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## Determination of hexavalent chromium in exhaled breath condensate and environmental air among chrome plating workers

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### Abstract

Chromium speciation has attracted attention because of the different toxicity of Cr(III), which is considered relatively non-toxic, and Cr(VI), which can cross cell membranes mainly as a chromate anion and has been classified as a class I human carcinogen. The aims of the present study were to measure soluble Cr(VI) levels in environmental samples, to develop a simple method of quantifying Cr(VI) in exhaled breath condensate (EBC), and to follow the kinetics of EBC Cr(VI) in chrome plating workers.

Personal air samples were collected from 10 chrome platers; EBC was collected from the same workers immediately after the work shift on Tuesday and before the work shift on the following Wednesday. Environmental and EBC Cr(VI) levels were determined by means of colorimetry and electrothermal absorption atomic spectrometry, respectively.

The method of detecting Cr(VI) in environmental air was based on the extraction of the Cr(VI)-diphenylcarbazide (Cr(VI)-DPC) complex in 1-butanol, whereas EBC Cr(VI) was determined using a solvent extraction of Cr(VI) as an ion pair with tetrabutylammonium ion, and subsequent direct determination of the complex (Cr(VI)-DPC) in EBC.

Kinetic data showed that airborne Cr(VI) was reduced by 50% in airway lining fluid sampled at the end of exposure and that there was a further 50% reduction after about 15 h. The persistence of Cr(VI) in EBC supports the use of EBC in assessing target tissue levels of Cr(VI).

### Keywords

Cr(VI); Speciation; Exhaled breath condensate; ETAAS; Chrome plating

### 1. Introduction

Chromium (Cr) is present in the environment in two main oxidation states: Cr(III) and Cr(VI). Whereas Cr(III) can be present in non-polluted environments, Cr(VI) is largely anthropogenic. Both forms are detectable in the workplace air of stainless steel production plants, chromium plating plants, chromium pigment factories, etc. [1]. Chromium speciation has attracted a great deal of attention because of the differential toxicity of its stable species: Cr(III) cannot usually

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cross cell membranes and its toxicity is considered to be relatively low [2,3], whereas Cr(VI) is transported through anion channels as chromate ( $\text{CrO}_4^{2-}$ ) [4], and its reduction by different intracellular components (such as glutathione, ascorbate, tocopherols and different enzyme cofactors) generates stable Cr(III) or unstable Cr(IV) and Cr(V) intermediates, all of which are capable of forming complexes with peptides, proteins and DNA, and generating oxidative stress in vitro and in vivo, though Cr(VI) oxidizing potential in humans is not known with certainty [5–8]. Cr(VI) has been recognised as a highly toxic elemental species on the basis of experimental and epidemiological evidence, and has been classified as a class I human carcinogen by the International Agency for Research on Cancer (IARC) [2].

In occupational settings, Cr(VI) exposure mainly occurs as a result of inhalation, and it is known that Cr(VI) compounds cause lung cancer [1]. The ex vivo monitoring of Cr species at the target organ level is important in occupational toxicology, because the measurement of total Cr in white and red blood cells [9] can only give information concerning systemic levels, which however reflect only exposure to soluble Cr(VI) and are influenced by absorption from all routes, including dermal exposure. Similar information can be obtained from Cr in urine, where Cr is completely reduced to the trivalent state [10]. However, the most toxic Cr species are represented by Cr(VI) compounds that are poorly absorbed and persist in the lung, where – when taken up by resident cells – can cause cancer.

We have recently demonstrated that total Cr is measurable in the exhaled breath condensate (EBC) of chrome plating workers, and that its EBC levels correlate with biomarkers of inflammation and oxidative stress, such as malondialdehyde (MDA) and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), thus suggesting that exhaled total Cr may reflect the lung dose responsible for toxic effects on the airways [11]. There are no published data concerning the Cr(VI)/Cr(III) equilibrium in the airway lining fluid, nor any data is available concerning its reducing power, although different concentrations of individual reducing agents (glutathione, ascorbic acid) may affect the reduction of Cr(VI) to Cr(III), and thus the levels of Cr(VI) that reach pulmonary tissue [12]. The measurement of Cr(VI) in EBC, which is also a suitable fluid for assessing the lung doses and effects of cobalt and tungsten in exposed workers [13], may therefore be a useful means of following the reduction pathways of Cr at target organ level and studying the mechanisms of pulmonary Cr(VI) toxicity.

Published methods of measuring Cr(VI) in environmental and water samples include colorimetry after the complexation of chromate ions with 1,5-diphenylcarbazide (DPC) or other molecules, with and without previous extraction and pre-concentration steps [14–18], and chromatographic techniques for the separation of different oxidation forms [19–21] and stripping voltammetry [22]. Furthermore, the website of the National Institute for Occupational Safety and Health (NIOSH) shows various protocols for determining Cr(VI) in environmental air [23], and a comparison of the different analytical methods [24]. The state of the art concerning the measurement of airborne Cr(VI) compounds in workplace aerosols and related samples has recently been reviewed [25]. Cr(VI) has never been assayed in EBC after exposure to hexavalent Cr compounds.

The aims of this study were to develop a simple liquid/liquid extraction and colorimetric method for monitoring soluble Cr(VI) in environmental samples without the need for more complex and expensive separation methods such as solid phase extraction (SPE), to measure EBC Cr(VI) levels in exposed workers, and to follow the kinetics of Cr(VI) exhalation using an even more precise and sensitive method based on ion pair extraction followed by electrothermal absorption atomic spectrometry (ETAAS).

## 2. Experimental

### 2.1. Subjects

The study involved 10 workers employed in a chrome plating plant who had normal spirometric indices and did not report any significant current or past respiratory disease; none of them had experienced any symptoms of acute respiratory illness in the 4 weeks preceding the study. Their demographic and clinical data are summarised in Table 1. Two EBC samples were collected: one immediately after the end of the work shift on Tuesday evening ( $t_0$ ) and the other before the beginning of the work shift on Wednesday morning ( $t_1$ ), about 15 h after the last Cr exposure.

All of the enrolled subjects gave their written informed consent to the procedures, which were approved by our local Institutional Human Ethics Committee. The biological material was sampled as laid down in the Declaration of Helsinki [26].

### 2.2. EBC collection

EBC was collected using a TURBO-DECCS (Transportable Unit for Research on Biomarkers Obtained from Disposable Exhaled Condensate Collection Systems) (ItalChill, Parma, Italy) as previously described [11]. Briefly, the subjects were asked to breathe tidally through the mouthpiece for 15 min, while sitting comfortably in the workplace office where the level of total Cr was below  $0.1 \mu\text{g}/\text{m}^3$ . The collecting temperature was  $-5^\circ\text{C}$ . We also measured total Cr in EBC of controls [11], but Cr levels were too low to detect the fraction of Cr(VI).

### 2.3. Instrumentation

Sonication was carried out in a Transsonic 460 ultrasonic bath (Elma, Singen, Germany). The solid phase (SPE) was extracted using a VacMaster<sup>TM</sup>-10 SPE Manifold (IST, Glamorgan, UK) connected to a vacuum pump by means of a pressure control valve. The PTFE valve liners came from IST (Glamorgan, UK), and the strong anion-exchange solid-phase extraction (SAE-SPE) cartridges were Supelclean<sup>TM</sup> LC-Florisil SPE Tubes (6 ml) from Supelco (Bellefonte, PA, USA). A DU 640 spectrophotometer (Beckman, Fullerton, CA, USA) was used for the colorimetric measurements, and a 220 Z atomic absorption spectrometer (VARIAN, Palo Alto, USA) with Zeeman effect background correction was used to monitor Cr in the biological samples.

### 2.4. Reagents

The standard certified Cr(VI) solution came from Fluka (Buchs, Germany), and the standard Cr(III) atomic absorption solution from Aldrich (Milwaukee, WI, USA) as tetrabutylammonium bromide 99% (TBAB), 4-methyl-2-pentanone and nitric acid 69.5%. The 1,5-diphenylcarbazide (DPC), acetonitrile, ammonium sulphate and ammonium hydroxide (all reagent grade) came from Sigma (St. Louis, MI, USA). Hydrochloric acid 37% (HCl), 1-butanol and absolute ethanol came from Carlo Erba (Milan, Italy).

### 2.5. Environmental measurements

**2.5.1. Environmental sample collection and extraction procedure**—Ambient monitoring was carried out by means of personal samplers. Briefly, airborne particulates were collected on PVC membrane filters (5.0  $\mu\text{m}$  porosity, 25 mm diameter, Supelco, Bellefonte, PA, USA) at a constant flow of  $3 \text{ l}/\text{min}^{-1}$  for 90–150 min in the morning or afternoon of the Tuesday work shift. The reducing power of the filters was excluded by incubating some of them with known Cr(VI) concentrations for 24 h (data not shown). The 7703 NIOSH method [23] for the extraction of total Cr by sonication of the membranes (10 ml of extraction buffer – 0.05 M ammonium sulphate, 0.05 M ammonium hydroxide – was used for every membrane)

was strictly followed. During the 24 h following the environmental air collection, Cr(VI) and total Cr were, respectively, measured by means of a colorimetric method and ETAAS with Zeeman effect background correction.

**2.5.2. Cr(VI) determinations**—The standards were freshly prepared from a certified Cr(VI) solution after dilution with water. Moreover, adequate amounts of standards were added directly to the membrane and extracted as the samples, with a recovery close to 100%. In the original NIOSH 7703 protocol, Cr(VI) was extracted from SPE cartridges with 9 ml of elution solution (0.5 M ammonium sulphate, 0.1 M ammonium hydroxide), but better Cr(VI) recovery was obtained by slightly changing the SPE procedure to include a cartridge washing step with methanol 3 ml, the cartridge loading of 3 ml of the aqueous extraction samples obtained from membranes after sonication, and the subsequent addition of 1 ml water to clean up the impurities. Finally, Cr(VI) was extracted from the cartridge with 9 ml of buffer elution 0.01 M ammonium sulphate and 0.01 M ammonium hydroxide and 100  $\mu$ l of HCl 37% and 3 ml of DPC complexation solution (25 mM of DPC in acetonitrile) were added to the SPE recovery samples. The determinations of the Cr(VI)–DPC complex were made by measuring solution absorbance at 540 nm.

The same analysis was carried out in parallel by changing the Cr(VI) colorimetric detection protocol. We found experimentally that the Cr(VI)–DPC complex tended to concentrate in some organic solvents such as 1-butanol (see Section 3), and so 9 ml of the elution solution with Cr(VI) and 3 ml of DPC standard solution (2 mM) in 1-butanol were added together with 400  $\mu$ l of HCl 37%. The samples were mixed thoroughly and, after five minutes, centrifuged ( $2000 \times g$ , 5 min) to separate the phases. The determinations of the Cr(VI)–DPC complex were made on the organic phase by measuring absorbance at 545 nm as the use of 1-butanol as solvent shifted the absorbance peak the complex from 540 to 545 nm.

**2.5.3. Cr(VI) determinations without SPE procedures**—The great affinity between the Cr(VI)–DPC complex and 1-butanol induced us to develop a simpler method of detecting Cr(VI) in environmental samples that avoided the SPE procedure. Because of the relatively high concentrations of Cr(VI) in the membranes, 1 ml of the extracted Cr(VI) was mixed directly with 1 ml of butanolic DPC solution (2 mM) without any previous extraction. In the mixing phase, we added 100–150  $\mu$ l of HCl 37% and directly measured the absorbance of the organic phase at 545 nm after centrifugation at  $2000 \times g$  for 2 min.

## 2.6. Cr(VI) and total Cr determinations in EBC

The protocol for measuring Cr(VI) in EBC was derived from published solvent extraction procedures for water samples [27]. Briefly, 1 ml of TBAB in 4-methyl-2-pentanone, 50 mM solution, and 50  $\mu$ l nitric acid 69.5% were added to 1 ml of EBC, and the biphasic system was thoroughly mixed for 10 min. After centrifugation at  $2000 \times g$  for 5 min, 800  $\mu$ l of the organic phase containing the ion couple between the chromate and tetrabutylammonium ion were mixed with 50  $\mu$ l of a concentrated aqueous solution of DPC (20 mM) in 60% ethanol and 7% nitric acid, and 1 ml of pure water. After mixing for 2–3 min, the two phases were separated by centrifuging the solution at  $3000 \times g$  for 1 min. Because of the low concentration of Cr(VI) in EBC, the Cr(VI) complexed with DPC in the aqueous phase was measured by means of ETAAS, which is about 2.5 times more sensitive than the colorimetric method used for the environmental analysis.

Total Cr in EBC was measured by means of ETAAS as previously reported [11].

## 2.7. Statistical analysis

The Bland–Altman method was used to compare the different methods of Cr(VI) detection [28]. Because of the relatively small number of studied subjects, parametric tests were used on logarithms to reduce the effect of outliers (dependent Student's *t*-test or ANOVA for repeated measures followed by Tukey's post hoc test) with a significant *p* value of 0.05. The data are expressed as geometric means (geometric S.D.), and also ranges.

## 3. Results

### 3.1. Detection of Cr(VI) in environmental samples

In order to test the liquid/liquid Cr(VI)/DPC extraction efficiency of 1-butanol and compare it with that of the colorimetric method suggested by the NIOSH 7703 protocol, in which DPC is dissolved in acetonitrile and directly added to the Cr(VI) solution [23], two different calibration lines were used in the range of 0–200  $\mu\text{g l}^{-1}$  of Cr(VI) in elution buffer (Fig. 1). Within this range, both methods showed a linear response ( $R = 0.999$ ), but the accumulation of Cr–DPC in 1-butanol enormously enhanced the signal: the slope of the regression lines passed from  $0.60 \pm 0.01$  for the standard method to  $1.83 \pm 0.01$  for butanol liquid/liquid extraction, and the limit of detection (LOD) calculated as 3 S.D. of the blank variability changed from about 1.5 to 0.5  $\mu\text{g l}^{-1}$ . The use of 1-butanol as solvent shifted the wavelength of maximum absorbance to 545 nm without changing the intensity of the peak (data not shown).

Fig. 2A shows the experimental detection of Cr(VI) in environmental samples (20 experimental points in the range of 0.1–7.0  $\mu\text{g/membrane}$ ) with and without SPE (see Section 2 for further details). The slope of the straight line in the regression fit was  $1.01 \pm 0.03$ , and the correlation coefficient 0.995 (Fig. 2A), whereas the Bland–Altman graph (Fig. 2B) showed only one point between 2 and 3 S.D. (as expected with 20 experimental points), and the mean deviation between the two methods was near to 0 (0.03  $\mu\text{g/membrane}$ ).

In relation to the selected workers, the morning exposure to total Cr [ $3.7$  (2.8)  $\mu\text{g m}^{-3}$ , range 0.8–14.1  $\mu\text{g m}^{-3}$ ] was not statistically different from the afternoon exposure [ $4.6$  (2.7)  $\mu\text{g m}^{-3}$ , range 1.4–23.4  $\mu\text{g m}^{-3}$ ]. This was confirmed by the measurements of Cr(VI), which passed from 2.5 (2.8) (range 0.5–10.3)  $\mu\text{g m}^{-3}$  in the morning to 3.3 (3.2) (range 1–17.6)  $\mu\text{g m}^{-3}$  in the afternoon. In terms of % Cr(VI) content, 67.8% (1.1) (range 56.7–76.6%) of the total Cr in the morning, and 70.1% (1.3) (range 43.9–100%) in the afternoon, was in the form of soluble Cr(VI).

On the basis of these results, the weighted average exposure of the workers during the work shift was 4.3 (2.6) (range 1.2–18.6)  $\mu\text{g m}^{-3}$  of total Cr, of which 2.9 (2.6) (range 0.8–13.8)  $\mu\text{g m}^{-3}$  was soluble Cr(VI). Soluble Cr(VI) accounted for 67.5% (1.1) (range 49.9–78.3%) of the total.

### 3.2. Detection of Cr(VI) in EBC

Various experiments were performed to validate the procedure for EBC Cr(VI) determinations described in Section 2.

- The signals of standard Cr(VI) alone and complexed with DPC in aqueous solution measured by means of ETAAS were similar (differences <5%, data not shown).
- About 90% of the Cr(VI) initially present in the solution was extracted as an ion pair with TBAB in water; the extraction efficiency was slightly lower in EBC (about 85%); the calibration curves in Fig. 3 shows that the extraction in both water and EBC was independent of the Cr(VI) concentration. On the contrary, <2% of Cr(III) was



extracted in the organic phase with TBAB, and its contribution to the final Cr(VI) signal was negligible up to  $50 \mu\text{g l}^{-1}$  (data not shown).

- The contribution of the contamination of DPC, nitric acid, TBAB and 4-methyl-2-pentanone to the blank signal was low, but not negligible (data not shown).
- To test the effects of other anions on the complexation procedures (i.e. competition effects of different ions forming the ion pair) and the ETAAS Cr(VI) signal, Cr(VI) was determined in two different drinking waters in which the concentrations of anions such as chloride, carbonate, nitrate and sulfate can be easily measured. The spectrum of a standard Cr(VI) concentration ( $5 \mu\text{g l}^{-1}$ ) was unaffected by the matrix (data not shown), and the simultaneous presence in solution of  $5 \mu\text{g l}^{-1}$  Cr(VI) and  $500 \mu\text{g l}^{-1}$   $\text{Fe}^{2+}$  (which tends to reduce Cr(VI) at acidic pH) reduced the ETAAS Cr(VI) absorption peak by only about 10%.
- The calibration curve of Cr(VI) in a pool of different EBCs (total Cr =  $0.1 \mu\text{g l}^{-1}$ ) showed an approximately 10% decrease in the Cr signal within the range 0– $5 \mu\text{g l}^{-1}$  (Fig. 3). Moreover, as in the case of water, the presence of up to  $50 \mu\text{g l}^{-1}$  of Cr(III) in EBC did not interfere with the Cr(VI) signal.
- Considering 3 S.D. of the blank, the LOD of EBC Cr(VI) was about  $0.2 \mu\text{g l}^{-1}$ .
- The coefficient of variation (CV) of the measurements of standard samples ( $0.8$ ,  $2$  and  $5 \mu\text{g l}^{-1}$ ) in water and a pool of EBCs was  $<10\%$  ( $2$  and  $5 \mu\text{g l}^{-1}$ ) and  $<30\%$  ( $0.8 \mu\text{g l}^{-1}$ ) for all of the intra- and inter-day determinations.

Fig. 4A shows total Cr in EBC, with the corresponding Cr(VI) concentrations. At  $t_0$ , total Cr was  $2.6$  ( $1.5$ ) (range  $0.8$ – $6.1 \mu\text{g l}^{-1}$ , significantly higher ( $p < 0.05$ ) than at  $t_1$  [ $1.5$  ( $2.0$ ), range  $0.4$ – $4.1 \mu\text{g l}^{-1}$ ], and Cr(VI) passed from  $0.9$  ( $3.4$ ) (range  $0.1$ – $2.9 \mu\text{g l}^{-1}$ ) at  $t_0$  with only one of the 10 EBC samples below the LOD, to  $0.2$  ( $3.6$ ) (range  $0.1$ – $2.1 \mu\text{g l}^{-1}$ ) at  $t_1$  ( $p < 0.01$ ), with six of the 10 EBC samples below the LOD. Where Cr(VI) was less than  $0.2 \mu\text{g l}^{-1}$ , half of the LOD ( $0.1 \mu\text{g l}^{-1}$ ) was assigned. The Cr(VI)/total Cr ratio (Fig. 4B) showed that EBC Cr(VI) levels were significantly lower at  $t_1$  [ $16.5\%$  ( $2.5$ ), range  $3.0$ – $51.2\%$  of total Cr] than at  $t_0$  [ $33.5\%$  ( $2.0$ ), range  $9.7$ – $79.0\%$ ;  $p < 0.05$ ] and in the environmental air ( $p < 0.01$ ); the difference between  $t_0$  and environmental air was also significant ( $p < 0.05$ ).

## 4. Discussion

Our study shows that it is possible to determine Cr(VI) in EBC and that the fractional contribution of Cr(VI) to total Cr decreased over time from the last exposure, thus ruling out its meaning as a simple marker of environmental contamination. This time-dependent decrease can in fact only be explained as an interaction between inhaled Cr(VI) and the pulmonary lining fluid, with a consequent reduction to Cr(III). The persistence of Cr(VI) in EBC reinforces the idea that the lower airways are the main target of Cr(VI) toxicity [10].

Cr(VI) can be detected in environmental samples using different approaches: soluble or total Cr(VI) can be distinguished by changing the extraction solution from the membrane buffer [29], and more sophisticated separation can be obtained by means of SPE or chromatography techniques [23]. However, as these are time-consuming and relatively expensive methods, we propose a very simple and rapid method of detecting soluble Cr(VI) based on the direct extraction of the Cr(VI)–DPC complex in 1-butanol and by-passing such extraction procedures as SPE. On the other hand, interferences may be problematic in workplaces where potentially interfering metals, like Fe in different oxidation states, could be present in much greater concentrations. Sample preparation procedures may be necessary in such cases. Our method has the advantage that Cr(VI) can be concentrated in the organic phase simply by changing the volume of the DPC/butanol solution: using a 3:1 ratio of sample:DPC/butanol solution (9:3

ml), tripled the increase in signal intensity and reduced the LOD by a factor of 3 (Fig. 1). This means that about 75% of Cr(VI) complexed with DPC was concentrated in the organic phase. Cr(VI) extraction of some pieces of membrane with the buffer suggested by Hazelwood et al. [29] in order to monitor the soluble and insoluble Cr(VI) compounds did not significantly change the results, the concentration of insoluble Cr(VI) in this study being considered negligible.

Fig. 2A and B also shows negligible interferences from other cationic transition elements in environmental samples. The results with and without the SPE step recommended in the literature to clean the samples from other cationic transition elements or impurities were perfectly consistent in the investigated range of concentrations, and this was confirmed by *in vitro* experiments in which  $500 \mu\text{g l}^{-1}$  of Cr(VI) were incubated with equal absolute quantities of cationic Fe, Pb, Ni, Cd, Cu and Co (data not shown). Another advantage of our method is that the SPE cartridges could be saturated by high concentrations of Cr(VI), whereas a direct colorimetric measure is linear in the range of the linearity of the spectrophotometer (Abs about 1.5). Further work is needed to compare our method with the ISO 16740 speciation method recently published [30].

In determining EBC Cr(VI), we adopted a different and more sensitive method that is useful for small concentrations and small volume samples (the LOD passes from  $0.5$  to  $0.2 \mu\text{g l}^{-1}$ ). Tetraalkylammonium (tetrapropyl or tetrabutylammonium) bromide tends to form ion pairs with metal cyanides, alkyl sulfonate, sulfur oxide, inorganic anions and oxyanions [31–34] and, in particular, tetrabutylammonium ions form stable ion pairs with chromate at acidic pH in aqueous solutions [17,27]. This ion pair can be selectively extracted in organic solvents as 4-methyl-2-pentanone or chloroform, or in aqueous two-phase systems [17,27]. We adapted the method of Noroozifar and Khorasani-Motlagh [27] to the small volumes of collected EBC (never more than 2 ml over 15 min), and the use of ETAAS also allowed good sensitivity in the generally diluted EBC samples. The percent recovery of Cr(VI) as an ion pair in 4-methyl-2-pentanone was in line with that observed in previous studies [17,27] despite the small volumes, and did not depend on the Cr(VI) concentration in the considered range (Fig. 3).

Moreover, the experiments using drinking water and  $\text{Fe}^{2+}$ , together with the results of interference studies published in the literature [27], make the developed method ideal for diluted samples such as EBC, although the slight decrease in the extraction efficiency of Cr(VI) as an ion pair and then as a complex with DPC (about 10% overall) suggested adding the standard directly to the pools of EBCs and treating them as samples. This can be explained as a probable interference of traces of EBC anions (salts and proteins) present upon the extraction of Cr(VI). The slight effect of the different components on the signal of the blank suggested using highly pure reagents to minimise these effects, but the blank absorbance was in any case acceptable: about 14 mAbs against the 6 mAbs of pure water.

The optimisation of the methods of measuring soluble Cr(VI) in environmental samples and EBC allowed us to study the kinetics of Cr(VI) in the airways. First of all, total Cr in EBC decreased 15 h after the last exposure, a finding that is in line with previous results observed before and after a weekend, and confirms that Cr can pass through airway lining fluid [11]: the kinetics of pulmonary lung desorption is relatively slow [35], and Cr accumulation has recently been observed in the bronchi and lungs of chromate workers [36]. Most importantly, the EBC Cr(VI) data confirmed that Cr(VI) was reduced to Cr(III) by airway lining fluid, but not completely so (Fig. 4A and B), even in workers exposed to smaller environmental Cr(VI) concentrations than the limits suggested by ACGIH [37], and those exposed to lower Cr concentrations than those reported in our previous study [11]. About 33.5% of Cr was still in the hexavalent form immediately after chromate exposure (about 67% of the environmental samples were hexavalent): 9/10 workers had clearly detectable EBC Cr(VI) levels, and 4/10

had detectable levels about 15 h after the last exposure. The persistence of Cr(VI) in the airways can justify the previously reported increase in biomarkers of inflammation in EBC [11], because unreduced Cr(VI) can be absorbed at pulmonary level and reveal its toxicity in pulmonary cells. We are currently attempting to relate the reduction kinetics of EBC Cr(VI) to individual susceptibility and EBC biomarkers of inflammation in a larger number of subjects.

## 5. Conclusions

In conclusion, we have developed a method for measuring EBC Cr(VI) based on ion pair extraction followed by ETAAS determination. The results of this study highlight the potential of EBC as a medium for assessing lung dose and effects after exposure to inhaled pneumotoxic substances (particularly transition elements) with different oxidation states. The integrated use of EBC and classic biological matrices such as urine and blood, which reflect systemic exposure, may therefore allow the fundamental completion of the biological monitoring of pneumotoxic compounds.

### Acknowledgements

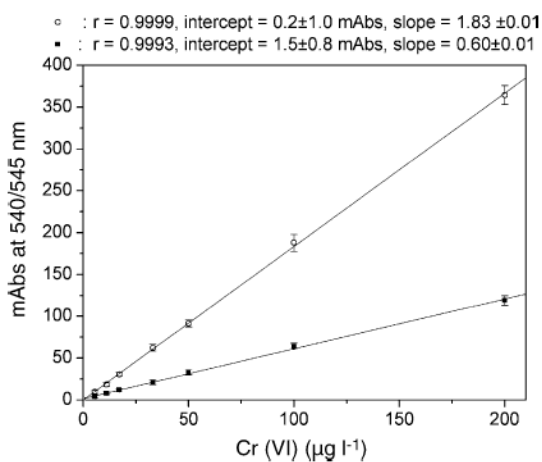
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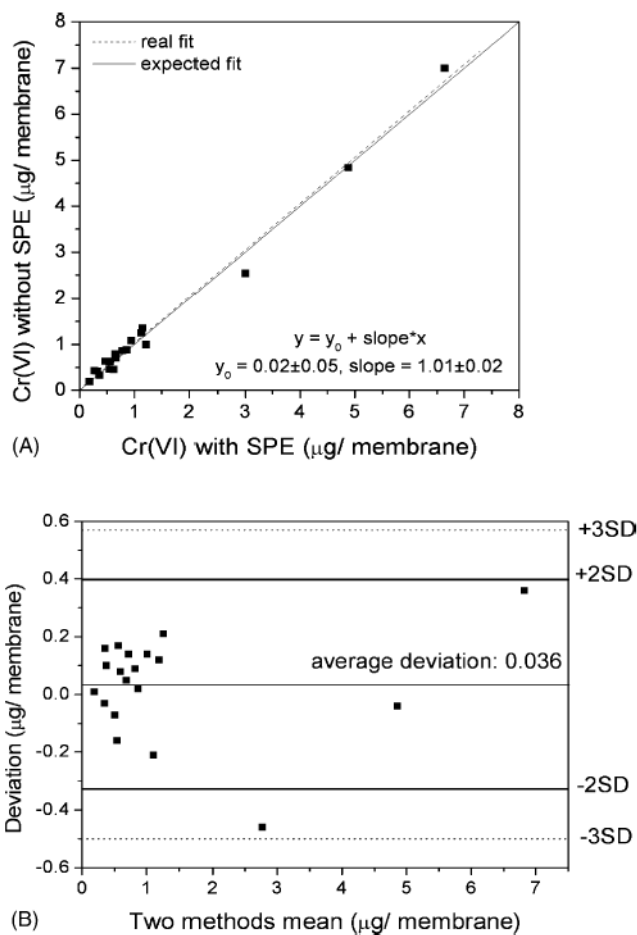
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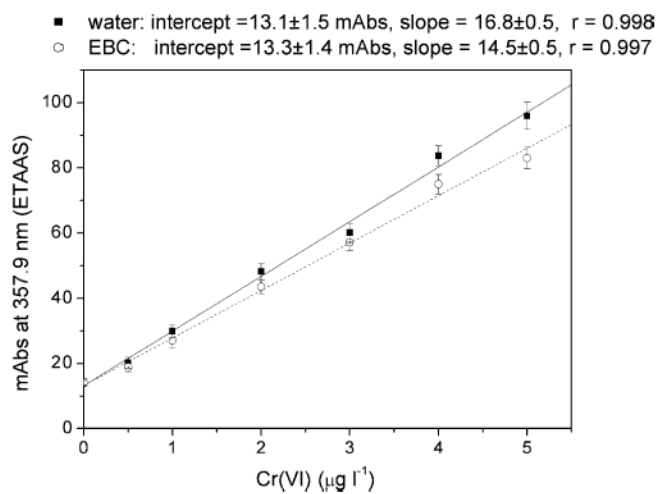
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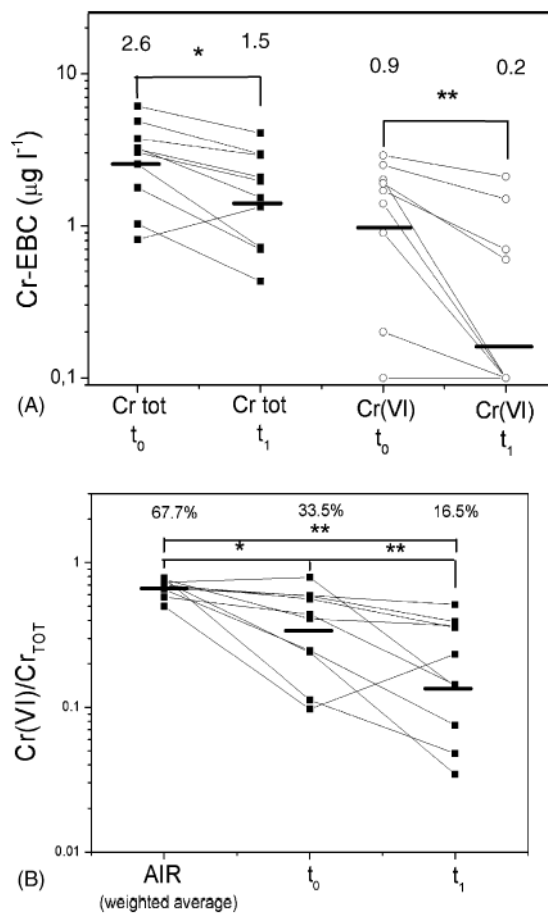
**Fig. 1.** Calibration lines of Cr(VI) in the range 0–200  $\mu\text{g l}^{-1}$  in elution buffer with the extraction of Cr(VI)–DPC in 1-butanol measuring absorbance at 545 nm (○), and following the NIOSH 7703 protocol measuring absorbance at 540 nm (\*). The figure also shows Pearson's  $r$ -value, the intercept and the slope of the lines with their S.D.s.



**Fig. 2.** (A) Correlation between the Cr(VI) concentrations determined with and without SPE in the environmental air samples, also showing the function of the fit with the values of the intercept with y-axis ( $y_0$ ) and slope. The expected fit is  $y = x$ . (B) Bland–Altman graph in which the “two methods mean” is the average of the values measured with and without SPE, and “deviation” the difference between these values. The figure also shows the lines representing  $\pm 2$  S.D. (—) and 3 S.D. (...).



**Fig. 3.** Calibration curve of Cr(VI) in the range  $0\text{--}5 \mu\text{g l}^{-1}$  after extraction with TBAB and complexation with DPC in water, and in a pool of EBC; the slopes of the curves and Pearson's  $r$ -value are also shown. The intercept represents the value of the blank in the absence of standard Cr(VI).



**Fig. 4.**

(A) Cr and Cr(VI) levels at the end of the Tuesday work shift ( $t_0$ ) and at the beginning of the Wednesday work shift ( $t_1$ ), and their geometric means (top of the graph). (B) The fraction of Cr(VI) in the environmental samples (AIR) at  $t_0$  and  $t_1$ , and their geometric means (top of the graph). \* $p < 0.05$ ; \*\* $p < 0.05$  with a paired Student's  $t$ -test (A) and repeated measures ANOVA followed by Tukey's post hoc tests (B).



**Table 1**  
Demographic and clinical data of the study subjects

	Chrome plating workers
Number of subjects	10
Sex (M/F)	10/0
Age (years)	39.3 ± 7.3
Smokers/ex-smokers/non-smokers	1/0/9
FVC (% predicted)	91.2 ± 8.3
FEV1 (% predicted)	92.5 ± 9.1
FEV1/FVC (%)	83.4 ± 9.2

The pulmonary parameters and age are expressed as mean values ± S.D. FVC: forced expiratory vital capacity; FEV1: forced expiratory volume in 1 s.