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Elevated DNA single-strand breakage frequencies in lymphocytes of welders exposed to chromium and nickel

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DNA damage (alkaline filter elution) and sister chromatid exchange (SCE) frequencies were measured in lymphocytes of 39 welders and 39 controls. The welders showed a significantly higher rate of DNA single-strand breakages and significantly elevated SCE values. These results are not in accordance with those of a former study in which only DNA-protein cross-links were measured. The different results may be explained on the basis of different exposure levels for chromium(VI) and nickel. Both methods are not specific but sensitive enough to measure genotoxic damage after occupational exposure to chromium(VI) and nickel in the range of threshold values for the workplace on a collective basis. Additionally, the results indicate that DNA single-strand breakage and DNA-protein cross-links show different increases depending on the exposure levels for chromium and nickel.

Introduction

The carcinogenic potency of chromium and nickel is well known. Hexavalent chromium and divalent nickel show positive effects in several genotoxic tests (1). Hexavalent chromium can easily pass the cell membranes, and it is reduced inside the cells to its trivalent form. Trivalent chromium, intermediates like Cr(V) and Cr(IV) and radicals are suspected to react with DNA and cause DNA damage (2–4). It is also known that divalent nickel ions are able to induce DNA damage in various cell systems (5,6). The nickel-induced DNA damage is probably induced by nickel catalyzed oxygen radicals and not by direct involvement of nickel compounds (7,8). Electric welders are often exposed to chromium and nickel containing welding fumes (1). There are few investigations of welders and workers, exposed to chromium and nickel, that measure the frequencies of DNA strand breakages and DNA–protein cross-links in surrogate tissues such as lymphocytes (9–11). In a previous investigation (11) of 39 welders we used the method of the alkaline filter elution and found an increased frequency of DNA–protein cross-links in the lymphocytes of the welders.

Using the sister chromatid exchange (SCE*) test, different results have been found in investigations of welders and workers exposed to chromium and nickel (12–14). In our previous investigation (11) the average SCE frequency in the lymphocytes of welders was significantly lower than the value of the control group.

In order to verify our previous results (11) we investigated another group of workers exposed to chromium and nickel. Again, we applied the SCE test and the method of the alkaline filter elution using polyvinylidene fluoride (HVLP) filters and polycarbonate (PC) filters with and without proteinase K.

***Abbreviations:** SCE, sister chromatid exchange; HVLP, polyvinylidene fluoride; PC, polycarbonate; GOT, glutamate-oxalacetatetranspeptidase; GPT, glutamate-pyruvatetranspeptidase; GGT, gamma-glutamyltranspeptidase; HEPES, 2-[4-(2-hydroxyethyl)-piperazine-1-yl] ethanesulphonic acid; BrdU, bromodeoxyuridine; MMA, manual metal-arc welding; RPMI, Roswell Park Memorial Institute medium; TLV, threshold limit value for chemical substances and physical agents; MAK, maximum workplace concentration; TRK, technical exposure limit.

Materials and methods

Subjects

Blood samples were taken from 39 fully-employed male welders and from 39 male control persons who were not known to be substantially exposed to carcinogens in their occupation or environment. The control group was standardized according to smoking habits and age distribution (±5 years). The same number of welders were investigated as in the previous study (11). Matched pairs were formed for statistical analyses.

Blood samples

Venous blood (25 ml) was taken in the morning from all workers and controls using monovettes (Sarstedt) containing 15 IU lithium heparin/ml blood. The blood samples were cooled to 5°C and transported to our Institute, with as little vibration as possible, within 5 h.

Further samples were taken from the welders to determine the values of chromium in erythrocytes and nickel in blood (monovettes containing EDTA) and the activity of glutamate-oxalacetatetranspeptidase (GOT), glutamate-pyruvatetranspeptidase (GPT) and gamma-glutamyltranspeptidase (GGT) in serum (monovettes containing beads).

Determination of nickel and chromium

The concentrations of chromium in the erythrocyte fraction and nickel in blood were determined by atomic absorption spectrometry (15).

Alkaline filter elution

We used the method of the alkaline filter elution according to Kohn *et al*. (16). Heparinized blood was diluted at a ratio of 1:3 (v/v) with Eagle's minimum essential medium in Hank's balanced salt solution and the lymphocytes were isolated on Percoll gradients (Biochrom KG, Seromed). If necessary, any erythrocytes remaining were lysed by incubation with 150 mM NH_4Cl, 10 mM $KHCO_3$, 1 mM Na_2–EDTA for 3 min at 0°C and lymphocytes were collected by centrifugation and then resuspended in phosphate buffered saline (PBS).

V79 cells (hamster fibroblasts) were cultivated in Dulbecco's minimum essential medium with 10% (v/v) horse serum, 10% (v/v) 2-[4-(2-hydroxy ethyl)-piperazine-1-yl] ethanesulphonic acid (HEPES), 7.5% sodium hydrogen carbonate and L-glutamine. Confluent growing V79 cells were harvested by trypsinization immediately before elution. Alkaline filter elution was carried out using a slight modification of the method described by Doerjer *et al*. (17). The samples were eluted through polycarbonate (PC) filters with a pore diameter of 2.0 μm (Nucleopore, Pleasanton, CA) and through polyvinylidene fluoride (HVLP) filters with a pore diameter of 0.45 μm (Milipore Corporation, USA). The elution through PC filters was carried out with (PC+) proteinase K (2 mg was added to a 4-ml lysis solution) and without (PC–). For each subject and filter type (PC+, PC–, HVLP) three samples were determined.

The elution buffer consisted of 2 M NaCl, 0.02 M Na_2–EDTA, 0.5 M H_3BO_3 and 0.65 M NaOH. The first 1 ml, corresponding to the tube volume, was discarded. The fractions were collected for 10 h. The eluates, filter extracts, and rinsed fractions of the filter holders and tubes were neutralized with 3 M NaH_2PO_4 and 3 M H_3PO_4, and their DNA content was determined fluorimetrically (18) after reaction with fluorochrome Hoechst 33258 (final concentration in the sample 0.5 μM). These results were used to calculate the DNA retention (D) of the filter as a percentage of the total amount of DNA; the elution rate (k) was calculated from $k = 2 - \lg D$. The relative elution

rates (k_{rel}) were calculated from the elution rates obtained for the V79 cells ($k_{rel} = K/K_{V79}$), which were determined simultaneously.

SCE test

Heparinized blood (400 µl) was incubated for 72 h at 37°C in 5 ml RPMI 1640 medium (Boehringer) with glutamine, antibiotics, 20% fetal calf serum (Flow), phytohaemagglutinin M (Gibco) and 10^{-5} M bromodeoxyuridine (BrdU). The treatment of the cultures with colcemid and hypotonic KCl solution, the fixation, the preparation of the chromosomes and the fluorescence plus Giemsa staining of the preparations were carried out according to the method of Perry and Wolff (19). The sister chromatid exchanges were determined by examining 25 complete second metaphases per subject. The SCE frequencies for each subject were taken as the average of the SCE frequencies counted per metaphase.

Statistical analysis

Statistical analyses (Wilcoxon test, Kruskal–Wallis test, Pearson and Spearman correlation, variance analysis) were performed on a personal computer using the SAS program. Results were significant if $P < 0.05$.

Results

In Table I the personal data of 39 welders and 39 controls are presented. The results of individual relative DNA elution rates for HVLP filters, PC filters with and without proteinase K and the SCE frequency are shown for each person. Additionally, the chromium concentration in erythrocytes, the nickel concentration in blood and the GGT activity in the serum of the welders are listed.

In Table II the average values of the alkaline filter elution rates, SCE frequencies and biomonitoring data are given for the welders and the controls. The mean concentration of chromium in erythrocytes of the welders was 4.3 µg/l ($n = 32$) and for nickel in blood it was 4.6 µg/l ($n = 33$).

There were no significant differences in the relative DNA elution rates through PC filters without proteinase K and HVLP filters between welders and controls. The mean relative DNA elution rate for PC filters with proteinase K was 1.40 for the welders and 0.82 for the controls and was significantly different ($P = 0.0001$). These results indicate a significantly elevated DNA single-strand breakage frequency and additional DNA–protein cross-links in welders. Furthermore, the mean SCE frequency of the welders was significantly elevated (6.22 versus 5.87; $P = 0.04$).

There were no significant correlations (Pearson and Spearman) between DNA elution rates, SCE frequencies and biomonitoring results. After dividing the welders in three categories of chromium and nickel exposure (Chromium in erythrocytes: A: ⩽ 1 µg/litre; B: 1.5 µg/litre; C: > 5 µg/litre; nickel in blood: A: ⩽ 2 µg/litre; B: 2–6 µg/litre; C: > 6 µg/litre) decreasing SCE values for the workers were observed with increasing chromium exposure (A: 6.41; B: 6.07; C: 5.89) and increasing SCE values with

Table I. Individual relative DNA elution rates, SCE frequencies and data of welders and controls
(A) Welders

No.	Age (years)	Smoking habits	ERPC–	ERPC+	ERHVLP	SCE	Cr (µg/l)	Ni (µg/l)	GGT (U/l)
1	23	NS	0.325	0.899	2.157	5.36	4.5	1.7	6
2	25	NS	0.474	1.233	1.338	6.52	0.6	4.6	5
3	27	NS	1.238	1.733	2.009	5.48			6
4	28	NS	1.905	3.156	5.013	5.88	0.1	1	12
5	33	NS	0.561	1.749	4.448	4.8		0.1	8
6	39	NS	0.55	0.431	3.279	6.08	0.3	0.5	14
7	42	NS	1.035	1.43	3.479	6.24	15	2.8	25
8	43	NS	0.884	1.283	1.82	5.28	3	0.8	12
9	45	NS	1.399	1.137	1.953	6.24			7
10	46	NS	0.814	1.728	2.769	6.52	5	1.7	6
11	50	NS	1.809	2.233	4.119	6.52	0.3	0.1	30
12	54	NS	1.621	2.021	4.24	8.08	1	0.1	35
13	26	S	0.648	1.048	2.468	6.72	6.5	1	28
14	29	S	0.957	1.248	3.237	6.52	0.8	7.1	5
15	29	S	0.768	0.958	2.027	6.88	5.5	2	51
16	33	S	0.745	0.954	1.842	5.6	0.2	3.4	11
17	34	S	0.457	0.459	1.562	5.8	0.7	0.3	15
18	34	S	0.809	1.106	1.436	5.88	38	0.2	14
19	35	S	0.908	1.195	2.702	7.04			31
20	35	S	1.1	1.818	3.018	5.64	8.8	2.6	6
21	35	S	1.071	1.72	2.45	6.32	0.6	12	10
22	36	S	1.155	2.373	5.133	4.76	3.3	4	6
23	36	S	0.823	1.147	2.565	5.72	2.1	3.7	12
24	37	S	0.893	0.915	1.554	5.32	6.7	0.7	20
25	37	S	0.663	1.149	2.277	6	8.7	0.3	28
26	38	S	0.629	0.983	2.086	7.48	1.7	0.3	17
27	38	S	1.191	1.261	3.863	5.24	3.6	0.5	18
28	38	S	1.057	1.234	1.947	6.52			10
29	38	S	0.742	0.976	2.652	6.08	2.1	0.2	15
30	40	S	1.342	3.476	6.362	4.44	7.3	0.1	12
31	44	S	1.02	1.659	4.08	6.24	2.3	3.6	12
32	46	S	0.645	0.76	1.543	5.84	1.8	1.6	8
33	47	S	1.077	1.138	4.129	6.48	0.1	66	17
34	47	S	0.324	1.692	3.443	6.28	0.5	4.4	15
35	48	S	1.275	1.754	2.439	8.88			24
36	50	S	0.907	0.78	1.587	7.32	4.4	14	29
37	52	S	0.782	1.223	3.276	6.88			17
38	54	S	0.414	0.942	1.974	6.84	1	6.1	17
39	56	S	0.961	1.433	2.995	6.96	1.9	3.2	30

increasing nickel exposure (A: 6.01; B: 6.00; C: 6.70). These results were not significant by the Kruskal–Wallis test. No influence could be seen with the alkaline filter elution assay.

The welders who worked >50% of their shift by manual metal-arc welding (MMA) revealed higher values of DNA elution rates through all three filter types (PC– 1.03 versus 0.86; PC+ 1.48 versus 1.34; HVLP 2.86 versus 2.85) and higher SCE values (6.41 versus 6.11) (not significant in the Wilcoxon test).

There was a slight positive correlation (Pearson) between working years and relative DNA elution rates through PC filters without proteinase K ($r = 0.32$; $P = 0.0498$; $n = 39$) and SCE values ($r = 0.38$; $P = 0.02$; $n = 39$).

The average age was 39.2 ± 8.6 years for the welders and 38.6 ± 9.8 years for controls. Only in the entire group ($n = 78$) there was a significant positive correlation between age and the SCE frequency in the Pearson ($r = 0.34$; $P = 0.002$; $n = 78$) and Spearman ($r = 0.35$; $P = 0.011$; $n = 78$) correlation analyses. There was no significant correlation between age and the relative DNA elution rates through all three filter types. The biomarker results did not significantly differ between smokers and non-smokers (Wilcoxon test).

Alcohol drinking habits (alcohol consumption versus no alcohol consumption) had some influence. In the group of welders, the relative DNA elution rates were significantly lower through PC filters with proteinase K (1.23 versus 2.30; $P = 0.002$) and HVLP filters (2.60 versus 4.22; $P = 0.031$) and the SCE frequency was significantly higher (6.38 versus 5.34; $P = 0.016$) in the Wilcoxon test for persons drinking alcohol ($n = 33$) in comparison with non-drinkers ($n = 6$). In the control group the SCE frequency was significantly higher (6.37 versus 5.69; $P = 0.034$) for non-drinkers ($n = 11$). The welders with a gamma-glutamyltranspeptidase (GGT) activity more than the threshold value of 25 U/l ($n = 7$) had significantly higher SCE frequencies in the Wilcoxon test (6.94 versus 6.04; $P = 0.023$). There was a significantly positive correlation between the GGT activity and the SCE frequency in the Pearson ($r = 0.52$; $P = 0.0006$; $n = 39$) and Spearman ($r = 0.50$; $P = 0.0011$; $n = 39$) correlation analyses. GGT activity had no influence on the DNA elution rates.

Discussion

It is known that hexavalent chromium contributes to the formation of DNA–protein cross-links and DNA single-strand

Table I. *continued*
(B) Controls

No.	Age (years)	Smoking habits	ERPC–	ERPC+	ERHVLP	SCE
1	23	NS	0.879	0.751	2.444	4.8
2	23	NS	0.784	0.875	2.072	6.2
3	29	NS	0.413	0.161	1.586	5.4
4	30	NS	0.919	0.63	1.586	4.84
5	32	NS	1.301	1.462	1.755	5.72
6	35	NS	0.834	0.702	1.801	5.48
7	40	NS	0.796	0.936	2.103	6.12
8	40	NS	0.67	0.567	2.101	4.12
9	46	NS	0.815	0.726	1.618	6.32
10	47	NS	0.968	0.74	3.172	6
11	55	NS	0.966	1.16	1.786	6.48
12	59	NS	0.743	0.924	1.985	6.56
13	25	S	0.618	0.557	1.801	4.88
14	26	S	1.02	0.954	1.738	5.48
15	28	S	1.01	0.896	1.4	5.44
16	30	S	0.641	0.537	2.058	6.08
17	30	S	1.251	1.345	3.04	8.68
18	32	S	0.75	0.792	1.538	7.08
19	33	S	1.231	1.017	2.54	5.24
20	33	S	1.02	0.951	3.543	5.12
21	33	S	0.96	1.181	4.729	5.8
22	34	S	0.656	0.582	2.197	5.12
23	35	S	1.031	1.148	2.546	5.04
24	35	S	0.901	0.982	6.402	6.52
25	36	S	0.498	0.37	1.58	5.36
26	36	S	0.914	0.87	4.164	5.72
27	37	S	0.543	0.532	1.788	5.6
28	37	S	1.087	0.86	4.828	5.36
29	38	S	0.56	0.366	1.626	6.04
30	40	S	0.898	1.199	2.159	7.92
31	42	S	1.211	1.356	3.42	7
32	45	S	1.218	1.086	3.028	5.28
33	46	S	0.859	0.611	3.491	5.36
34	48	S	0.585	0.489	1.448	5.72
35	50	S	0.919	0.9	2.178	5.24
36	52	S	0.574	0.511	1	5.4
37	53	S	0.782	0.54	2.09	6.88
38	55	S	1.22	1.132	2.408	7.48
39	57	S	0.884	0.655	2.2	6.4

S, smokers; NS, non-smokers; ERPC–, relative DNA elution rates through polycarbonate filters without proteinase K; ERPC+, relative DNA elution rates through polycarbonate filters with proteinase K; ERHVLP, relative DNA elution rates through polyvinylidene filters; SCE, sister chromatid exchange frequency; Cr, chromium concentration in erythrocytes; Ni, nickel concentration in blood; GGT, glutamate-oxalacetatetranspeptidase activity.

Table II. Mean values and standard deviations of results obtained of 39 welders and 39 controls

Parameter	Welders (mean ± SD)	Controls (mean ± SD)	P^a
Chromium concentration in erythrocytes (μg/l)			
$n = 32$	4.3 ± 7.0		
Nickel concentration in blood (μg/l)			
$n = 33$	4.6 ± 11.4		
Relative DNA elution rate (PC filters without proteinase K)			
$n = 39$	0.92 ± 0.37	0.87 ± 0.23	0.73
$n = 27$ (S)	0.87 ± 0.26	0.88 ± 0.24	0.91
$n = 12$ (NS)	1.05 ± 0.54	0.80 ± 0.32	0.44
Relative DNA elution rate (PC filters with proteinase K)			
$n = 39$	1.40 ± 0.63	0.82 ± 0.30	0.0001
$n = 27$ (S)	1.31 ± 0.59	0.83 ± 0.30	0.0003
$n = 12$ (NS)	1.59 ± 0.70	0.80 ± 0.32	0.0029
Relative DNA elution rate (HVLP filters)			
$n = 39$	2.85 ± 1.18	2.43 ± 1.00	0.08
$n = 27$ (S)	2.76 ± 1.17	2.63 ± 1.25	0.54
$n = 12$ (NS)	3.05 ± 1.21	2.00 ± 0.45	0.0225
SCEs/cell			
$n = 39$	6.22 ± 0.88	5.87 ± 0.91	0.04
$n = 27$ (S)	6.28 ± 0.90	5.97 ± 0.97	0.09
$n = 12$ (NS)	6.08 ± 0.84	5.67 ± 0.76	0.37

[a]Wilcoxon test.
S, smokers; NS, non-smokers.

breakages *in vitro* and *in vivo* (1,2). Nickel also may cause such DNA damage (5,6). Chromium and nickel induced DNA–protein cross-links seem to be stable over long periods of time, whereas chromium-induced DNA single-strand breakages are rapidly repaired (20–22).

In our study, we found significantly elevated DNA single-strand breakage frequencies and additional DNA–protein cross-links in the lymphocytes of welders exposed to chromium and nickel. Also, the SCE frequencies were significantly elevated in comparison with a standardized control group.

There are only few investigations that have looked for DNA–protein cross-links and DNA single-strand breakages in lymphocytes of workers exposed to chromium and nickel. Vangala *et al*. (9) found elevated DNA single-strand breakages in a group of workers exposed to chromium. Costa and colleagues (10,23) described significantly increased DNA–protein cross-link frequencies in a group of manual metal arc-welders. However, Gao *et al*. (24) detected no difference in the DNA single-strand breakage frequencies of workers with low chromium exposure.

In our study, we determined the chromium concentrations in erythrocytes and the nickel concentrations in blood to calculate the exposure of the welders. The average concentration of chromium in erythrocytes of the welders was 4.3 μg/l ($n = 32$) and of nickel in blood it was 4.6 μg/l ($n = 33$). These values were much higher than the mean environmental background levels of 0.5 μg/l chromium in erythrocytes and 0.2–0.4 μg/l in blood (25). There are only a few reports that describe the relationship between chromium concentrations in erythrocytes and hexavalent chromium concentrations in the air at the workplace of welders. According to these data, the average chromium concentration in the erythrocytes of welders in our investigation could correspond to a chromate concentration of ~100 μg CrO_3/m^3 in the air, and the average nickel concentration in blood to a concentration of 300 μg Ni/m^3 in the air (26,27).

In our previous investigation (11) of welders exposed to chromium and nickel we detected only significantly elevated DNA–protein cross-links in comparison with the controls, and no hint of DNA strand breakages. We determined the chromium and nickel concentrations in the urine of the welders, which could have averaged an exposure of 70–80 μg CrO_3/m^3 and <100 μg Ni/m^3 in the air (26,27).

Thus, in our actual investigation the chromium and nickel exposure of the welders was much higher than in our previous research or in that of other reports. This might be the reason for the elevated rate of DNA single-strand breakage frequencies that we found. Costa *et al*. (28) report on the saturation amount of DNA–protein cross-links in lymphocytes of exposed workers who had chromium concentrations in erythrocytes of 7–8 μg/l, with this corresponding to a chromate exposure of 30 μg CrO_3/m^3 in air (26,27). This could lead to the conclusion that at lower chromium concentrations, DNA–protein cross-links are mainly formed, whereas at higher concentrations, DNA strand breakages predominate. The different results of our two investigations might be a result of different chromium exposure levels, which lead to DNA–protein cross-links at lower concentrations and DNA single-strand breakages at higher concentrations. To summarize, if DNA–protein cross-links have a prominent carcinogenic effect, the relative carcinogenic effect of low chromium exposures in the range of 30–50 μg CrO_3/m^3 in air might be greater than at higher chromium exposures.

There might be a similar dose-dependent effect of nickel. Ciccarelli *et al*. (29) treated cell nuclei of rats with nickel carbonate at variable concentrations and detected DNA–protein cross-links at all levels. DNA single-strand breakages were only detectable at higher nickel carbonate concentrations.

In our investigation, the relative DNA elution rates did not significantly correlate with exposure against chromium and nickel, age and smoking habits. In different investigations there was no consistent influence of smoking habits on DNA single-strand breakage and DNA–protein cross-link frequencies (10,11,30–34).

We found significantly higher SCE frequencies in the lymphocytes of welders in comparison with controls. Previous investigations have shown different results. Knudsen *et al*.

(35) and Jelmert *et al*. (36) found decreased average SCE frequencies in lymphocytes of welders exposed to chromium and nickel. However, Nagaya *et al*. (37) found no difference in SCE frequencies between workers exposed to chromium and controls.

In our previous investigation (11) the SCE frequencies of 39 welders were significantly lower than those of the controls. In the actual investigation, the significantly elevated SCE frequency might be a consequence of higher chromium and nickel exposure, but there are indications that both compounds may cause different effects in the SCE test. Katsifis *et al*. (38) found an antagonistic effect of chromium(VI) and nickel(II) in the SCE test *in vitro*.

We found significant positive correlations (Pearson and Spearman) between the age and the SCE frequencies in the entire group of welders and controls, which is in accordance with reports of Soper *et al*. (39) and Sarto *et al*. (13). Other authors have not confirmed the influence of age (40–42).

Biomonitoring data and smoking habits had no significant influence on the SCE frequency in our actual study, but in most studies smokers have higher SCE values (e.g. 40–43).

There are few reports that describe elevated SCE frequencies depending on alcohol consumption (40,42,43). We found significantly higher SCE frequencies in lymphocytes of welders, with a GGT activity that was more than the threshold value of 25 U/l, in welders with increased alcohol consumption compared with welders with a normal GGT activity.

In conclusion, both of our investigations show that the results of the alkaline DNA filter elution and SCE test are not specific for exposure against chromium and nickel. On the other hand, the methods are sensitive enough to indicate DNA damage in lymphocytes of welders on a collective basis after chromium and nickel exposure in the range of US and German occupational limits (TLV and MAK/TRK) (26,44). The method of the alkaline DNA filter elution seems to be more sensitive for detecting genotoxic effects than the determination of SCE frequencies. Additionally, the results indicate that DNA single-strand breakages and DNA–protein cross-links show different values depending on the exposure levels of chromium and nickel. DNA–protein cross-links are measurable even at low exposures and DNA single-strand breakages seem to relatively increase at exposures of 30–50 µg CrO_3/m^3 and higher. These results suggest that DNA–protein cross-links that occur as a result of low chromium exposure may have a prominent carcinogenic effect and that actual TLV an MAK/TRK values seem to be too high to prevent health damage in welders.

In further studies, the dose-dependent influence of chromium on the results of the alkaline DNA filter elution should be verified. The question remains open as to whether there is a dose-dependent effect of nickel on the alkaline DNA filter elution rates and SCE frequencies, and a possible antagonistic effect between chromium and nickel on the SCE frequencies.

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