Biological Monitoring for Chromium Exposures in the Workplace

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The main objective of this study was to assess chromium exposure and to promote understanding of biological monitoring. Workplace activities observed involved production in a leather tannery. The urine Cr and serum Cr level in the specimens of non-occupational controls and occupational workers were determined by graphite furnace atomic absorption spectrophotometry (GFAAS). This technique includes a dilution of serum with 12 mM ultra nitric acid and 1% Triton X-100. The results showed that total serum Cr levels in the exposed occupational workers were significantly higher than those in unexposed non-occupational controls ($p < 0.05$), and that there is a risk of chromium poisoning to the workers engaged in leather tannery worksites. The total urine Cr levels in the exposed workers were no different than in controls ($p > 0.05$). The etiology of chromate poisoning is also briefly mentioned.

Key words — biological monitoring; chromium; GFAAS; serum; tannery worker

Introduction

Biological monitoring and biological effect monitoring are methods for assessing the absorption of certain substances to which individuals may be exposed in the workplace. The biological monitoring of toxic metals in urine and serum has become a matter of wide interest owing to the toxicity of these metals and their influence in controlling the course of biological processes.¹⁾

Chromium was discovered by Vauquelin in 1797 as a component of lead ore.²⁾ It is an essential trace element for carbohydrate and lipid.^{3,4)} Chromium(II), (IV) and (V) compounds exist, but (III) and (VI) are the most common oxidation states, especially in aqueous chemistry.⁵⁾ Trivalent chromium is believed to be poorly absorbed. Little toxic effect is attributed to trivalent chromium when present in very large quantities. Hex-

avalent chromium has been found to be biologically active.^{6,7)} It is more rapidly absorbed and is more corrosive and irritating.⁸⁾ Therefore, adverse effects of chromium compounds are mainly due to the hexavalent form of the element, whereas trivalent compounds seem to be less hazardous to occupationally exposed workers.⁹⁾ Contact with, inhalation or ingestion of hexavalent chromium compounds may result in a variety of diseases such as allergic dermatitis, skin and mucous membrane ulcerations, perforation of the nasal septum, allergic asthmatic reactions and bronchial carcinomas. Hexavalent chromium levels have also been linked to diabetes and vascular diseases.¹⁰⁾

Chromium and its compounds are widely used in various kinds of industry, mainly in tanning, pigment, chromium electroplating and metallurgy industries.¹¹⁾ In the leather tanning industry, workers are usually exposed

to a harmful environment because of the chromium trioxide and dichromates being used in the tanning process. Under these circumstances, chronic long-term accumulation or a single acute exposure constitutes a health hazard.

Experimental

Reagents and Standards — All inorganic acids (Suprapur grade) were obtained from Merck, Darmstadt. Ultrapure water ($18.0 \text{ M}\Omega \text{ cm}^{-1}$), prepared with a Millipore water purification system, was used throughout this study.

Stock solution (1000 ppm) of Cr was prepared from Merck Titrisol standard solution ($1.000 \pm 0.002 \text{ g}$) by adding 5 ml concentrated nitric acid and then diluted with ultrapure water to 1 l. Standard solution (1 ppm) was prepared by appropriate dilution daily and stored at 4°C . Triton X-100 solution, 1% (v/v) was prepared by diluting 1 ml of Triton X-100 to 100 ml with ultrapure water.

Containers — All sample containers, autosampler cups *etc.*, were acid washed with 10% (v/v) nitric acid for 24 h before rinsing with copious amounts of ultrapure water and shaking dry prior to use.

Sample Collection and Pretreatment — Urine and serum sampling were done once every three months from August 1994 to July 1995. The urine specimens were collected from 55 adults (24 males and 31 females), and the serum specimens from 20 adults (7 males and 13 females). All had been working in the leather tanning industry with business careers ranging from 3–20 years, and were within the age range of 18–65 years. The factory used for this study is located in Ping-Tung Industrial Park, Taiwan, Republic of China. Controlled normal urine and serum specimens were obtained from 58 healthy adults (37 males and 21 females), and 30 healthy adults (21 males and 9 females), respectively. They were within the age range of 20–25 years old, and all were undergraduate students in the Chemistry Department of Kaohsiung Medical College.

Urine specimens were collected in a non-

contaminated administration room, isolated from the production area of the factory. Samples from each subject were collected during his/her 8-h shift.¹²⁾ They were filtered through a $0.45 \mu\text{m}$ Millipore membrane filter, and ultrapure concentrated nitric acid (0.5 ml per 100 ml of urine) was added to acidify the sample for future analysis. The acidified urine specimen was either kept at 4°C for no longer than 3 weeks, or frozen at -20°C for a longer storage period prior to analysis.¹³⁾

Five milliliters of venous blood was collected by new sterile needles in Vacutainers[®] (Becton-Dickinson, Rutherford, New Jersey, U.S.A.). Following overnight fast, the blood samples were drawn from each subject and kept for 2 h at room temperature. After centrifuging, the serum samples were separated.

Microwave Oven Procedure — Microwave dissolution is particularly suitable for rapid preparation of samples for instrumental analysis. Its use as a means of preparing analytical solutions for atomic absorption is well-documented.¹⁴⁾

Various acid mixtures were tested to find the best decomposition procedure. A triacid solution $\text{HNO}_3\text{--H}_2\text{SO}_4\text{--HClO}_4$ (volume ratio 3:1:1) proved to be the best selection. Twenty ml of urine sample and 20 ml of the triacid solution were transferred into a 80 ml pressure-resistant polytetrafluoroethylene (PTFE) bottle. The sample was digested using either 300 W for 4 min or 600 W for 2 min, to remove the interfering matrix within the samples.¹⁵⁾ The serum samples were diluted with Triton X-100 (1:1) for these analyses.

Analytical Conditions — The atomic absorption spectrophotometer setting and furnace programs used with the pyrolytically coated graphite tubes (GBC Science Equipment Part No. G19310.21) and pyrolytical graphite platform (L'vov platform) are shown in Table I.

Injection — The chromium level for treated urine and serum samples was determined by a GBC 908 graphite furnace atomic absorption spectrophotometer, coupled with an automatic sample loader PAL3000. A $20 \mu\text{l}$ aliquot was injected in a pyrolytically coated graphite tube

TABLE I. Graphite Furnace Parameters

Parameter	Chromium
Lamp current (mA)	8.0
Wavelength (nm)	357.9
Slit width (nm)	0.5
Argon flow rate (l/min)	3.5
Drying temp. (°C)	250
Ashing temp. (°C)	800
Atomization temp. (°C)	2400

with a pyrolytic graphite platform.

Results and Discussion

Quality Assurance of the Method

Accuracy of the analytical reliability was checked with Trace Metal 7878 APG Setpoint™ QC Standard and Standard Reference Material 2670 Elevated Level (Analytical Products Group, Inc., U.S.A.). Table II summarizes the analytical data obtained by graphite furnace atomic absorption spectrophotometry (GFAAS), and the results indicated that the method being used in this study was reliable for analysis.

Sensitivity

Using the certified chromium value of the SRM 2670 (85.00 µg/l), the measured absorbance (peak area) obtained was in the vicinity of 4.5 for 20 µl of serum. This corresponds to a characteristic mass (m_0) of 1.66 pg/0.0044 absorbance.

Chromium Concentration in Urine Specimens

Table III lists the urine chromium concentration in the group of exposed workers and

TABLE II. GFAAS Analysis for Standard Chromium Samples (µg/l)

	Trace metal 7878	SRM 2670 Elevated level	C.V. %
Measured value	65.45 ± 4.83	87.63 ± 4.71	7.4
Certified value	68.81 ± 6.18	85.00 ± 6.00	8.9

C.V. is the coefficient of variation. Each value is the mean ± S.D. of 5 runs by GFAAS. The Trace Metal 7878 APG Setpoint™ QC Standard and Elevated Reference Standard Urine SRM 2670 (NIST) were used to check the analytical accuracy and precision.

TABLE III. Chromium Concentrations in Urine of Tannery Workers and Controls

Group	Mean (µg/l)	Range (µg/l)	Median (µg/l)	p value
Control subjects (n=58)	2.9 ± 2.1	0.1–8.5	2.5	
Tannery workers (n=55)	3.8 ± 2.5	0.2–9.8	3.6	p > 0.05

Each value is the mean ± S.D.

nonexposed controls. The mean value of chromium in the urine of the 55 exposed workers was 3.8 ± 2.5 µg/l and the mean value of chromium in urine of 58 nonexposed controls was 2.9 ± 2.1 µg/l. Despite the somewhat higher level in the exposed workers, there was no significant difference between them ($p > 0.05$). This higher chromium concentration can be explained as follows:

Some of the workers usually developed diabetes mellitus, in fact, 7 of them (4 male and 3 female) were found to have this disease. Table IV lists the chromium concentrations in the urine of these afflicted workers. Bukowski *et al.*¹⁶⁾ reported that the chromium level in the urine of individuals with diabetes mellitus was about two times higher than that of non-diabetics.

Chromium Concentration in Serum Specimens

Table V lists the serum chromium concentration in the group of exposed workers and nonexposed controls. The results showed

TABLE IV. Chromium Concentrations in Urine of Tannery Workers with Diabetes Mellitus

Age	Sex	Working years	Cr in urine (µg/l)
37	M	8	5.9
51	M	3	2.5
58	F	3	1.6
40	F	6	1.1
46	M	8	3.4
50	M	13	3.2
58	F	16	4.1

M, male; F, female.

TABLE V. Chromium Concentrations in Serum of Tannery Workers and Controls

Group	Mean ($\mu\text{g/l}$)	Range ($\mu\text{g/l}$)	Median ($\mu\text{g/l}$)	<i>p</i> value
Control subjects (<i>n</i> =30)	0.3 ± 0.1	0.1–3.6	1.5	
Tannery workers (<i>n</i> =20)	4.9 ± 2.1	2.3–9.6	4.5	<i>p</i> <0.05

that for the control subjects, the serum chromium normal range was 0.1–3.6 $\mu\text{g/l}$, with a mean value of 0.3 ± 0.1 $\mu\text{g/l}$, and the range of serum chromium in tannery workers was 2.3–9.6 $\mu\text{g/l}$, with a mean value of 4.9 ± 2.1 $\mu\text{g/l}$. The mean value of workers from the present study was also compared with those of Randall and Gibson⁸⁾ (0.49 $\mu\text{g/l}$) and Simpson and Gibson¹⁷⁾ (0.50 ± 0.07 $\mu\text{g/l}$), both of which were significantly lower (*p*<0.001). Thus the serum chromium concentration found in the workers was not only significantly higher than that in the controls (*p*<0.05), but also higher than other reported literature values. According to Kaohsiung Medical College Hospital a suggested total chromium level in serum of less than 0.14 $\mu\text{g/l}$ is considered normal value.

The 20 workers in the tannery workplace were therefore possibly directly exposed to chromate dust or solution mist. According to on-site observation, there was no local ventilation provided on the surface of the chromate baths for the tanning process where the workers operated. Though the actual level of airborne chromium in the factory was not determined, it is reasonable to believe that in the atmosphere of the workplace it was much higher than a normal living environment, so that most of the tannery workers in the present study were at risk of occupational intoxication. Poisoning generally occurs either through inhaling chromates or through the inhalation of chromates or of contaminated airborne dust.

This study also revealed that the serum chromium concentration in exposed workers

TABLE VI. The Concentrations of Cr in Serum of Tannery Workers

Sample code No.	Worker age	Sex	Working Years	Cr in serum ($\mu\text{g/l}$)
1	31	F	3	4.6
2	37	M	8	4.5
3	51	M	3	4.0
4	33	F	3	2.8
5	36	M	10	6.9
6	32	M	2	2.5
7	58	F	3	2.3
8	58	F	16	8.6
9	44	F	8	4.6
10	40	F	6	4.0
11	44	F	9	6.2
12	44	F	8	3.7
13	44	F	8	4.4
14	46	M	8	3.3
15	40	M	20	8.4
16	33	F	6	4.8
17	50	M	13	9.6
18	45	F	4	2.7
19	50	F	3	3.4
20	54	F	13	6.7

M, male; F, female.

increased proportionally with their working years (Table VI), possibly due to the accumulation of chromium in the human body which caused the concentration in blood to be higher than that in urine. Therefore, the serum chromium level can be used as an indicator for long-term exposure to chromate.

The difference between the results of the present study as compared with literature results was analyzed by the Mann-Whitney test, using the *p* value as a judgment of significance. The sample population showed that worker is not related to age or sex, which is in agreement with the study by Pan *et al.*¹⁸⁾ The comparison between different values reported in the literature and this study on chromium concentration in the serum of healthy individuals using GFAAS is shown in Table VII. The normal range of serum chromium of the control subjects in the present study was 0.1–3.6 $\mu\text{g/l}$, with a mean value of 0.3 ± 0.1 $\mu\text{g/l}$ which was slightly higher than the values of Ericson *et al.*¹⁹⁾ (0.18 ± 0.07 $\mu\text{g/l}$) and Scher-

TABLE VII. Comparison of Different Literature Values^{a)} for the Chromium Concentration in Serum of Healthy Individuals

Source	No. of specimens	Mean±S.D. ($\mu\text{g/l}$)	Range ($\mu\text{g/l}$)	Reference
Veillon <i>et al.</i> (1984)	15	0.11 ± 0.07	0.05–0.29	22
Schermaier <i>et al.</i> (1985)	19	0.13 ± 0.08	0.06–0.38	20
Ericson <i>et al.</i> (1986)	16	0.18 ± 0.07	0.13–0.31	19
Randall and Gibson (1987)	52	0.15	0.12–0.20	8
Chappuis <i>et al.</i> (1992)	27	0.10 ± 0.04	0.02–0.19	21
Present study	30	0.3 ± 0.1	0.1–3.6	

a) All the analysis were done by GFAAS.

maier *et al.*²⁰⁾ ($0.13 \pm 0.08 \mu\text{g/l}$). Those values of Chappuis *et al.*²¹⁾ ($0.10 \pm 0.04 \mu\text{g/l}$) and Veillon *et al.*²²⁾ ($0.11 \pm 0.07 \mu\text{g/l}$) were much lower than found in this study. But Schermaier *et al.*²⁰⁾ and Randall and Gibson⁸⁾ reported a mean chromium value of about $0.13 \mu\text{g/l}$ and $0.15 \mu\text{g/l}$ in normal control serum, respectively.

The slightly higher serum chromium for the controls in the present study than those reported in the literature can be attributed to the fact that, all control subjects in the former were students majoring in chemistry, who might have a higher possibility of coming into contact with chromium compounds during their course of study than others. Randall and Gibson⁸⁾ selected people not exposed to chromium in their workplace as controls, and Ericson *et al.*¹⁹⁾ used laboratory personnel; Schermaier *et al.*²⁰⁾ and Veillon *et al.*²²⁾ did not report the background of their controls.

The etiology of chromate poisoning is also briefly mentioned. Chromium and its compounds can be absorbed into the blood in different ways, and transferred to various tissues and organs *via* blood circulation, thus resulting in many diseases. Clinical manifestations usually included perforation of nasal septum, carcinogens, and gastrointestinal tumors. The common disease in the leather

tanning worksite is dermatitis. Once the leather tanning worker is irritated by chromium or one of its compounds, protective clothing no longer prevents a relapse. Consequently, the only way for a preventive cure is to completely avoid further exposure to chromate and its compounds.

Conclusion

The purpose of this work was to assess biological monitoring, for practical application in determining chromium levels in blood samples from workers at a leather tannery.

The analytical performance obtained by the atomic absorption technique used here was satisfactory, and the technique was amenable to GFAAS. The first set of results were announced to workers and precautions were taken. Factory employees must be attentive to industrial hygiene practices and employ safety measures, *e.g.*, adequate ventilation and use of respirators.

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