Biomonitoring of occupational exposure: Neutron activation determination of selected metals in the body tissues and fluids of workers manufacturing stainless steel vessels

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Occupational exposure was examined for 20 workers dealing with welding, polishing, and assembling of stainless steel vessels. Instrumental neutron activation analysis was used for determination of selected elements in hair and nail, whereas urinary Cr and Mn, blood Mn and serum Cr were determined by radiochemical neutron activation analysis. Increased levels of Cr in hair, nails, serum and urine, Mo in hair, and Mn in blood were found in the exposed group compared to controls. Accuracy of the results was proven by analysis of reference materials and by comparison of element levels in controls with reference values for non-exposed persons.

Introduction

Workers in the stainless steel construction industry are exposed to numerous substances with known neurotoxicity, genotoxicity, carcinogenic, allergenic and immunological effects.1 The main health risk is associated with inhalation of welding fumes and airborne particulate matter (APM), which contain elevated levels of steel-alloying elements, such as Cr, Ni, Mo, Mn, V.

There is a growing need to harmonize activities in the field of occupational health methodology and approaches to risk assessment2,3 in which direct and/or biological monitoring can be used. Results of direct monitoring, i.e., the assessment of exposure from concentrations of toxic substances in the workplace may be misleading. Various mechanisms of intake and/or absorption may lead to a different body burden, when multiple mechanisms of intake are to be considered, and especially when short-term and long-term effects are to be distinguished.4 Therefore, in the assessment of the health risk arising from environmental, occupational and accidental exposure to toxic metals and other substances, the use of biological monitoring is steadily increasing,5–7 because our knowledge of various factors that influence levels of biological indicators of exposure8–10 and those that affect reference values for occupationally non-exposed populations has considerably increased in recent years, especially for toxic trace elements in the body tissues and fluids.11

Both approaches were used to examine occupational exposure of workers of a plant manufacturing stainless steel storage and production vessels for the pharmaceutical, food and chemical industries. Results of multi-elemental analysis of workplace APM performed by INAA, which showed exceeding of maximum admissible limit for Cr in the workplace air, have already been published.12 In this work the biological monitoring involved the determination of levels of Cr, Mn, Mo, Ni and V in hair and nails by instrumental neutron activation analysis (INAA), and levels of Cr in serum and urine and Mn in blood and urine by radiochemical neutron activation analysis (RNAA) in exposed and control persons.

Experimental

Exposed and control persons

Details on both groups have already been given elsewhere.12 Briefly, the group of exposed workers (average exposure 16 y) consisted of 18 men and 2 women (average age 33.1 y), of which 52 % were smokers. They were occupationally exposed to welding fumes and/or APM originating from shaving, polishing and assembling of vessels made of austenitic stainless steel containing on average 18% of Cr, 9–10% of Ni, 2–2.5% of Mo, 1–2% of Mn and traces of V (~0.01%). Most of the workers were not specialized in one type of work, but were rather changing their activities during a week and/or during a shift. Thus no subgroup of welders, polishers, etc. exposed exclusively to welding fumes or APM could be distinguished.

The control group was formed by 20 men (average age of 45.5 y, 47% of smokers) employed in an agricultural enterprise located about 5 km from the plant producing stainless steel vessels.

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Sampling and sample handling

Both groups of workers were sampled in the middle of the week, prior to the beginning of a work shift, after all persons took a shower. Hair and nail samples were obtained by clipping with stainless steel scissors. The IAEA recommended procedure was employed for hair washing, whereas an adapted procedure described elsewhere was used for nail cleaning. Spot samples of urine of a volume 50 to 100 ml were collected in acid leached polystyrene vials. Blood was obtained from a cubital vein using a Teflon cannula, which was first flushed with 15 ml of blood collected for immunological tests. Then 5 ml of blood was collected in polyethylene (PE) cryogenic vials (Nalgene) which were pre-cleaned as given below. From this volume, 1 to 2 ml were immediately transferred to another Nalgene vial for determination of manganese, while the remaining blood was centrifuged to obtain serum for chromium determination. Serum aliquots of 1 to 1.5 ml were placed into pre-cleaned vials made of synthetic quartz (Suprasil AN, Heraeus). The quartz vials were closed with an acid-leached Teflon stopper. All manipulations on blood sampling and handling were performed in the stream of air obtained from a mobile filtration unit with ULPA filters (Holten) providing a Class 10 environment. The urine, blood and serum samples were deep-frozen prior to further treatment.

All collection vials used for sampling and sample handling were pre-cleaned by leaching in dilute sub-boiled nitric acid for 24 hours and washing with de-ionized water in a clean laboratory providing a Class 100 environment.

Irradiation

For INAA, samples and standards were prepared for irradiation as follows. Hair (75 to 150 mg) and nail (25 to 75 mg) samples were packed in disk shaped polyethylene PE capsules of a 20-mm diameter made by heat sealing of PE acid-leached foils of 0.15 mm thick. Aliquots of 0.5 ml of urine for the determination of Mn and Cr by RNAA were placed in acid-cleaned PE and quartz vials, respectively. Frozen blood samples for the determination of Mn by RNAA were irradiated in the collection PE vials, whereas the serum samples for the determination of Cr by RNAA were freeze-dried in the quartz vials prior to irradiation. Multi- and single element standards for INAA and RNAA, respectively, were prepared in the same geometrical shape as the samples by weighing out 20 to 50 µl aliquots of solutions containing known concentrations of elements. For INAA, this has been achieved by depositing the solutions onto disks of chromatographic paper Whatman, air drying and subsequent heat sealing in the PE disk capsules, whereas for RNAA the standards were made to the sample volume by de-ionized water.

The samples and standards were irradiated in VVR-15 nuclear reactor of the Nuclear Research Institute, Rež, plc at fluence rates of $1 \times 10^{14}$ and $3 \times 10^{13}$ n·cm$^{-2}$·s$^{-1}$ for thermal and fast neutrons, respectively. Short-time irradiation (1 min) in PE capsules and/or vials was carried out in a pneumatic facility with the transport time of 4 seconds. The samples and standards were irradiated in PE “rabbits” individually, together with neutron flux monitors (5 µg of gold prepared as the standards). For long-time irradiation (20 h) in quartz vials, the samples and standards were packed together in an Al irradiation can.

Radiochemical separation

Chromium in urine and serum: After 1 to 2 weeks of decay, the outer surface of quartz vials were cleaned by leaching in hot aqua regia and cooled in liquid nitrogen. After opening of the vials, urine was washed out with 5 to 10 ml of distilled water, while serum was solubilized in 5 ml of fuming nitric acid in a beaker. Radiochemical separation of $^{51}$Cr was carried out using an adapted procedure according to GREENBERG and ZEISLER. To both sample types, 1 ml of a $K_2CrO_4$ solution containing 4 mg of Cr per ml (inactive carrier) and 3 ml of concentrated $H_2SO_4$ were added. Then, the samples were mineralized in a Kjeldahl decomposition flask by repeated additions of 1 ml aliquots of concentrated $HNO_3$ during heating the flask over a gas burner until a clear solution was obtained and white fumes of $H_2SO_4$ appeared. Then, $Cr(III)$ was oxidized to $Cr(VI)$ by addition of 1 ml of concentrated $HClO_4$ and heated till white fumes of $HClO_4$ and subsequent change of the color of the solution from green to orange. After cooling down to laboratory temperature, 1 ml of a 0.05 mol·l$^{-1}$ $KMnO_4$ solution and 25 ml of a 2.5 mol·l$^{-1}$ HCl were added and $Cr(VI)$ was extracted in a separatory funnel for 1 minute by two 10 ml-portions of a 5% (w/v) solution of tribenzylamine (TBA) in chloroform. Combined chloroform fractions were scrubbed with 5 ml of water for 30 seconds (to remove co-extracted $^{65}$Zn) and Cr was stripped into water phase with 5 ml of 3 mol·l$^{-1}$ $NH_4OH$ for 90 seconds. After washing the separatory funnel with 1 ml of water, the 6-ml fractions with separated $^{51}$Cr were placed in plastic vials for counting. The chemical yield of separation that varied in the range of 90–95% was determined by reactivation of 25-µl aliquots of the water phase, which contained 16.66 µg of Cr inactive carrier, i.e., an amount in sufficient excess to Cr present in the samples.

Manganese in urine and blood. An adapted procedure published earlier was employed, which is briefly described as follows. After 1 to 2 minutes of