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Evaluation of chromium in red blood cells as an indicator of exposure to hexavalent chromium: An *in vitro* study

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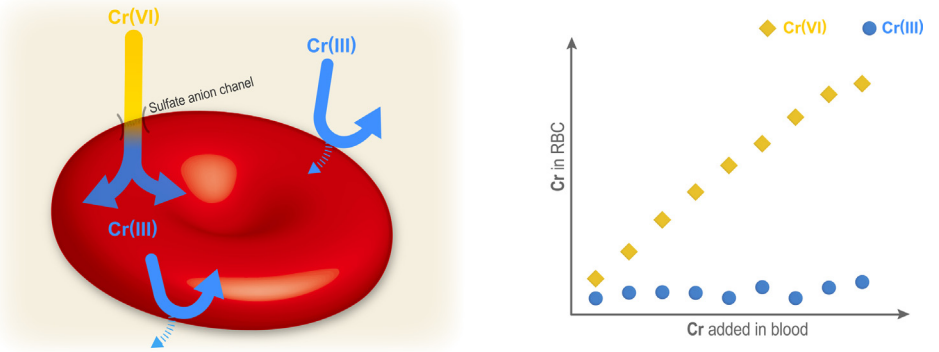
HIGHLIGHTS

- Our *in-vitro* study evaluates the selectivity of RBC in accumulating Cr(VI).
- A monotonic relationship exists between Cr(VI) added to blood and the Cr in RBC.
- Various parameters were tested, with negligible effects found on the relationship.
- Cr in RBC may be a good biomarker of recent exposure to Cr(VI).

GRAPHICAL ABSTRACT



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ABSTRACT

Chromium(VI) compounds are classified as carcinogenic to humans. Whereas chromium measurements in urine and whole blood (i.e., including plasma) are indicative of recent exposure, chromium in red blood cells (RBC) is attributable specifically to Cr(VI) exposure.

Before recommending Cr in RBC as a biological indicator of Cr(VI) exposure, *in-vitro* studies must be undertaken to assess its reliability. The present study examines the relationship between the chromium added to a blood sample and that subsequently found in the RBC.

After incubation of total blood with chromium, RBC were isolated, counted and their viability assessed. Direct analysis of chromium in RBC was conducted using Atomic Absorption Spectrometry.

Hexavalent, but not trivalent Cr, was seen to accumulate in the RBC and we found a strong correlation between the Cr(VI) concentration added to a blood sample and the amount of Cr in RBC. This relationship appears to be independent of the chemical properties of the human blood samples (e.g., different blood donors or different reducing capacities).

Abbreviations: AA, ascorbic acid; ACGIH, American Conference of Governmental Industrial Hygienists; BEI, biological exposure indicator; CrA, chromium in atmosphere; CRI, collision reaction interface; CrIE, chromium in erythrocyte (in RBC); ECHA, European chemicals agency; GEQUAS, the German external quality assessment scheme (for analyses in biological materials); GF-AAS, graphite furnace atomic absorption spectroscopy; ICP-MS, inductively coupled plasma-mass spectrometry; PRC, plasma reduction capacity; QMEQAS, Quebec multielement external quality assessment scheme; RBC, red blood cells or erythrocytes; SVHC, substance of very high concern; TLV-TWA, threshold limit value-time weighted average.

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GF-AAS
Biomonitoring

Even though *in-vivo* studies are still needed to integrate our understanding of Cr(VI) toxicokinetics, our findings reinforce the idea that a single determination of the chromium concentration in RBC would enable biomonitoring of critical cases of Cr(VI) exposure.

1. Introduction

Chromium was first discovered in 1780 by the French chemist, Nicolas Louis Vauquelin, in a mineral sample of 'Siberian red lead'—now known as crocoite (lead chromate). Chromium is a silver, lustrous and very hard metal that can take a high mirror polish, and it is odorless, tasteless and malleable (Weeks, 1932). Chromium compounds are valued as pigments due to their vivid green, yellow, red and orange colors. The metal is also widely used for its catalytic properties. Chromium is used in stainless steel, contributing to resistance to oxidation, and when combined with nickel or vanadium (and tungsten), contributes to ductility and resistance to temperature, respectively. Chromium can exist in oxidation states ranging from −2 to +6. Of these, pure metal (Cr(0)), trivalent (Cr(III)) and hexavalent chromium (Cr(VI)) can all be encountered in the working environment. Metallic chromium dust may pose a health risk in the workplace but the risk can easily be reduced through the use of respiratory protection and/or technical devices. Cr(III) is an essential dietary nutrient for humans and is considered nontoxic (Nurminen, 2004). Upon skin contact, both Cr(III) and Cr (VI) may cause irritation and frequently produce allergic reactions (affecting 1–3% of the general population) (Hansen et al., 2003; Thyssen and Menne, 2010; Yoshihisa and Shimizu, 2012). This contact dermatitis could easily be prevented by wearing protective clothing and gloves to avoid dermal exposure. Among the forms of chromium encountered in workspace, only Cr(VI) compounds are recognized as carcinogenic and are classified as SVHC by the ECHA (IARC, 1990; INRS, 2006). Monitoring of this species is therefore of particular importance.

Industrial operations such as refractory steel processing, stainless steel production, welding, chrome plating, tanning (Hedberg et al., 2015) and chromite ore-processing may expose employees to these elements. In metrological approaches, monitoring of exposure to these metals combines analysis of the air with analysis of biological environments such as urine or blood. In Europe, the recommended indicative limiting values for professional exposure are atmospheric chromium (CrA) concentrations of 2 mg/m^3 (8-h average) for Cr(0), for the inorganic chromium compounds (Cr(II)) and for the (insoluble) inorganic chromium compounds (Cr(III)) (European Commission, 2006). In France, professional exposure limit values (VLEP) also exist for the compounds of Cr(VI) (0.001 mg Cr/m^3) and Cr trioxydes (0.05 mg Cr/m^3) (JO, 2012). In the United States, the ACGIH (American Conference of Governmental Industrial Hygienists) recommends the use of limiting values based on actual occupational exposure: the TLV (Threshold Limit Value). The TLV-TWA (Threshold Limit Value—Time Weighted Average) exposure limit value corresponds to a concentration accumulated over either an 8-h workday or a 40-h working week. The ACGIH recommends a value of 0.5 mg Cr/m^3 for chromium metal and for the compounds of Cr(III), a value of 0.05 mg Cr/m^3 for Cr(VI) and a value of 0.01 mg Cr/m^3 for insoluble Cr(VI) (ACGIH, 2013).

While analysis of CrA is well-documented from a methodological point of view, and is therefore used for regulatory purposes, there is a lack of data concerning the analysis of chromium in biological samples, even though chromium in these samples reflects the real exposure experienced by a worker.

In recent decades, a number of surveillance techniques have been developed that allow internal exposure to Cr(VI) to be monitored through analysis of biological fluids such as urine, blood and plasma. A number of investigations have helped to establish the relationship between airborne chromium and levels of chromium in blood, plasma and urine (Alexopoulos et al., 2008; Gube et al., 2010; Matczak et al., 1995; Miksche and Lewalter, 1997; Mutti et al., 1979; Mutti et al., 1984; Stridsklev et al., 2004; Tola et al., 1977). Urinary chromium can be regarded as a reliable marker of internal chromium exposure and is sufficiently sensitive for biological monitoring of exposure levels below the occupational limits. However, on the basis of urinary chromium alone, it is not possible to distinguish between exposure to Cr(VI) and exposure to Cr(III). Only chromium in erythrocytes is diagnostic of internal exposure to Cr(VI). This is because hexavalent chromium, unlike Cr(III) complexes, is able to cross the cell membranes of red blood cells (RBC) via anion carrier proteins (Kortenkamp et al., 1987). Chromium in lymphocytes has also been suggested to be a good indicator of Cr(VI) exposure (Lukanova et al., 1996).

Before validating the use of chromium in RBC and/or lymphocytes as an eventual Cr(VI) exposure biomarker, in vitro verification experiments should be performed. The main goal of this study is to check for linearity between the amounts of Cr(VI) added to blood samples and the concentrations of Cr measured in erythrocytes. We tested several parameters including the chromium oxidation state (oxidation states 3 and 6), chromium counterion (K^+, Na^+ and NH_4^+), chromium concentration (1 μg/L to 1 g/L), incubation temperature (4–37 °C), incubation time (up to 24 h), added ascorbic acid (AA) concentration (0–100 mg/L) and different blood donors. To study the influence of these parameters, whole blood samples were spiked with various quantities of highly-water-soluble Cr(VI) and Cr(III) compounds and the chromium levels in the RBC and lymphocytes were then determined. Only data for the RBC are presented in this paper.

2. Experimental methods

2.1. Apparatus

A Varian AA280Z atomic absorption spectrophotometer, equipped with a Zeeman background corrector, was used for atomic absorption measurement of chromium at 357.9 nm and with a slit-width of 0.5 mm. A hollow cathode Cr lamp (SCP Science, 030-150-244) was operated at 3 mA. Uncoated graphite tube cuvettes were purchased from Schunk Kohlenstofftechnik (Germany).

The Vi-CELL Cell Viability Analyze system, composed of the analyzer instrument and software (Beckman Coulter, Miami, USA), was used to determine cell concentration and viability. This system provides an automated means of performing the trypan blue dye exclusion method. The Vi-CELL instrument was calibrated using the Beckman Coulter Vi-CELL concentration control, following standard Beckman Coulter procedures. Each measurement consisted of the acquisition of fifty individual images per sample. Vi-CELL reagent packs were used as instructed in the Beckman Coulter, Inc instruction manual.

2.2. Reagents and solutions

All chemicals used in the study were of analytical grade or higher. Nitric acid was used to prepare 0.2% HNO_3 (v/v) with