

Stainless steel manual metal arc welding fumes in rats

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ABSTRACT Forty two male Wistar rats were exposed to manual metal arc (MMA) stainless steel (SS) welding fumes generated by an automatic welding device for "nose-only" exposure. The exposure simulated an actual MMA/SS welding environment as closely as possible. For the retention study, the duration of exposure was one hour per workday for one, two, three, or four weeks and for the clearance study four weeks. The retention and clearance of the chromium, nickel, and iron found in MMA/SS welding fumes in the rats' lungs were studied as was the distribution of the metals to other organs. Instrumental neutron activation analysis (INAA) was used for the multi-element chemical activation analyses. The concentrations of chromium and nickel in the blood and the urine were determined by atomic absorption method (AAS). The retention of exogenous iron was determined by a magnetic measuring method. The results indicated that the lungs were the target organs of soluble hexavalent chromates. The half times of lung clearance for Cr, Ni, and Fe were 40 ± 4 d, 20 ± 2 d, and 50 ± 10 d. When the lung clearance curves are compared, the half times of Cr and Fe lung clearance are similar but nickel disappears faster. The distribution and clearance patterns of chromium to other organs differ from those obtained after single intravenous or intratracheal injections of alkaline chromates.

Alloyed steels resistant to corrosion have replaced non-alloyed steels in many applications during the past decade and hence more chromium and nickel compounds are now found in metal aerosols at industrial workplaces. For instance, stainless steel welding fumes contain both chromium compounds and nickel compounds. The characterisation of manual metal arc (MMA) stainless steel (SS) welding fumes has recently been given much attention¹⁻⁵ (S Kimura *et al*, paper presented at AES 60th annual meeting, Detroit, April 1979). The total concentration of chromium in MMA/SS welding fumes varies between 3% and 10%: water soluble hexavalent alkaline chromates (CaCrO_4 , $\text{K}_2\text{Cr}_2\text{O}_7$) comprise 60-95% of the total concentration of chromium¹⁻⁵ (S Kimura *et al*, 1979).

The concentration of nickel in MMA/SS welding fumes varies from approximately 0.5% to 1%. It has been assumed that the fume particles of iron oxide contain an alloyed element of nickel in a poorly water soluble form.^{1,4,5}

The presence of water soluble hexavalent chromium in MMA/SS welding fumes has led scien-

tists to estimate the exposure of MMA/SS welders to chromium. The determination of the concentration of chromium in the urine has been used to obtain an estimation of the individual's exposure.⁶⁻⁸ The composition of MMA/SS welding fumes and the structure of the chromium compounds and the nickel compounds are complicated, however, and the biological kinetics of the different inorganic components of MMA/SS welding fumes are not known.

The kinetic models of some chromium compounds have been studied, mainly after intravenous or parenteral administration.⁹⁻¹¹ Chromium compounds differ in their organ distribution and excretion; the difference depends on the valence of the chromium (Cr) found in the compound. The hexavalent form of Cr is taken up by various organs including the red blood cells; the trivalent form of Cr is bound in plasma. Within the organism the hexavalent form of Cr is relatively rapidly reduced to the trivalent form.

The fate of Cr compounds in the tissues after inhalation has received little attention, even though the respiratory tract is the most important route of occupational exposure. Baetjer *et al*¹² reported the lung clearance and the distribution patterns of Cr in guinea pigs after intratracheal injection.

Some rat studies have been published about the distribution of the metallic components of different types of welding fumes inhaled by the rats.¹³⁻¹⁵

We have studied the lung retention and lung clearance of the Cr, nickel (Ni), and iron (Fe) found in MMA/SS welding fumes in the rat and the distribution of Cr, Ni, and Fe from the lungs to other organs in the rat. The exposure to welding fumes closely simulated the exposure that actually occur in an MMA/SS welding environment.

Materials and methods

Forty two male Wistar rats (300 ± 15 g) were exposed to MMA/SS welding fumes. The rats, which were in good health, were free of respiratory infections. Their feed consisted of commercial rodent chow (Ewas, Astra-Ewas) and tap water ad libitum. The rats were divided into four groups for the study of fume retention and into nine groups for the study of fume clearance. For the retention study, the duration of exposure was one hour per workday for one, two, three, or four weeks and for the clearance study four weeks. The rats of the retention study were decapitated 24 hours after the last exposure. The rats of the clearance group were decapitated one, three, or eight hours, or one, four, eight, 14, 28, 56, or 106 days after the last exposure. Two controls were decapitated on each occasion.

The lungs, kidneys, liver, spleen, and brain were set aside for instrumental neutron activation analysis

(INAA). Contamination was avoided by using pre-washed plastic cups, and polyethylene bags and capsules were used to remove and store the organs. The samples were quickly rinsed with deionised water so that blood and airborne particles would be removed from their surfaces. All plastic ware was washed in 2N HNO₃, EDTA (6 g/l) and rinsed with deionised water before use. The blood samples were bled into dry heparinised tubes. Instrumental neutron activation analysis was used to obtain multi-element chemical analysis of the lungs, kidneys, liver, spleen, and brain. The determinations of the concentrations of Cr and Ni in the blood and in the urine were carried out by atomic absorption (AAS). The retention of exogenous iron (Fe_{ex}) was determined by a magnetic measuring method.¹⁶⁻¹⁷

SYSTEM OF EXPOSURE

Figure 1 shows the exposure chamber developed for use in experiments on exposure to different metal aerosols. The volume of the chamber is 71 l. The chamber is fitted with sockets for 57 removable transparent rat holders for "nose-only" exposure. The fume was generated by an automatic arc welder developed for this particular purpose. A portion of the fume was isokinetically sampled and diluted into the exposure chamber. The welded material was stainless steel (AISI 304), and rutile electrodes (Esab OK 63-30 4 mm in diameter) were used. The total mass concentration of the fume was 43 mg/m³. On average, the fume contained 3.7% Fe, 3.6% Cr,

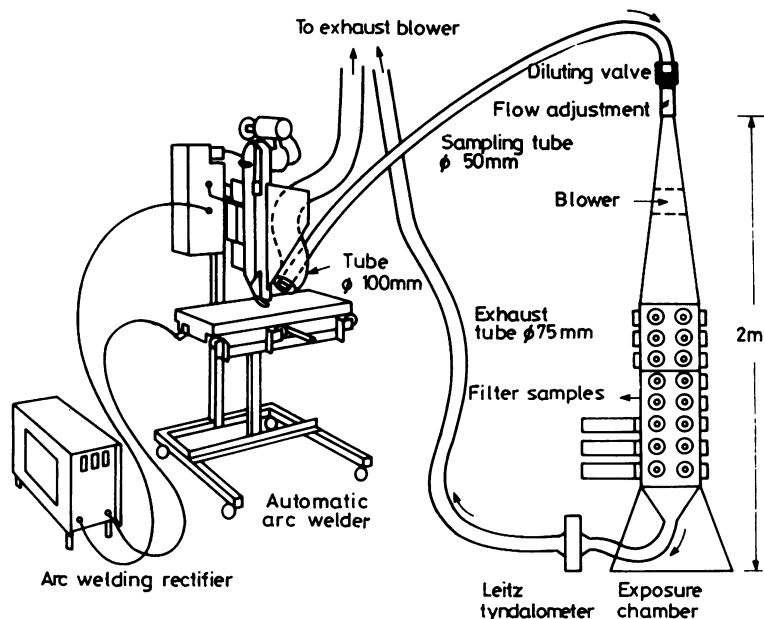


Fig 1 Exposure system for automatic arc welding with coated electrode.

0.4% Ni, and 0.002% Co. Throughout the exposure period, the mass concentration was checked with dust samples collected on Millipore filters (pore size $0.8 \mu\text{m}$, 37 mm in diameter). The chemical composition of the fumes was determined by the instrumental neutron activation method. The welding fume particles, which are originally very small ($0.3\text{--}0.6 \mu\text{m}$), tend to form chains several microns in length.¹

MEASUREMENT OF EXOGENOUS IRON BY THE MAGNETIC TECHNIQUE

Stainless steel welding fumes contain iron, some of which is in magnetic form (magnetite). Because endogenous lung iron is non-magnetic, the amount of exogenous lung iron may be measured by the magnetic measuring technique.^{16,17} The iron content of the examined lungs was measured with a SQUID magnetometer in a magnetically shielded room located at Helsinki University of Technology. The sensitivity of the measurements is 0.3 nAm^2 , which corresponds to $0.1 \mu\text{g}$ of magnetite or $0.3 \mu\text{g}$ of fume iron.

ATOMIC ABSORPTION SPECTROMETRIC DETERMINATION

The blood analyses were done by electrothermal atomic absorption spectrometry (Perkin-Elmer 4000 with HGA 400), and the samples were injected manually into graphite tubes. The ramp heating programme was used for the determinations of both chromium and nickel. The atomising temperature was 2500°C .

The blood samples were diluted with nitric acid (1 part blood: 5 parts HNO_3) to reach pH 2. The reference samples were prepared from the blood samples of unexposed rats. Chromium was analysed directly from the dilution, but the samples were first centrifuged for the analysis of nickel. The method for the analysis of Cr was similar to the method described by Tola *et al.*⁷

All the nickel seemed to remain in the supernatant; burnt blood samples yielded the same results as the direct acid dilution. The precision of the method, which was calculated from 10 consecutive injections of the same dilution, was 4% for Cr and 2% for Ni.

NEUTRON ACTIVATION ANALYSIS

The organ samples were lyophilised, and 60–160 mg of dried sample was sealed into a quartz ampoule, which was then irradiated for 30 hours together with a liquid standard and National Bureau of Standards reference materials (SRM 1577 and 1571). The irradiation took place in the neutron flux ($10^{13} \text{ n cm}^{-2} \text{ s}^{-1}$) of a Triga Mark II reactor. After a period of decay lasting three to six weeks, the gamma-activities were measured for three hours by an Ortec

25% Ge(Li) detector (resolution 2.1 keV at 1335.5 keV) connected to an automatic gamma-spectrometer (Nokia LP 4900). Chromium, iron, cobalt, zinc, selenium, and rubidium were determined quantitatively. The reliability of the analysis was checked by analysing reference material of National Bureau of Standards bovine liver or orchard leaves (SRM 1577 and 1571). According to the results obtained with the reference materials, the determinations were reliable throughout the study.

The instrumental neutron activation method detection limit was 1.1 for Cr, 16 for Fe, and 0.7 for Ni, all expressed as $\mu\text{g/g}$ dry weight.

Results

LUNG RETENTION AND CLEARANCE

The retention and clearance patterns of Cr, Ni, and Fe_{ex} in the lungs are shown in figs 2a and 2b. A linear relationship was observed between the duration of exposure and the concentrations of Cr, Ni, and Fe_{ex} in the lungs. The average concentrations of Cr in the lungs 24 hours after the maximum exposure

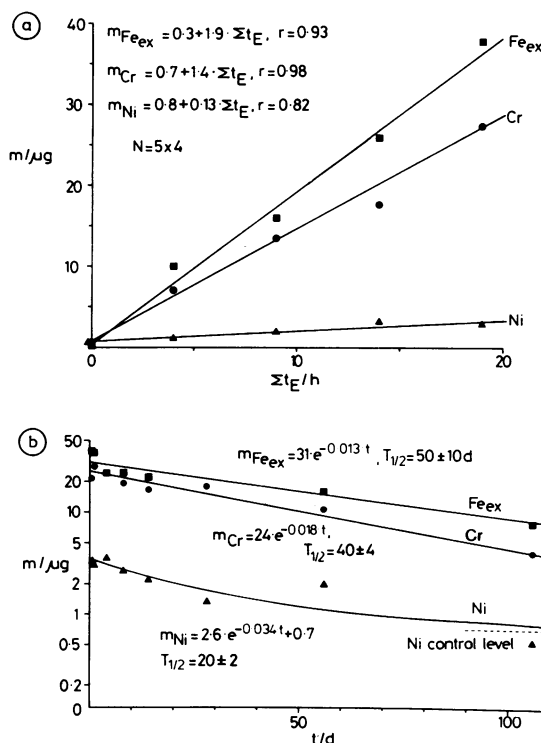


Fig 2 (a) Retention pattern and (b) clearance pattern of chromium (Cr), nickel (Ni), and exogenous iron (Fe_{ex}) in the lungs of rats exposed to MMA/ISS welding fumes.

ure (19 h) was 57 ppm, which corresponds to a total lung Cr content of 27 µg. Typical concentrations for Ni and Fe_{ex} were 6.7 ppm and 76 ppm, respectively; thus the Ni and Fe_{ex} contents of the lungs were 3.3 µg and 36 µg.

The biological half times of lung clearance (single exponential function fit) were 40±4 d for Cr, 20±2 d for Ni, and 50±10 d for Fe_{ex} (fig 2b). The lung clearance was faster in the beginning of the clearance period, but a multiexponential clearance model could not be applied because the number of points was limited. When the lung clearance curves are compared, the half times of the lung clearance of Cr and Fe_{ex} are similar, whereas Ni disappears faster.

CHROMIUM AND NICKEL IN THE BLOOD AND THE URINE

The concentration of Cr in the whole blood was constant (0.53±0.05 µmol/l) during the period of exposure, but it decreased rapidly ($T_{1/2}=6.0\pm 0.4$ d) after the exposure ceased (fig 3). The estimated maximum Cr content of the blood was 0.5 µg. The concentration of Ni in the blood was below the detection limit (0.05 µmol/l). The amount of Fe_{ex} in the blood was less than 1 µg.

Because it proved difficult to collect the urine samples without contamination, the analyses of Cr and Ni in the urine were not exact. The urine analyses performed, however, indicated that the immediate concentration of Cr in the urine after any period of exposure was 1.1±0.4 µmol/l. The urinary concentration of Ni was 0.31±0.10 µmol/l.

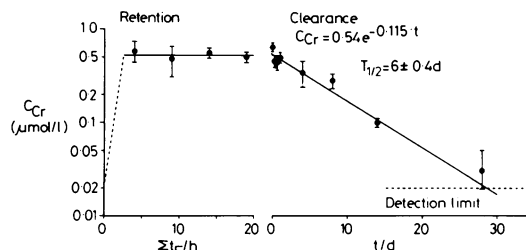


Fig 3 Concentration of chromium (Cr) in blood as a function of duration of exposure and duration of clearance.

The diurnal excretion rate into the urine was approximately 0.23 µg/d for Cr and 0.07 µg/d for Ni. When the concentrations of Cr and Ni within the exposure chamber are compared, it can be seen that Ni is excreted into the urine faster than Cr.

The estimated amount of Cr excreted into the urine throughout the total period of exposure was 6 µg. The corresponding amount for Ni was 1.8 µg.

CONCENTRATIONS OF CHROMIUM AND NICKEL IN OTHER ORGANS

In the liver the concentration of Cr did not increase significantly during the first week of exposure. The concentration of Cr in the liver then began to increase, and it reached the maximum level about three weeks after the last exposure. The concentration of Cr in the liver returned to the level of the controls in two months (table 1). The maximum content of Cr in the liver was approximately 3 µg.

Table 1 Mean values of the concentrations of Cr and Ni in different rat organs as a function of cumulative duration of exposure/clearance after the termination of exposure. The concentrations are expressed as µg/g dry weight

Cumulative duration of exposure (h)	Concentration of chromium ±SD (µg/g)		
	Liver	Kidneys	Stomach
4	0.19±0.12	0.45±0.33	1.08±0.26
9	0.0	0.32±0.15	1.54±0.30
14	0.08	0.69±0.10	1.1 ±0.6
19	0.3±0.4	0.45±0.19	0.9±0.3
Controls (n=15)	0.16±0.17	0.16±0.18	0.75±0.28
Duration of clearance (d)			
0.04	0.11±0.11	0.63±0.59	1.3±0.6
0.13	0.29±0.25	0.71±0.13	1.3±0.7
0.33	0.43±0.26	0.50±0.42	1.1±0.4
1	0.31±0.38	0.45±0.19	0.9±0.3
4	0.39±0.43	0.82±0.34	0.7±0.12
8	0.32±0.12	0.63±0.48	0.8±0.4
14	0.83±0.52	0.62±0.41	0.5±0.14
28	0.80±0.39	0.14±0.05	2.0
56	0.15±0.12	0.30±0.02	1.1±0.3
106	0.07±0.05	-	-
Concentration of nickel ± SD (µg/g)			
Controls	1.0±0.9	0.5±0.3	1.6±0.7
Exposed rats	Not significantly raised		

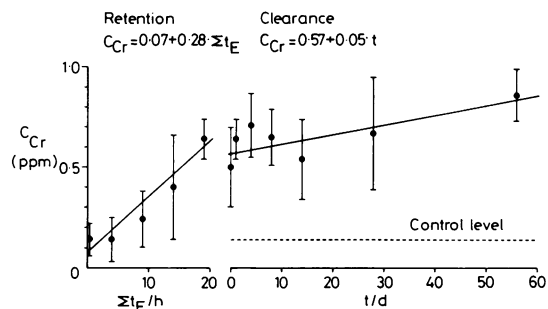


Fig 4 Chromium (Cr) content of spleen as a function of duration of exposure and duration of clearance.

In the kidneys the concentration of Cr increased to its maximum level during the first week of exposure, it then decreased to the level of the controls about 30 days after the last exposure (table 1). The maximum content of Cr in the kidneys was approximately 0.6 μg .

In the spleen the concentration of Cr increased during the period of exposure, and it continued to increase for two months after the termination of exposure. This continuing increase indicates that Cr from the red blood cells accumulates in the spleen (fig 4). The maximum content of Cr in the spleen was 0.12 μg .

In the stomach the concentration of Cr increased slightly during the exposure, but no clear accumulative trend could be discerned (table 1).

The concentrations of Ni in the liver, kidneys, and spleen corresponded to concentration of the controls. No Fe_{ex} could be detected in the organs examined.

In the brain the concentrations of Cr, Ni, and Fe_{ex} did not differ from the corresponding values in the control animals.

Discussion

The data provided by this study are relevant to human exposure to MMA/SS welding fumes and the subsequent distribution of metals such as chromium and nickel to organ tissues. The exposure conditions simulated the actual MMA/SS welding environment as closely as possible.

If one assumes that the ventilation rate of rats is 0.1 l/h then the rats in our study inhaled a maximum of 164 μg of Cr, 170 μg of Fe, and 18 μg of nickel. If one estimates that the rats' lungs retained 30% of the contaminants contained in the welding fumes inhaled the total theoretical retentions are 50 μg for Cr, 55 μg for Fe, and 5.6 μg for Ni (table 2).

The maximum lung content of Cr measured was

27 μg . The corresponding content was 38 μg for Fe and 3.4 μg for Ni (table 2). The results indicate that the lungs are the main target organ of the Cr compounds found in MMA/SS welding fumes. The water soluble hexavalent alkaline chromates found in welding fumes are therefore chemically transformed into insoluble Cr compounds in the respiratory tract.

When the lung clearance curves were compared, the half times of Cr and Fe_{ex} lung clearance were similar, but nickel disappeared faster from the lungs. The excretion of Ni into the urine was also faster than that of Cr, even though the Ni compounds found in MMA/SS welding fumes have been assumed to be in a poorly water soluble form. The half time of $\text{K}_2\text{Cr}_2\text{O}_7$ after intratracheal injection into guinea pigs was 40 ± 5 d.¹² Thus the lung clearance pattern of the Cr compounds found in MMA/SS welding fumes resembles that of pure alkaline chromates. After the rats had been given $\text{Na}_2^{51}\text{CrO}_4$ intravenously, the lung retention and clearance patterns differed from those of our study.¹⁰ The Cr content of the lungs measured one hour after intravenous injection was about 15% of the total single chromate dose, and Cr was eliminated from the lungs very rapidly (the half time of lung clearance was about 3 d).

In the whole blood the content of Cr was constant at 0.5 μg throughout the entire exposure period, and the half time of Cr clearance was 6 d. One hour after intravenous injection of chromate into rats, the accumulation of Cr measured in the whole blood was high, about 25% of the total injected dose, and the half time of Cr clearance was about 13 d. Conversely, the content of Cr in the plasma at the same point in time was low, about 7% of the injected dose, and Cr was rapidly eliminated from the plasma ($T_{1/2} = 2$ d).¹⁰ Thus the clearance rate of Cr from the whole blood after the long term inhalation of the Cr

Table 2 Retention, clearance, and distribution of chromium (Cr), nickel (Ni), and iron (Fe) compounds found in MMA/SS welding fumes, in rats

	Cr (μg)	Fe (μg)	Ni (μg)
Estimated amount of inhaled metal	164	170	18
Total estimated metal retention	50	55	5.6
Estimated excretion into urine for entire period of exposure	6	—	1.8
Maximum metal content measured in:			
Lungs	27	38	3.4
Liver	3	1	Below detection limit
Kidneys	0.6	1	„
Spleen	0.12	1	„
Whole blood	0.5	Below detection limit	1

compounds found in MMA/SS welding fumes was faster than that which occurred after the single intravenous injection of pure chromate. Further inhalation studies are necessary so that the distribution of Cr between the plasma and the blood cells can be discerned.

In the liver the concentration of Cr began to increase three weeks after the initial exposure. The maximum content (3 μg) was reached three to four weeks after the last exposure. The content of Cr decreased to the level of controls two months after the termination of exposure. After the single intravenous injection of chromate, the concentration of Cr in the liver increased immediately to a high level and was thereafter eliminated rapidly ($T_{1/2}=5$ d).¹⁰ It may be assumed, therefore, that the mechanism of Cr deposition in the liver differs in these two studies. Our results suggest that the concentration of Cr measured in the liver after inhalation is mainly due to Cr that has been translocated from the lungs, whereas after the single intravenous injection of Cr into the lungs Cr was transported either by plasma proteins or by red blood cells.

In the kidneys also the accumulation and the clearance patterns of Cr differed from those observed after a single intravenous injection.¹⁰ In our study the content of Cr in the kidneys reached its maximum level (0.6 μg) during the first week of exposure. This relatively constant level, which was maintained until two weeks after the termination of exposure, then decreased to the level of the controls in two weeks. The total amounts of Cr and Ni excreted into the urine during the exposure period were estimated at 6 μg and 1.8 μg , respectively. Further investigations are necessary to obtain a more exact understanding of the excretion patterns of Cr and Ni.

In the spleen the content of Cr continued to increase after the termination of exposure (the maximum content of Cr was 0.12 μg). This apparently indicates that Cr liberated from red blood cells accumulates in the spleen. A similar tendency for chromates to accumulate in the spleen was observed after the intratracheal injection of $\text{K}_2\text{Cr}_2\text{O}_4$ into guinea pigs¹² as well as after the intravenous administration of Na_2CrO_4 into rats.¹⁰

Our results have partially shown the kinetics of the most interesting compounds found in MMA/SS welding fumes that occur in some organs of the rat. It appears that the kinetics of both Cr compounds and Ni compounds depend on the route of intake, but a more thorough understanding of the toxicokinetics of Cr compounds and Ni compounds in the organism may be attained only after further research. The relatively low concentration of Ni

compounds in MMA/SS welding fumes and the detection limit of the analytical methods of analysis used prevented us from acquiring a precise understanding of the kinetics of Ni in various organs.

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Br J Ind Med 1983 40: 229-234
doi: 10.1136/oem.40.2.229

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