# Use of molecular epidemiological techniques in a pilot study on workers exposed to chromium

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**Abstract**

***Objectives*—Molecular epidemiological techniques, capable of detecting damage to DNA, were used to see if such damage occurred in the lymphocytes of a group of workers exposed to chromium. The two aims of this pilot study were to see if these new techniques might make useful biological monitoring tools for workers exposed to chromium and also, to help assess whether the current occupational exposure limit for chromium (VI) was sufficiently protective in this specific working situation.**

***Methods*—Volunteer groups of 10 workers exposed to chromium and 10 non-exposed workers provided urine and blood samples towards the end of the working week. Chromium concentrations were measured in whole blood, plasma, lymphocytes, and urine. Lymphocytes were used to examine two forms of DNA damage in the two groups; these were the level of DNA strand breakage and, the production of 8-hydroxydeoxyguanosine.**

***Results*—Chromium concentration in whole blood, plasma, and urine of workers exposed to chromium was significantly raised ($P < 0.01$) compared with non-exposed controls, but in isolated lymphocytes, there was only a modest but significant ($P < 0.05$) increase in chromium in the group exposed to chromium. There was no difference in the levels of DNA strand breaks or 8-hydroxydeoxyguanosine between the groups. Air monitoring for chromium was not undertaken but current levels for the group exposed to chromium were reported to be around 0·01 mg/m³, which is 20% of the current United Kingdom occupational exposure limit.**

***Conclusions*—We were unable to detect any damage in lymphocytic DNA due to exposure to chromium. This may have been due to the low chromium exposure (<20% of the United Kingdom occupational exposure limit), the ability of plasma to detoxify chromium (VI) to chromium (III) before it reached the lymphocytes, or perhaps the insensitivity of the molecular techniques used. It is now important to test these and other such techniques on groups exposed to levels closer to the United Kingdom occupational exposure limit.**

(*Occup Environ Med* 1994;51:663–668)

Keywords: cancer, chromium, molecular epidemiology

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Accepted 23 May 1994

The inhalation of aerosols containing chromium is the major route of exposure to chromium compounds in those industries where an increased risk of lung cancer has been shown.[1 2] The airborne chromium concentration should, therefore, directly reflect the risk of lung cancer and other effects associated with exposure to chromium. The control of airborne chromium concentration, through air monitoring, has been used extensively to reduce risk.[1] Monitoring the airborne chromium concentration in the working environment is the simplest method of maintaining chromium containing compounds below an agreed concentration. In the United Kingdom, the current occupational exposure limits are 0·5 mg/m³ for chromium(II) and chromium(III) (as occupational exposure standards) and 0·05 mg/m³ for chromium(VI) (as a maximum exposure limit (MEL).[3] Monitoring ambient chromium concentrations may not necessarily indicate the amount of chromium absorbed into the body.[4] This is due in part to the various valency states of airborne chromium used in industry, the relative solubilities of different chromium compounds and the extent of protection afforded by respiratory protective equipment.[1] It is generally agreed that chromium(VI) is the valency state responsible for lung cancer induced by chromium and that it differs from chromium(III) compounds in its absorption and ability to cross cell membranes.[5–7] Biological monitoring, as a means of assessing uptake, has been used to complement air monitoring and since the late 1960s, urinary chromium and blood chromium, have been used for biological monitoring of exposure in some industries.[8–11] Also, erythrocytes[12] and lymphocytes[13] have been investigated to assess exposure to chromium(VI). Thus, biological monitoring and monitoring of biological effects on workers exposed to different valency states of chromium are becoming increasingly important as many people feel they might give a better estimate of risk.

Recently, molecular epidemiological studies of exposed human populations that used various genotoxic markers have been used as a new approach to assess exposure to carcinogens, where population surveys have been conducted at the level of DNA alterations.[14] These techniques may be able to identify a risk of cancer long before cancer develops.[15] Lymphocytes, which are an easily accessible target cell population, have often been used in such studies.[14 16–18] As lymphocytes are readily available nucleated cells, they may have the potential to be used to predict DNA damage in cells in putative target organs, such as the

lung. As a new approach, the quantitative relation of such indicators to the risk of developing cancer has not been determined and as screening tools their specificity and sensitivity have yet to be determined.[19] There is a general consensus that genotoxic effects are concerned with early stages in the multistep process of carcinogenesis, particularly for genotoxic carcinogens such as chromium(VI). Our previous studies have shown that chromium(VI) is able to induce DNA strand breaks in human lymphocytes both in vitro and in rat lymphocytes in vitro and in vivo.[20] Furthermore, investigation of the formation of the oxidised deoxynucleoside 8-hydroxydeoxyguanosine (8-OHdG) in isolated DNA after treatment with chromium(VI) or chromium(V) compounds suggested that the mechanism of toxicity might involve the production of hydroxyl radicals, probably through Fenton chemistry.[21] Therefore, it was logical to investigate the outcome of real occupational chromium exposure on such DNA alterations in exposed humans as it may provide scope for these new techniques to be used for monitoring of exposed workers for biological effects.

In this paper, we present the first report exploring some of these techniques to investigate any association between chromium uptake (biological monitoring) and DNA damage (monitoring of biological effect) associated with exposure to chromium.

## Materials and methods

### STUDY POPULATION

Twenty volunteer subjects (15 men and five women) from a plant that produces bichromate in England participated in the study. Ten subjects were employed in chromate production and other work areas that produced materials that contain chromium. Ten control subjects who worked in the same plant but were not occupationally exposed to materials that contain chromium formed a comparison group—for example clerical and other professional staff. A questionnaire on general health, smoking and alcohol consumption, and occupational history, was given to all participants by an interviewer. The questionnaire also elicited information on potentially important confounders that could induce DNA damage, such as a radiographic examination during the last month and recent medication. The question on medication was also intended to elicit information about recent illness, including viral infection that may have been a confounder. Table 1 shows the characteristics of the study population. Age and alcohol consumption were comparable between the two groups, but sex and smoking were not (table 1).

*Table 1 Description of study population*

| | *Exposed* | *Control* |
|---|---|---|
| Subjects (n (m/f)) | 10(10/0) | 10(5/5) |
| Mean (SD) age (y) | 44 (7) | 40 (10) |
| Smoking: | | |
| Current | 6 | 2 |
| Ex | 1 | 2 |
| Non | 3 | 6 |
| Alcohol consumption (U/week (SD)) | 23 (37) | 10 (15) |
| Mean (SD) duration of employment (y) | 15 (7) | 13 (9) |

### MATERIALS CONTAINING CHROMIUM TO WHICH WORKERS WERE EXPOSED

The chromium production plant investigated in this study has a long history of production. Although the production process has stayed much the same there have been plant and process modifications over the years that will have altered exposure patterns.[22] The plant produces a wide range of chromium products, such as sodium dichromate, chromic acid, potassium dichromate, and chrome tanning agents. The primary production of sodium dichromate is based on the roasting of crushed and ground chromite ore (containing insoluble trivalent chromium oxide) with sodium carbonate. This produces sodium chromate (soluble and hexavalent). Further processing, including crystallisation and acidification, produces sodium dichromate, the starting material for many other substances that contain sodium. Other products are made on site from the reaction of sodium dichromate with other materials. Thus, although many of the compounds are hexavalent there will also be several trivalent compounds in the working environment.

At the time of this pilot study, no hygiene measurements were carried out, however, we were provided with general background monitoring information on airborne chromium concentrations in the plant, obtained from both personal and area samplers, and these ranged from 0·001 to 0·055 mg Cr(VI)/m$^3$, but most areas were well below 0·01 mg Cr(VI)/m$^3$. This concentration is around one fifth of the current United Kingdom MEL (0·05 mg Cr(VI))/m$^3$).

### COLLECTION OF BLOOD AND URINE

Blood (16 ml) and spot urine (40 ml) specimens were collected during the morning at the end of the working week. Blood was withdrawn from a vein in the arm with an Abbocath-T20G Teflon catheter (Abbot Ireland, Sligo, Republic of Ireland) and equally distributed into two sets of 10 ml Monoject lithium-heparin coated tubes (Sherwood Medical, Ballymoney, Northern Ireland). All specimens were coded and placed in a cooled ice box. The subjects also provided spot urine specimens in 20 ml sterile plastic containers (Bibby Sterilin, Stone, Staffordshire) that were also kept in the ice box. The subjects were instructed to change out of their work clothes and wash their hands before supplying the spot urine specimen (to limit chromium contamination). All samples were coded and placed in a cooled ice box that was transported from the plant to the laboratory on the day of collection.

### ISOLATION OF LYMPHOCYTES AND PLASMA

Lymphocytes were isolated by a hypotonic lysis method immediately after the specimens reached the laboratory. Precooled hypotonic

solution (9 ml 0·87% $NH_4Cl$-10 mM Tris-HCl, pH 7·2) was added to disposable tubes (Cel-Cult, Sterilin, Hounslow) that were kept on ice. Blood (3ml) was then added to each tube, gently mixed and left on ice for 30 min to lyse the red blood cells. Tubes were then centrifuged (1300 rpm) for 20 min at 4°C and the pellet produced was resuspended in the hypotonic solution (3 ml). This suspension was spun again, as described above, for 10 min to wash the lymphocyte preparation and the supernatant aspirated. The lymphocytes were then counted with a Neubauer haemocytometer. The plasma was separated by centrifugation (at 2000 rpm for 10 min) and then placed into disposable plastic tubes.

ANALYSES OF DNA STRAND BREAKAGE AND DNA ADDUCTS IN LYMPHOCYTES

Breaks in DNA strands were measured with a fluorometric alkaline unwinding assay that was based on the method described by Birnboim and Jevcak[23] with slight modifications.[20] The method is highly sensitive (sufficient to detect one strand breakage/chromosome) and has the advantage over alkaline elution techniques in that it is not influenced by effects on the conformation of the DNA coil.[24] The DNA adduct, 8-OHdG, was measured in lymphocyte DNA with high performance liquid chromatography (HPLC) coupled with electrochemical detection as previously described by Faux *et al.*[25]

ANALYSES OF CHROMIUM CONCENTRATIONS IN WHOLE BLOOD, PLASMA, URINE, AND LYMPHOCYTES

Total chromium concentrations were measured with an electrothermal atomic absorption spectrophotometer with Zeeman background correction (Varian Zeeman Spectra AA-300). Methods for measuring the chromium concentrations in blood, plasma, and lymphocytes were based on that described by Schermaier *et al*[26] with a detection limit of 0·025 μg/l. Measurement of urinary chromium concentrations was carried out by a technique described by Halls and Fell[27] with a detection limit of 0·26 μg/l of biological sample. The creatinine concentrations in the urine samples were analysed based on the Jaffé reaction with a Pye Unicam SP-550 UV/VIS spectrophotometer. Seronorm 115 trace element urine control material (containing 9·4 mmol creatinine/l) was used for quality control of measuring creatinine.

The sample size was small and we could not show that the data was normally distributed therefore, a non-parametric statistical test (Mann-Whitney) and linear regression analysis were used for data analysis with a Minitab statistics package.

*Table 2 Mean (SEM) chromium concentrations in body fluids and isolated lymphocytes*

| *Biological matrix* | *Exposed* | *Control* |
|---|---|---|
| Whole blood (μg/l n = 10) | 5·50 (1·20)** | 0·73 (0·13) |
| Plasma (μg/l, n = 10) | 2·8 (0·38)** | 0·65 (0·12) |
| Urine (μg/l, n = 10) | 7·1 (1·1)** | 0·55 (0·08) |
| Urine (μg/g creatinine, n = 10) | 5·97 (1·1)** | 0·76 (0·13) |
| Lymphocytes (μg/$10^{10}$ cells, n = 9) | 1·01 (0·08)* | 0·76 (0·03) |

*P < 0·05; **P < 0·01.

*Table 3 Mean (SEM)% breaks in DNA strands and formation of 8-OHdG in isolated human lymphocytes in vivo*

| *DNA alterations* | *Exposed (n = 9)* | *Control (n = 6)* |
|---|---|---|
| D-S DNA† | 59 (7)* | 50 (5) |
| 8-OHdG/dG | 0·037 (0·007)* | 0·041 (0·006) |

*P > 0·05.
†Double stranded DNA remaining.

## Results

ASSESSMENT OF INTERNAL CHROMIUM EXPOSURE

Chromium concentrations in whole blood, plasma, and urine specimens from subjects exposed to chromium were significantly higher (P < 0·01) than those of the controls (table 2). Chromium concentrations in isolated lymphocytes from exposed subjects were only marginally higher (P < 0·05) than the controls (table 2).

DETECTION OF DNA ALTERATIONS IN ISOLATED HUMAN LYMPHOCYTES

Breaks in DNA strand and 8-OHdG measured in isolated lymphocytes showed no significant difference between the exposed and control groups (table 3).

RELATION BETWEEN CHROMIUM CONCENTRATIONS IN BODY FLUIDS AND IN ISOLATED LYMPHOCYTES IN EXPOSED WORKERS

Linear regression analysis for the data of the group exposed to chromium showed that no significant linear relation (P > 0·05) was found when the chromium concentration in lymphocytes was compared with the chromium concentration in whole blood, plasma, and urine. When these data were converted to a ratio of whole blood chromium concentration to plasma chromium concentration, there was a significant linear relation between the chromium concentration in lymphocytes and the ratio ($r$ = 0·69; fig 1). This ratio was previously suggested as a reliable index for the assessment of exposure to chromium(VI).[28] The relation between chromium concentrations in urine and in

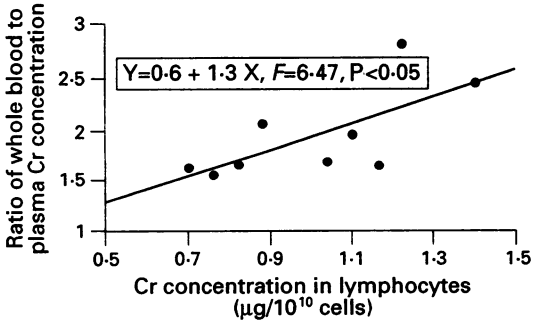


*Figure 1 Relation between chromium concentration in lymphocytes and ratio of whole blood to plasma chromium concentration. The data used for this regression analysis were from the group exposed to chromium (n = 9).*

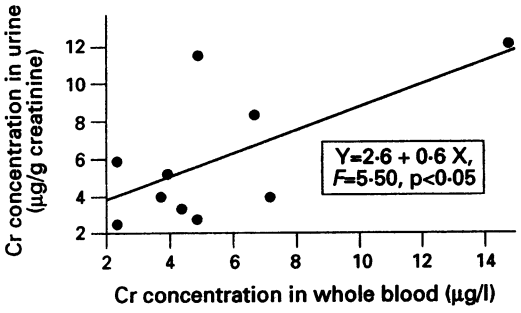


*Figure 2 Relation between chromium concentrations in urine and whole blood from the group exposed to chromium (n = 9).*

whole blood, plasma, and lymphocytes was also investigated with linear regression analysis. The relation was marginally significant ($P < 0.05$) only between urine and whole blood ($r = 0.64$; fig 2).

CONSIDERATION OF POSSIBLE CONFOUNDING VARIABLES ON DNA DAMAGE IN ISOLATED LYMPHOCYTES AND CHROMIUM CONCENTRATIONS IN SAMPLES FROM EXPOSED WORKERS

Although there was no evidence of a difference in the extent of DNA damage, either in DNA strand breaks or the formation of 8-OHdG, between controls and the exposed group, there seemed to be an increased effect of smoking on both DNA strand breaks and 8-OHdG. The difference, however, was not significant. Chromium concentrations were also higher in the lymphocytes, whole blood, and urine of the exposed smokers although again, the difference was not significant (table 4). The increased DNA damage could reflect this extra chromium burden, or, it could reflect DNA damage caused by smoking itself. Due to the smaller numbers of smokers in the control group, no such comparison could be made for this group.

## Discussion

The findings show that chromate production workers have a significantly higher chromium concentration in whole blood, plasma, and urine than do non-exposed workers (control group). Our finding of a mean (SEM) urinary chromium concentration of 7·1 (1·1) µg/l (5·97 (1·1) µg/g creatinine) is broadly in accordance with the range of findings reported in other groups of workers exposed to chromium. In particular, a mean urinary chromium concentration has been reported as 31·5 µg/l (creatinine concentrations not reported) in a group of chromate production workers.[1 6] In chromate pigment production workers, urinary chromium has been reported as 6–121 µg/g creatinine, 20–214 µg/l for blood, and 25–105 µg/l for serum.[9] In stainless steel welders, urinary chromium concentration was 1·0 and 5–15 µg/g creatinine in two studies.[11 29] A group of chromium platers had an average urinary concentration of 9 µg/g creatinine.[30] Thus, it can be seen that in comparison with most of these reported studies, the chromium concentrations in our study are low (table 2). The low urinary concentrations are consistent with the low estimated airborne concentrations of chromium that the workforce are exposed to (< 0·01 mg/m³). This reasoning is based on a presumed relation between airborne chromium exposure and urinary chromium concentration where it has been calculated that a urinary chromium concentration of 33 µg/g creatinine corresponds to an airborne exposure of 0·05 mg/m³.[31] This is, of course, without the use of personal protection. In our study, respiratory protection was used in some locations.

Mean chromium concentrations in whole blood, plasma, and urine from the unexposed control group were 0·73 µg/l, 0·65 µg/l, and 0·76 µg/g creatinine (0·55 µg/l) respectively (table 2), which are in good agreement with previous reported findings.[9 11 32] The Health and Safety Executive reported whole blood chromium concentrations at <1·0 µg/l for apparently unexposed healthy people.[33] Our findings indicate that the chromium concentrations in the body fluids of controls were all within the accepted normal range. The results of these chromium concentrations in the control group confirm two points. Firstly, that the techniques used for the analysis of chromium, in these biological matrices were feasible and secondly, that the actual concentrations measured, which were well within the published range of unexposed populations, strongly suggested that there was no uptake of chromium from occupational sources in our control population.

Chromium concentrations in isolated lymphocytes were modestly, but significantly ($P < 0.05$) increased (1·3-fold; ratio 1·14/0·74) in the group exposed to chromium compared with the control group whereas increases in whole blood, plasma, and urine were increased 7·5, 4·3, and 7·9-fold respectively (all $P < 0.01$). This contrasts with Coogan *et al* who reported that lymphocytes accumulate chromium to a greater extent than red blood cells in vitro and in vivo.[13] An animal study also showed that chromium was accumulated selectively in lymphocytes after intratracheal treatment with sodium dichromate at 0·44 mg Cr/kg.[28] The present finding does not necessarily contradict the animal study, as the difference might be explained by the very low chromium exposures in the workplace in this study compared with the above two studies. In the workplace, the relatively smaller amount of chromium(VI) absorbed may also have been due to its reduction to chromium(III) in the plasma thus preventing

*Table 4 Mean (SEM) chromium concentrations in biological matrices of smokers and non-smokers from the group exposed to chromium*

| *Biological matrix* | *Smokers (n = 6)* | *Non-smokers (n = 4)* |
|---|---|---|
| Lymphocytes (µg/$10^{10}$ cells) | 1·14 (0·09)* | 0·85 (0·08) |
| Whole blood (µg/l) | 5·87 (1·80) | 4·95 (1·20) |
| Plasma (µg/l) | 2·80 (0·55) | 2·80 (0·55) |
| Urine (µg/g creatinine) | 6·26 (1·80) | 5·55 (1·10) |

$P > 0.05$. *n = 5.

entry into the lymphocytes. Lewalter *et al* have reported that absorbed chromium(VI) can penetrate erythrocytes,[12] but this penetration is dependent on the chromium(VI) concentration absorbed, as human plasma is capable of spontaneous reduction of chromium(VI) to chromium(III) up to 200 $\mu$g/l. This may be due to reducing agents, such as ascorbic acid, in the plasma.[12] A recent study reported that plasma reducing capabilities for chromium(VI) ranged from 25 to 33 $\mu$g/l, however, these reducing capabilities did not correlate with plasma ascorbic acid concentration.[34]

There was no significant effect of chromium exposure on damage to DNA, measured as DNA strand breaks and formation of 8-OHdG, in the present study. This accords with the findings of an animal study in which a single intratracheal dose of sodium dichromate (0·04 mg Cr/kg), approximately corresponding to an exposure level at the MEL of 0·05 mg/m$^3$ over an 8 h working day, was not found to induce increased DNA strand breaks in rat lymphocytes compared with controls.[20] This is also consistent with the results of chromium concentration in lymphocytes. Thus, these present findings further suggest that the current MEL may provide protection against DNA damage and that the measurement of chromium concentration in lymphocytes is relevant when these cells are used for assessing genotoxic effects induced by chromate.

Linear regression analysis for the group exposed to chromium showed that chromium concentration in urine was not correlated to the concentration in plasma and lymphocytes. There was only a weak association between urine and whole blood chromium concentrations (fig 2). Measurement of urine chromium concentration alone was not sufficient to evaluate the real internal exposure to chromium,[29] especially when exposure contains different valency species of chromium. Minoia and Cavelleri have reported that a clear relation between exposure and urinary total chromium concentration after a shift exists in workers exposed to Cr(VI), and urinary concentration in workers exposed to high concentrations of chromic sulphate (Cr(III)) were lower. A linear relation was established when whole blood and plasma chromium concentrations were converted to a ratio and then compared with chromium concentration in the lymphocytes (fig 1). This agrees with previous findings that both lymphocytes and red blood cells can selectively accumulate chromium(VI), and the ratio of blood to plasma chromium may be a useful indicator of exposure to chromium(VI).[12 13 28 35]

Smoking as a possible cause of DNA damage was investigated in this study (table 4). Slightly increased chromium concentrations in lymphocytes, whole blood, and urine were found in cigarette smokers compared with non-smokers from the exposed group (table 4). These increased concentrations were not significantly higher when compared with those of non-smokers, which agrees with findings reported by Bukowski *et al*.[36] In our study it should be noted that we have only classified subjects into smokers and non-smokers. Cigarette tobacco contains chromium from 0·24 to 6·3 mg/kg[2]. The smokers in this investigation were not heavy smokers; most smoked between 10 and 20 cigarettes/day. A small effect of smoking on DNA damage was found in the group exposed to chromium, measured as increases in DNA strand breaks and formation of 8-OHdG. Breaks in DNA strands induced by smoking and formation of 8-OHdG have previously been investigated.[37-39] Clearly, these results make it vital that investigators should always consider the effect of smoking in any studies of chemically induced DNA damage.

According to our present findings, it would seem that the results that show no DNA damage to circulating peripheral lymphocytes, may give a certain measure of security to the chromate production workers under the present airborne exposure conditions (generally below 0·01 mg Cr(VI)/m$^3$) and with whatever respiratory protection is customary. This is well below the United Kingdom MEL of 0·05 mg Cr(VI)/m$^3$.[2] It may also indicate that the use of lymphocytes for biomonitoring of chromium exposure at such low air concentrations of chromium may be inappropriate. Thus, in the light of this pilot study and the implications discussed above, it is important that priority should be given to the study of populations with higher exposure to chromium, closer to the United Kingdom MEL than that of the present study.

We thank the workforce and management of British Chrome and Chemicals for their participation in the study and the medical staff for organising and collecting the biological samples. We would also like to thank Dr J K Chipman and Professor R Colman (School of Biochemistry, University of Birmingham) for use of the high performance liquid chromatograph and fluorimeter respectively. The study was partly supported by Scott Moncrieff Fund, the Medical School of the University of Birmingham.

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