour incidence between the different groups given corn oil and between the different groups given the chemical initiator 3-MC. The grossly observed lung tumour multiplicity was confirmed by histopathological analysis: 3-MC/GMA-SS low, 5.85 ± 0.76 ($P < 0.0001$) and 3-MC/GMA-SS high, 6.00 ± 0.87 ($P < 0.0001$), compared with 3-MC/sham, 2.15 ± 0.32. Based on histopathology, lung tumour incidence (preneoplastic or neoplastic lesions, combined) was 21.9 ± 3.4% and 85.0 ± 4.1% for the groups given corn oil or the chemical initiator 3-MC, respectively, and no differences were found among the different groups given corn oil or among the different groups given the initiator 3-MC. The number of microscopically observed lung lesion types (primarily preneoplastic epithelial proliferations and adenomas, pre-neoplasia: adenomas within pre-neoplasia:adenoma: adeno-carcinoma:carcinoma) were reported as: corn oil/sham, 5:2:2:0:0; corn oil/GMA-SS low, 1:0:3:1:0; corn oil/GMA-SS high, 0:1:4:0:0; 3-MC/sham, 16:5:34:0:1; 3-MC/GMA-SS low, 61:17:70:4:0; 3-MC/GMA-SS high, 65:9:93:6:1. The group given initiator 3-MC and the high dose of GMA-SS welding fumes had a significantly increased ($P < 0.01$) incidence of malignant tumours (7 out of 29 mice had adenocarcinomas or carcinomas) compared with the 3-MC/sham group (1 carcinoma-bearing mouse out of 26 mice). The group given initiator 3-MC and the low dose of GMA-SS welding fumes had 4 adenocarcinomas, but 2 were present in a single mouse [the Working Group noted that the significance was not relevant in this case] (Zeidler-Erdely et al., 2013). [The Working Group concluded that GMA-SS welding fumes act as a lung tumour promoter in male A/J mice initiated with the chemical carcinogen 3-MC. The Working Group noted the non-physiological route of exposure.]

The effect of GMA-SS welding fumes as a lung tumour promoter was evaluated in a two-stage initiation–promotion model of lung tumorigenesis. Groups of 30 age- and weight-matched male A/J mice (age, 5–6 weeks) were given the chemical initiator 3-MC (10 μg/g bw) dissolved in corn oil or corn oil alone (vehicle) by intraperitoneal injection (Falcone et al., 2017). One week after initiation, mice were exposed to GMA-SS welding fumes (4 hours per day, 4 days per week, for 9 weeks) at a target concentration of 40 mg/m^3 (estimated total, 360 μg) or filtered air by whole-body inhalation, as described in Section 3.1.1. The exposure was estimated to be equivalent to approximately 14 weeks in a 75-kg human working an 8-hour shift using a calculation similar to that described in Section 3.1.1. Grossly observed lung tumour multiplicity and incidence were determined 30 weeks after

initiation. Survival was approximately 95% at 30 weeks for all groups. Tumour incidence was greater than 96% for both groups given initiator 3-MC. Mice initiated with 3-MC and exposed to GMA-SS welding fumes (3-MC/GMA-SS) had significantly increased lung tumour multiplicity (16.11 ± 1.18) compared with 3-MC/air groups (7.93 ± 0.82; $P < 0.0001$); no significant difference was found between the corn oil groups (corn oil/air, 0.32 ± 0.10; corn oil/GMA-SS, 0.45 ± 0.13). Tumour incidences were 29% and 38% in the corn oil/air and corn oil/GMA-SS groups, respectively, and were not significantly different. Similar results for total tumour number were also found across all five individual lung lobes (left:apical:cardiac:diaphragmatic:azygos): corn oil/air, 3:3:0:3:0; corn oil/GMA-SS, 5:1:0:5:2; 3-MC/air, 78:30:25:67:30; 3-MC/GMA-SS, 150:68:63:110:60 ($P < 0.009$, increase for all five lobes compared with 3-MC/air). The increase in grossly observed lung tumour multiplicity was confirmed by histopathological analysis: 3-MC/air mice had 90 total lesions (20 bronchioloalveolar adenomas and 70 bronchioloalveolar hyperplasia) versus 153 (34 bronchioloalveolar adenomas and 119 bronchioloalveolar hyperplasia) in the 3-MC/GMA-SS group ($P < 0.05$, increase). Abnormal morphological changes in the lung included significantly increased severity scores for lymphoid infiltrates in the corn oil/GMA-SS and 3-MC /GMA-SS groups compared with the respective controls. The authors noted that, compared with their oropharyngeal aspiration initiation–promotion study (Zeidler-Erdely et al., 2013), the tumour promoter effect was similar in the two studies despite a significantly lower total lung burden and dose rate via inhalation (Falcone et al., 2017). [The Working Group concluded that GMA-SS welding fumes act as a lung tumour promoter in male A/J mice initiated with the chemical carcinogen 3-MC. The Working Group noted the use of a single dose.]

3.2 Rat

Intrabronchial implantation

[The Working Group reviewed a 34-month intrabronchial implantation study of pellets loaded with the particulate fraction of MMA-SS welding fumes in male and female Sprague-Dawley rats (Berg et al., 1987). The study was judged to be inadequate for the evaluation because of the limited methodology, the rapid decline of the health of the rats, and the poor survival rate of the rats including controls.]

3.3 Hamster

Intratracheal instillation

Four groups of 35 male Syrian golden hamsters (age, ~6 weeks) were exposed to metal inert gas stainless steel (MIG-SS; 2.0 mg) or MMA-SS (2.0 or 0.5 mg) welding fumes, or saline vehicle (0.2 mL), by intratracheal instillation once per week for 56 weeks. A fifth group similarly treated with calcium chromate ($CaCrO_4$; 1.0 mg) was used as a positive control. The hamsters showed signs of respiratory distress after each intratracheal instillation. The exposures were reduced to once every 4 weeks after week 26 in the group given the high dose of MMA-SS welding fumes due to increased morbidity and mortality. Carcinogenic effects were evaluated after an additional 44 weeks after the last exposure (the experiment was terminated at week 100) and histopathology was performed on the respiratory tract, liver, and kidneys. At termination of the experiment, one lung tumour, a well-differentiated combined epidermoid and adenocarcinoma, was found in the group given the high dose of MMA-SS welding fumes. A single anaplastic tumour (that was likely to be a carcinoma, as noted by the study authors) was found in the lung of a hamster given the low dose of MMA-SS welding fumes, which died after 1 year of treatment. No lung tumours were found in

the three other groups (Reuzel et al., 1986). [No histopathological scoring or survival data were reported and the methodology was limited. The study authors concluded that these two tumours suggest a carcinogenic action of MMA-SS welding fumes because no lung tumours were observed in a group of 429 male and 363 female historical control hamsters. The origin of the historical controls was not specified by the study authors. The Working Group suggested caution in drawing such a conclusion based on a single potentially malignant tumour in each dose group of the hamsters exposed to MMA-SS welding fumes. Overall, the Working Group judged the study to be inconclusive.]

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