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Serum and Urine Chromium as Indices of Chromium Status in Tannery Workers[1] (42510)

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Abstract. Serum and urinary Cr levels of a selected group of men exposed to CrIII in four Southern Ontario tanneries were compared with those of men not exposed to Cr. Fasted blood samples were obtained from 72 tannery workers (TW; mean age ± SD = 36 ± 12 years) and from 52 controls (CS; mean age ± SD = 41 ± 13 years). Serum Cr levels as determined by graphite furnace atomic absorption spectrophotometry were significantly higher ($P = 0.0001$) for TW (median 0.49 ng/ml, range 0.37–0.81) than for CS (median 0.15 ng/ml, range 0.12–0.20). Urine samples were collected from 49 TW and 43 CS on a Friday PM and from 42 TW on a Monday AM. Urinary creatine (Cre) was determined by the Jaffe reaction. For Friday samples, the median urinary Cr/Cre ratio was significantly higher ($P = 0.0001$) for TW (median 0.83 ng/mg, range 0.48–1.82) than for CS (median 0.18 ng/mg, range 0.13–0.26). For TW, Cr/Cre was correlated with serum Cr ($r = 0.72$, $P = 0.0001$). Neither urinary Cr/Cre nor serum Cr was correlated with length of employment in the tanning industry. There were significant differences in serum Cr levels and urinary Cr/Cre ratios among TW employed in different areas of the tanneries. For TW, the median urinary Cr/Cre ratio for Monday morning samples was significantly lower than for Friday afternoon samples ($P = 0.03$). These data indicate that CrIII is absorbed and that serum and urine Cr in tannery workers may be indices of Cr exposure and status.

Chromium is an essential trace element involved in glucose and lipid metabolism (1). It is often used for industrial purposes such as the leather tanning process. The toxicological effects of industrial exposure to Cr compounds are well known and include allergic dermatitis, skin ulcers, perforation of the nasal septum, and increased incidence of bronchogenic carcinoma (2).

Most of these health hazards have been ascribed to exposure to hexavalent Cr compounds because they are more rapidly absorbed and are more corrosive and irritating than other valency states. In the leather tanning industry, the tanning compounds contain, almost exclusively, trivalent chromium, a form considered to be very poorly absorbed (2, 3). Hence no routine biological monitoring for industrial exposure to chromium in the leather tanning industry has been required. Instead a time-weighted average exposure to total chromium and hexavalent Cr in air is commonly used (4).

One of the reasons for the lack of routine biological monitoring for industrial Cr exposure is the difficulty associated with the analysis of Cr in fluids and tissues (5). Reported values for serum and urine Cr have declined dramatically during the last two decades as improvements in sample collection, instrumentation, and analytical techniques have occurred (5).

In part, because of the uncertainties in the methodology, most investigators have dismissed the use of serum and/or urine Cr levels as biological indices of chronic Cr exposure or status (5). Consequently, in this study we have compared the serum and urine Cr levels of a group of Southern Ontario tannery workers with a group unexposed to Cr in the work place to investigate whether serum and/or urine Cr levels provide an index of Cr exposure in the workplace. To our knowledge, this is the first study which reports serum Cr levels in tannery workers.

Materials and Methods. *Subjects.* Seventy-two men aged 36 ± 12 years (mean ± SD) working in tanneries in four Southern Ontario cities and 52 men aged 41 ± 13 years not exposed to Cr in the workplace from the Guelph and Toronto areas were recruited on a voluntary basis for the study. Subjects were matched for age, race, and socioeconomic status. The socioeconomic index (SEI) of the subjects was determined from their occupation

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according to the scale of Blishen and McRoberts (6). The SEI was 34.6 ± 8.8 (mean ± SD) for the tannery workers and 37.9 ± 12.2 for the control subjects. These mean SEI values correspond to the skilled labor class.

The study was approved by the Human Ethics Committee at the University of Guelph. Written consent was obtained from the subjects after the nature of the study had been fully explained to them.

Subjects completed a questionnaire on relevant demographic, general health information, and family medical history. The subjects were apparently healthy, with no history of insulin or noninsulin dependent diabetes or coronary heart disease. None of the subjects took chromium and/or yeast supplements.

Mean length of employment for subjects in the tanning industry was 10.6 years with a range of 12 months to 48 years. Areas of work in the tanneries included the chrome tan, wringing, sorting, buffing dust, and split and shave departments.

Sample collection and preparation. Following an overnight fast, peripheral venipuncture blood samples were drawn using siliconized needles (Monovette, Sarstedt, St. Laurent, P.Q.) and Cr-free plastic vacutainers (Monovette, Sarstedt). Samples were taken on Tuesday mornings with subjects in the sitting position. Sample preparation following blood collection was carried out in a laminar flow cabinet (Canadian Cabinets Co., Ltd., Model HE4-97-T) whenever possible. Powder-free vinyl gloves (Surgikos, Canada, Peterborough, Ontario) were worn when handling the samples. The blood was allowed to clot in the collection containers for 2 hr at room temperature and then centrifuged twice for 15 min. The serum was separated subsequently using acid-washed glass Pasteur pipets (Fisher, Toronto, Ontario), stored in 1.5-ml trace element-free sample cups (Sarstedt), and frozen at −20°C for later analysis. Glass Pasteur pipets and sample cups were soaked overnight in 10% nitric acid, rinsed six times in deionized glass distilled water, and then dried before use.

Spot urine samples were collected from 49 tannery workers and from 43 control subjects on a Friday afternoon and from 42 tannery workers on the following Monday morning. Sterile Cr-free specimen jars (125 ml, Cat. No. 14-375-110A, Fisher) were used for urine collection and all subjects were asked to wash their hands prior to voiding. Aliquots (5 ml) of the urine samples were stored in sterile polypropylene test tubes (Sarstedt) at room temperature for Cr analysis. A second aliquot of each urine sample was stored at −20°C for creatinine analysis.

Serum analysis. The serum samples were analyzed for Cr by one of the authors (J.R.) in the laboratory of Dr. Claude Veillon, Research Chemist, Vitamin and Mineral Nutrition Laboratory, United States Department of Agriculture (Beltsville, MD) using the method of Veillon *et al.* (7). All analytical work was carried out in a Class 100 hood.

Approximately 1 ml of each serum sample was weighed into 10 × 100-mm quartz glass test tubes (Thermal American Fused Quartz Co., Montville, NJ) using sterile polyethylene pipets (Bio-Rad Laboratories (Canada), Mississauga, Ontario). The quartz glass test tubes had been boiled previously for several hours in 10% nitric acid, rinsed six times in deionized distilled water (Millipore Corporation, Bedford, MA), and dried by lyophilization. The dried tubes were silanized with a mixture of 10% dimethyldichorosilane (Pierce Chemical, Rockford, IL) in toluene (Fisher, Fairlawn, NJ), rinsed with methanol (Fisher) and then with deionized water.

The serum samples were prepared for ashing by adding 10 μl of 0.186 g/ml high purity magnesium nitrate (Alfa Products, Danvers, MA) as an ashing aid. Subsequently, the serum samples were frozen and then lyophilized in a freeze dryer designed to minimize contamination from stainless steel (Model FD-6-UP, FTS Systems, Inc., Stone Ridge, NY). Following lyophilization, the test tubes containing the samples were placed under inverted quartz glass beakers. The latter were placed on top of quartz glass plates in the bottom of a muffle furnace (51894, Lindberg, Watertown, WI). The temperature of the furnace was set initially at 100°C for 1 hr and then raised 50°C every hour to 250°C. After 1 hr at 250°C, the temperature was again raised to 480°C, at which temperature the samples were ashed overnight. Following ashing, 0.1 *N* hydrochloric acid (prepared by isothermal distillation (8)) equivalent to the volume of each serum sample was added to dissolve the ash.

Several samples (six or seven) of reference

bovine serum No. 7292 (9) were ashed at the same time as the test serum samples as a check on precision and accuracy of the method. A standard curve using a Cr standard prepared by dissolving elemental Cr in HCl was made up in the ashed bovine serum to compensate for any matrix effects.

Analysis of serum Cr was determined by flameless atomic absorption spectrophotometry (Model 5000, Perkin–Elmer, HGA 500, Norwalk, CT) using the furnace parameters and operating conditions of Anderson *et al.* (5). Argon was used as the purge gas and only pyrolitically coated furnace tubes were used. The dissolved ash (25 μl) was pipetted manually into the furnace. Eppendorf pipets (Brinkman, Toronto, Ontario) with no exposed stainless steel parts and trace element-free pipet tips (Bio-Rad Laboratories (Canada)) were used.

Of the 124 samples, 20 were ashed in duplicate as a check on the reproducibility of the method. The reference bovine serum used as a control gave a value of 0.31 ± 0.04 ng/ml (mean ± SD) compared to the value assigned by Veillon *et al.* (10) of 0.30 ± 0.05 ng/ml (mean ± SD). The detection limit for the determination of serum Cr was 0.05 ng/ml.

Urinary Cr analysis. The unashed urine samples were analyzed for Cr by graphite furnace atomic absorption spectrophotometry using a Varian Spectra AA 30 GTA 96 furnace and autosampler (Varian Canada, Georgetown, Ontario). Each sample was tested for approximate concentration before analysis. The method of premixed standard additions was used as described by Veillon *et al.* (11). Each sample (1 ml) was pipetted into each of four 2-ml sample cups (Fisher) which had been rinsed three times with deionized glass distilled water. To one sample cup was added 10 μl of 1 *N* HCl (G. Frederick Smith (GFS) Chemical Co., Columbus, OH) whereas 10 μl of Cr standard of appropriate concentration was added to each of the three remaining cups. The Cr standards (GFS) were made up in 1 *N* HCl. Each addition was mixed several times with a disposable transfer pipet (Bio-Rad Laboratories (Canada)) and was then loaded into the autosampler.

Depending on the concentration of Cr in the sample, between 10 and 35 μl was automatically dispensed into the furnace. In order to facilitate proper delivery of the urine into the graphite tube, 2 μl of 1 *N* HCl was taken up into the capillary tube of the autosampler prior to the urine. On delivery into the graphite tube, the acid served to rinse out the capillary tube.

The program for the furnace and the operating parameters were similar to those used by Veillon *et al.* (11). Argon was used as the purge gas and only pyrolitically coated tubes were used. The drying time was adjusted depending on the sample size (i.e., 35-μl injection, 60-sec drying time; 10-μl injection, 40-sec drying time). Results were displayed graphically and the values were computed by the DS-15 data station. Each point on the standard additions curve was carried out in duplicate. In cases where readings were not precise, the analysis was repeated.

Two urine pools, one of low Cr concentration and one of high Cr concentration, were used as checks on accuracy and precision. The values (means ± SD) for these pooled urine samples were 0.20 ± 0.06 and 1.12 ± 0.12 ng/ml, respectively, compared to 0.22 and 0.96 ng/ml determined in the laboratory of Dr. Claude Veillon, USDA. The detection limit for the determination of urinary Cr was 0.05 ng/ml.

Urinary creatinine. Cre was determined on the thawed urine samples using a Rapid Stat diagnostic kit (Lancer Division of Sherwood Medical, St. Louis, MO). This procedure is based on the Jaffe reaction. The accuracy and precision of the method was assessed by using a reference material, Monitrol ES Level 1 Chemistry Control (Dade Chemical Co., Miami, FL).

Air sampling and analysis. Area air samples were collected from 3 locations in each of the tanneries for 3 working days. Samples were collected for 4-hr periods at a flow rate of 2 liters/min according to methods outlined by NIOSH (12). Air samples were analyzed for hexavalent Cr according to NIOSH Method 7600 (12). For total Cr determinations of the air samples, the filters were ashed in a plasma low temperature asher (LTA-504, LFE Corporation, Waltham, MA), reconstituted in 0.1 *N* nitric acid (GFS Chemical Co.), and analyzed by flame atomic absorption spectrophotometry on a Varian Spectra AA 30.

Statistical analysis. Data for serum Cr and

TABLE I. SERUM Cr IN TANNERY WORKERS AND CONTROLS

	Tannery workers ($n = 72$)	Control subjects ($n = 52$)	P^a
Serum Cr[b] (ng/ml)	0.49 (0.37–0.81)	0.15 (0.12–0.20)	0.0001

[a] Kruskal–Wallis test.
[b] Median and 25th–75th quartile.

urinary Cr, and urinary Cr/Cre ratios for both groups were not normally distributed and variances were heterogeneous so that nonparametric statistics employing ranks were used, the median being used to indicate central tendency. Attempts to normalize the data by means of various transformations were not successful. A skewed distribution has been reported by other investigators for biochemical indices of men subject to industrial Cr exposure (13, 14). In this study, the Kruskal–Wallis test was used to assess differences in serum Cr, urinary Cr, and urinary Cr/Cre ratios between tannery workers and controls. Similarly, the Kruskal–Wallis test was used to assess differences in air Cr levels, serum Cr, urinary Cr, and urinary Cr/Cre among areas of employment in the tanneries.

The paired t test was used to assess differences between urinary Cr and the Cr/Cre ratio for Friday afternoon and Monday morning samples. Spearman's rank correlation coefficients were calculated to assess the relationships between serum Cr and urinary Cr and the Cr/Cre ratio and between duration of exposure and the biological parameters.

Results. As shown in Table I, the median serum Cr value for tannery workers was significantly higher ($P = 0.0001$) than that for the control subjects. Serum Cr values were correlated with age only for control subjects ($r = 0.29$, $P = 0.03$) but not with height or weight for either group. Serum Cr levels in tannery workers were correlated with urinary Cr/Cre ratios for the Friday afternoon samples ($r = 0.72$, $P = 0.0001$) and weakly correlated with the Monday morning samples ($r = 0.45$, $P = 0.003$). Figures 1a and 1b present scatter plots for the data for serum Cr and urinary Cr/Cre ratios for Friday afternoon and Monday morning urine samples, respectively.

Median urinary Cr value and the median Cr/Cre ratio for urine samples collected on Friday afternoon were significantly greater ($P = 0.0001$) for tannery workers compared to controls (Table II). There were no significant correlations of urinary Cr or Cr/Cre with age, height, or weight for either the tannery workers or the control subjects. Median urinary Cr ex-

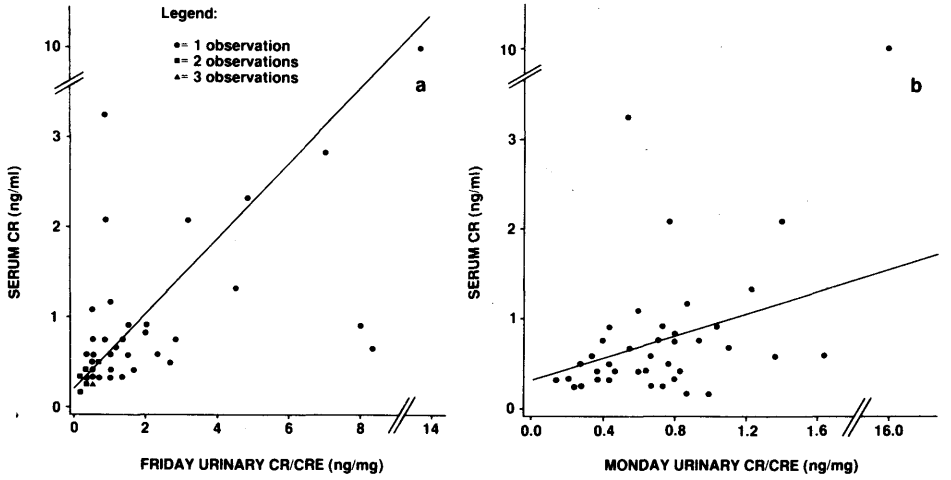

FIG. 1. (a) Scatter plot of serum Cr and Friday afternoon urinary Cr/Cre ratio for tannery workers ($r = 0.72$, $P = 0.0001$). (b) Scatter plot of serum Cr and Monday morning urinary Cr/Cre ratio for tannery workers ($r = 0.45$, $P = 0.003$).

TABLE II. URINARY Cr AND Cr/Cre IN TANNERY WORKERS AND CONTROLS

	Tannery workers ($n = 49$)	Control subjects ($n = 43$)	P^a
Urinary Cr[b] (ng/ml)	0.96 (0.62–2.75)	0.24 (0.16–0.31)	0.0001
Urinary Cr/Cre[b] (ng/mg)	0.83 (0.48–1.82)	0.18 (0.13–0.26)	0.0001

[a] Kruskal–Wallis test.
[b] Median and 25th–75th quartile.

cretion and the Cr/Cre ratio for the tannery workers were significantly lower on Monday morning than on Friday afternoon (Table III).

There were no significant correlations between length of employment in the tannery and serum Cr, urinary Cr, or Cr/Cre ratios, respectively. However, as shown in Table IV, there were significant differences in the median serum Cr and in the median urinary Cr/Cre ratio for both Friday and Monday samples among tannery workers employed in different areas of the tanneries. Men handling wet hides in the chrome tan and wringing departments had significantly higher median serum Cr ($P < 0.05$) and median urinary Cr/Cre ($P < 0.05$) than those working in other areas of the tanneries.

Time-weighted average exposure to total air Cr was 1.7 ± 0.5 $\mu g/m^3$ (mean ± SEM). There were no significant differences in total air Cr either among the tanneries or among the working areas of the tanneries.

Discussion. The median serum, urine Cr, and urinary Cr/Cre levels in the tannery workers of this study were markedly higher than those of the unexposed controls. These data suggest that chromium arising from industrial exposure in the leather tanning industry has been absorbed by the tannery workers.

To date, there appears to be no other data on serum Cr values of tannery workers. Furthermore, their use as an index of chronic industrial exposure to Cr has not been extensively investigated. In part, this has arisen from the early observation that intravenously injected ^{51}Cr apparently disappeared rapidly from the blood and was not believed to be in equilibrium with body tissues (3). Based on this evidence, serum Cr was dismissed as an index of long-term Cr status (5, 15). Instead it was associated with recent Cr exposure or recent Cr intake. Indeed Anderson *et al.* (5) noted a significantly elevated mean serum Cr (0.38 ng/ml) in 76 subjects supplemented with $CrCl_3$ for three months compared to 0.13 ng/ml before supplementation.

In this study, serum Cr levels in the tannery workers were positively correlated with urinary Cr/Cre ratios of both Friday afternoon (postshift; $r = 0.72$, $P < 0.01$) and Monday morning (basal; $r = 0.45$, $P < 0.01$) urine samples. These results are of particular interest because they suggest that serum, as well as urinary Cr/Cre ratios, may indeed serve as indices of more long-term Cr exposure and status. These findings are consistent with the recent observations of Lim *et al.* (16) who have characterized the distribution and kinetics of intravenous ^{51}Cr III in human subjects. These investigators have revealed that plasma Cr is in equilibrium with two clearly defined pools with a medium and slow exchange rate, respectively, as well as a third compartment with a fast turnover rate. The slow compartment has the largest pool size (25 μg), whereas the medium and fast compartments contain about 0.8 and 0.13 μg Cr, respectively.

Our results have also demonstrated a dose–effect relationship between serum Cr and urinary Cr/Cre ratios and work area of the tannery. Those tannery workers handling wet hides in the chrome tan and wringing departments had significantly higher serum and urinary Cr/Cre ratios ($P < 0.01$) than those working in other areas of the tannery (Table IV).

The paucity of data on serum Cr levels of both subjects exposed to Cr in the work place

TABLE III. URINARY Cr AND Cr/Cre IN TANNERY WORKERS FOR FRIDAY AND MONDAY

	Friday PM ($n = 42$)	Monday AM ($n = 42$)	P^a
Urinary Cr[b] (ng/ml)	0.87 (0.56–2.57)	0.80 (0.51–1.16)	0.008
Urinary Cr/Cre[b] (ng/mg)	0.84 (0.49–1.58)	0.68 (0.41–0.87)	0.03

[a] Paired t test.
[b] Median and 25th–75th quartile.

TABLE IV. SERUM Cr AND URINARY Cr/Cre RATIO IN WORKERS FROM DIFFERENT AREAS OF THE TANNERIES

Area of tannery	Serum Cr[a] (ng/ml)	Urinary Cr/Cre[a] Friday (ng/mg)	Urinary Cr/Cre[a] Monday (ng/mg)
Chrome tan, wringing departments	1.04*	2.75*	0.78*
	(0.63–2.27)	(1.51–5.95)	(0.70–1.24)
	$n = 20$	$n = 13$	$n = 11$
Blue sort, split & shave, buffing dust departments	0.44**	0.61**	0.52**
	(0.35–0.65)	(0.32–1.48)	(0.37–0.82)
	$n = 27$	$n = 19$	$n = 17$
Upper leather finish, plant services, Supervisor	0.39**	0.54**	0.67**
	(0.25–0.56)	(0.45–0.72)	(0.38–0.94)
	$n = 25$	$n = 17$	$n = 13$

Note. Numbers in the same column with different superscripts are significantly different ($P < .05$).
[a] Median and 25th–75th quartile.

and those unexposed is associated with the difficulties of the analysis of subnanogram levels of Cr. Until recently, there has been no general agreement on normal values for serum and plasma Cr. Values reported from 1970 to 1978 ranged from 3 to 10 μg/ml whereas most recent values are between 0.10 and 0.20 ng/ml. These recent Cr levels are very close to the detection limits (0.05 ng/ml) of even the latest atomic absorption spectrophotometer. Hence it is not surprising that investigators have failed to demonstrate that subjects with marginal Cr depletion, as indicated by an improvement in glucose tolerance following Cr supplementation, have lower serum Cr levels than those showing no response to Cr supplementation. These inherent methodological difficulties have therefore discouraged the investigation of serum Cr levels per se as indices of Cr status. Instead, changes in serum Cr levels following a glucose load have been advocated as a measure of Cr status (17, 18). For instance, in subjects with adequate Cr stores, Cr is believed to be released from tissues following a glucose load, resulting in increased serum Cr levels. However, equivocal results have been found using this method, irrespective of whether the subjects have shown an improvement in glucose tolerance following Cr supplementation (19–23). Indeed in the most recent study of Anderson *et al.* (5), no change in serum Cr concentration was noted following an oral glucose load, even in the Cr-supplemented group. Hence, it appears that change in serum Cr following a glucose load is not a reliable index of Cr status.

Similar methodological difficulties exist for the analysis of urinary Cr levels. Nevertheless, because urine is the major excretory pathway of absorbed Cr, accounting for about 80% of ingested Cr (19), several investigations of urinary Cr as an index of industrial Cr exposure and status have been attempted (24–29). For instance Gylseth *et al.* (24) and Welinder *et al.* (25) evaluated urinary Cr excretion in stainless steel welders and reported a high degree of correlation between the concentration of inhaled Cr (as total Cr) and the Cr concentration in the urine immediately after work. However because urinary Cr concentrations fell to apparently normal levels 3 days after exposure (24, 26), they were suggested to monitor only an acute index of industrial Cr exposure. More recent studies using more sensitive analytical techniques have documented elevated urinary Cr levels of exposed workers after 31 (25) and 40 (27) days of holiday as well as following 4.5 years of retirement (25).

The absence of any significant correlation between serum Cr, urinary Cr, and Cr/Cre ratios for both Friday afternoon and Monday morning urine samples and the length of employment for the tannery workers is not unexpected because many other factors affect level of Cr exposure besides duration. For instance, area of work (Table IV) was shown to be a significant factor affecting serum Cr and urinary Cr/Cre ratios in this study. Other factors which may contribute to the amount of industrial Cr absorbed include use of safety measures (gloves and masks), personal hy-

giene, and accidental ingestion (27). In addition, those with long years of service in the tanning industry are often moved into management positions.

The time-weighted average exposure to total air Cr did not differ among the areas in the tanneries. Furthermore, all levels of total air Cr were well below the threshold limit value of 0.50 mg/m^3 as proposed by the Occupational Health and Safety Division of the Ontario Ministry of Labour (4). Nevertheless, serum Cr and urinary Cr/Cre ratios were significantly elevated for the tannery workers compared to unexposed controls. These findings are in contrast to those reported by Tola *et al.* (29) who did not observe any elevation in urinary Cr excretion levels when hexavalent air Cr levels were below the threshold limit value of 0.50 mg/m^3 of air.

The elevated serum and urinary Cr levels in the tannery workers studied here could not be attributed to Cr VI absorption from the air as analyses of air samples collected from three different work areas of each tannery revealed Cr VI levels to be below the detection limit. Hence our results indicate that trivalent Cr compounds used in the tanning industry in Southern Ontario are indeed absorbed. Furthermore, serum and urine Cr levels of tannery workers appear to be indices of Cr exposure and status. At the present time the possible health hazards due to occupational exposure to Cr III have been ignored because the rate of Cr III absorption (0.4%) has been considered insignificant in industrial settings.

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