Scand J Work Environ Health 1983;9(3):259-264

<https://doi.org/10.5271/sjweh.2411>

Issue date: Jun 1983

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by [Littorin M](#), [Hogstedt B](#), [Stromback B](#), [Karlsson A](#), [Welinder H](#),
[Mitelman F](#), [Skerfving S](#)

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No cytogenetic effects in lymphocytes of stainless steel welders

by Margareta Littorin, MD,¹ Benkt Högstedt, MD,^{1,2} Bodil Strömbäck,³ Anita Karlsson,² Hans Welinder, PhD,¹ Felix Mitelman, MD, PhD,³ Staffan Skerfving, MD,¹

LITTORIN M, HÖGSTEDT B, STRÖMBÄCK B, KARLSSON A, WELINDER H, MITELMAN F, SKERFVING S. No cytogenetic effects in lymphocytes of stainless steel welders. *Scand j work environ health* 9 (1983) 259–264. In 24 manual metal arc stainless steel welders (means: exposure time 19 years, 100 electrodes/d, air chromium level 81 µg/m³, urinary chromium 47 µmol/mol creatinine) and 24 matched referents, lymphocytes in peripheral blood were analyzed for cytogenetic effects. No statistically significant differences were observed as to frequency of cells with breaks and fragments (1.5 % for the welders, 1.9 % for the referents); gaps and isogaps (1.8 vs 2.0 %); interchanges, dicentrics, rings and markers (0.8 vs 0.5 %); total number of cells with structural aberrations (4.1 vs 4.4 %); hyperdiploidy (0.3 vs 0.2 %); or total number of cells with aberrations (4.4 vs 4.6 %). Neither were there any differences in the frequencies of micronuclei (7.8 vs 7.9 per mille) or sister chromatid exchanges (11 vs 12 per cell) in lymphocytes of peripheral blood.

Key terms: chromium, chromosome aberrations, micronuclei, nickel, sister chromatid exchanges.

Hexavalent (VI) chromium is mutagenic in different test systems in vitro (1, 12, 18, 23, 34, 37, 38). Certain nickel compounds have mutagenic properties in mammalian test systems in vitro (11, 24, 25, 33, 34, 35). An increased frequency of chromosome aberrations in peripheral lymphocytes has been reported for workers engaged in chromium production (3). Certain chromium (VI) and nickel compounds are carcinogenic in animals and man (11, 12, 18, 24, 26, 33, 35). Chromium (VI) and nickel are present in stainless steel welding fumes (20, 32, 36). Such fumes, especially from manual metal arc welding, have been shown to be mutagenic in vitro in bacteria

and mammalian cells (8, 15, 21), as well as positive in the mammalian spot test (14). Chromium has been suspected to be a causative agent (8, 15). The mutagenic effect seems to be diminished or eliminated in the case of welding fumes (8), as well as in the case of chromium (7, 19, 28) in the presence of rat liver microsome fraction or human erythrocyte lysate (28). Microsomal preparation from rat lung was however a poor inactivator of chromium mutagenicity (28). Recently a retrospective cohort study of mortality suggested an association between lung cancer and stainless steel welding (31). In addition a slight excess of lung cancer deaths was noted among welders with mixed exposure (29). Similarly a proportionate mortality study reported that welders and flamecutters had an excess of lung and urinary bladder cancer (22).

In the present communication we report a study of the cytogenetic effects in vivo in lymphocytes from peripheral blood of stainless steel manual metal arc welders and matched referents.

¹ Department of Occupational Medicine, University Hospital, Lund, Sweden.

² Department of Occupational Medicine, County Hospital, Halmstad, Sweden.

³ Department of Clinical Genetics, University Hospital, Lund, Sweden.

Reprint requests to: Dr M Littorin, Department of Occupational Medicine, University Hospital, S-221 85 Lund, Sweden.

Subjects and methods

Subjects

Welders. Twenty-four male manual metal arc welders from six industries in different parts of Sweden were studied. They were selected because of long and intense welding on stainless steel (table 1). The electrodes and materials used during the last five to ten years contained about 20 (range 12–27) % chromium, 10 (range 0–23) % nickel (electrodes up to 60 % in a few cases and for shorter time periods), and mostly about 3 (range 0–10) % molybdenum. In an interview the average number of electrodes used per day during the last decade was estimated by 19 of the welders to be about 100 (table 1). This figure was "checked" by the counting of electrodes

used by 17 welders during a workday in which air levels were measured (see the Methods section). The average number used then was 98 (range 35–196). The diameter of the electrodes was between 3.25 and 5.0 mm, in a few cases 2.5 mm. The corresponding current used was 100 to 250 A, in a few cases 80 A. Some of the welders under study used welding shields, and others used respiratory protective devices when working in narrow spaces, while others used no such equipment. All industries had general ventilation; some also had local exhaust systems.

Referents. For each welder a referent was matched according to sex, age (within 5 years), smoking (table 1), socioeconomic class, living area, and drug consumption. Both subjects in one pair used a beta-blocker as antihypertensive treatment. A rough match on alcohol consumption was also made. None of the referents had any occupational (or other) experience with the handling of stainless steel (or other known mutagenic/carcinogenic agents). Their occupations were electrician (one), dairy worker (two), municipal worker (four), office clerk (two), policeman (one), postman (one), railway clerk (four), sawmill worker (six), and butcher (three).

Table 1. Age, smoking habits, and exposure data for the welders and the referents. (TWA = time-weighted average)

	Welders	Referents
Number	24	24
Age (years)		
Mean	44	44
Range	33–64	32–63
Smoking habits ^a		
Smokers	9	9
Exsmokers ^b	6	6
Nonsmokers (never smoked)	9	9
Exposure		
Time (years)		
Mean	19	—
Range	7–41	—
Number of electrodes/d	100	—
Total chromium air levels		
Mean ($\mu\text{g}/\text{m}^3$ TWA)	81	—
Range ($\mu\text{g}/\text{m}^3$ TWA)	4–415 ^c	—
Hexavalent chromium air levels		
Mean ($\mu\text{g}/\text{m}^3$ TWA)	55	—
Range ($\mu\text{g}/\text{m}^3$ TWA)	5–321 ^d	—
Chromium in urine		
Mean ($\mu\text{mol}/\text{mol}$ creatinine)	47	1.5
Range ($\mu\text{mol}/\text{mol}$ creatinine)	5–155 ^e	< 0.4–7.0

^a Two pairs could not be matched as to smoking. In one pair one subject had never smoked; one subject had smoked earlier.

^b Stopped smoking more than one year ago. In one case however only six months had passed since the subject had stopped smoking.

^c N = 22.

^d N = 17.

^e N = 23.

Methods

Medical examinations. The welders and referents were interviewed for medical and occupational history. Special attention was paid to viral infections and exposure to ionizing radiation, heavy metals, and organic solvents. Routine blood analyses were performed. In addition renal function was studied in some detail. In no subject was there evidence of significant disease (except for the two cases of treated hypertension already mentioned).

Chromium in air. The exposure to chromium in air was determined during one workday (Monday). Representative air samples were collected for each welder in 2-h periods on four cellulose acetate filters (Millipore, pore diameter 0.8 μm) by use of a portable pump (MSA or Casella; 2 l/min). Chromium was analyzed by flameless atomic absorption spectrometry (Perkin-Elmer 403) after the filter was treated with

water (soluble chromium VI) and aqua regalis (total chromium). The time-weighted average exposure for one work-day was calculated (table 1).

Chromium in urine. Urine samples were collected during the workday. In this study the samples used were obtained immediately after the end of work (table 1). The analysis of chromium in urine was made with a direct flameless atomic absorption spectrometric method. The detection limit was 0.008 $\mu\text{mol/l}$. The method error was 0.02 $\mu\text{mol/l}$ in the range 0–0.2 $\mu\text{mol/l}$ and 0.1 $\mu\text{mol/l}$ in the range 0.6–2 $\mu\text{mol/l}$. The accuracy of the method was confirmed by good results in repeated interlaboratory checks.

Cytogenetic studies. Lymphocytes were obtained from venous blood samples taken in the afternoon at the same time from each pair. The samples were shipped by air to the laboratory. Latency time before the start of cultivation was about 16 h. Each cytogenetic parameter was analyzed by one observer, who was unaware of the exposure data.

Chromosome aberrations were assessed by a routine microculture method described earlier (9). Cultivation time was 72 h. For each individual 100 metaphases were analyzed. The aberrations were scored according to Evans et al (5).

Micronuclei were analyzed in lymphocytes cultured for 96 h in the presence of phytohemagglutinin (4, 30). After centrifugation the cells were suspended in an equal amount of medium, smeared on a slide, stained according to May–Grünwald–Giemsa (without previous hypotonic treatment or fixation), and scored for micronuclei. For each of 40 subjects (includes 19 pairs) 1,000 lymphocytes were screened.

The sister chromatid exchange frequency was studied in 9 welders and 12 referents (eight pairs included). The sister chromatid exchanges were analyzed in phytohemagglutinin-stimulated peripheral blood lymphocytes cultured for 72 h and stained with the Giemsa technique (27). 5-Bromodeoxyuridine was present during the entire culture period at a final concentration of 0.1 mmol/ml of medium. From each individual 21–25 metaphases

CELLS WITH ABERRATIONS (%)

Fig 1. Structural chromosome aberrations (including gaps) in lymphocytes of 24 welders and 24 matched referents (controls). Arrows indicate medians. Closed symbols denote smokers, open ones nonsmokers and exsmokers.

in cells with 46 chromosomes were analyzed.

Statistical analysis. Wilcoxon matched-pairs signed-ranks test (for the welder-referent pairs) and the analysis of covariance (with regard to exposure, age and smoking) were used. All the p-values reported are two-tailed.

Results⁴

The frequencies of different types of chromosome aberrations did not differ between the welders and referents (when analyzed statistically in pairs or by covariance). This statement applies to the frequency of cells with breaks and fragments (1.5% for welders, 1.9% for referents), gaps and isogaps (1.8 vs 2.0%), interchanges, dicentric, rings and markers (0.8 vs 0.5%), total number of cells with structural aberrations (4.1 vs 4.4%), hyperdiploidy (0.3 vs 0.2%), and total number of cells with aberrations (4.4 vs 4.6%) (fig 1).

⁴ A table containing detailed individual data on exposure and cytogenetics can be obtained from the authors.

Fig 2. Micronuclei in lymphocytes of 20 welders and 20 referents (controls). Arrows indicate medians. Closed symbols denote smokers, open ones nonsmokers and exsmokers.

Fig 3. Sister chromatid exchanges (SCE) (average number per cell) in 9 welders and 12 referents (controls). Arrows indicate medians. Closed symbols denote smokers, open ones nonsmokers and exsmokers.

When analyzed by covariance, there was a statistically significantly ($p < 0.007$) higher frequency of cells with interchanges, dicentrics, ring chromosomes, and marker chromosomes (asymmetrical or incomplete symmetrical chromosome-type aberrations) in smokers as compared to nonsmokers plus exsmokers. However the frequency of cells with other types of aberrations did not differ, neither did the total number of cells with aberrations.

No difference in micronuclei was observed between the welders and referents (means 7.8 vs 7.9 per mille) (fig 2), and there was no effect of smoking.

There was no difference in the frequency of sister chromatid exchanges between the

welders and referents (1 vs 12 per cell) (fig 3), and no difference between the smokers and nonsmokers.

Discussion

Intense and long-time exposure to manual metal arc welding fumes from stainless steel did not give rise to cytogenetic effects in blood lymphocytes. No cytogenetic damage in welders was found, in comparison to referents, in a similar study from Finland (10).

Only one of the cytogenetic parameters studied showed an effect of smoking. This is not quite in accordance with earlier studies and could be due to the small

number of subjects studied or to the fact that the subjects were not heavy smokers (less than one pack of cigarettes per day).

The lack of cytogenetic effects between the welders and referents in our study might be explained in several ways. Ingredients in manual metal arc welding fumes from stainless steel may not be mutagenic in man. The workroom exposure to mutagenic agent(s) may be too low, or there may be an insufficient distribution of mutagen(s) to the lymphocytes, eg, due to retention in the lungs (2, 13, 16) or to metabolic inactivation of mutagen(s) or to a mopping up of chromium by the red blood cells (6, 17). In addition, mutagenic agent(s) may reach the lymphocytes but for some reason do not generate cytogenetic damage in this type of cell, or cytogenetically damaged lymphocytes may be efficiently eliminated from the blood stream. The lack of cytogenetic damage in lymphocytes could, however, still be in accordance with a mutagenic/carcinogenic effect in the lungs or other organs. Of course cytogenetic studies of this kind do not exclude the possibility of mutagenic effect, eg, point mutations.

Acknowledgments

The study was supported by grants from the Swedish Cancer Society and the Swedish Work Environment Fund.

The statistical analysis was performed by B Gullberg, BSc. The best assistance in the fieldwork was given by the staffs of those health care units serving the industries engaged in this study.

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Received for publication: 29 October 1982