

TOXICOLOGY

Nephrotoxicity effects of the wood preservative chromium copper arsenate on mice: Histopathological and quantitative approaches

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Abstract

Chromium copper arsenate (CCA) was used for the protection of wood building materials until the restriction by EPA in 2002.

During a short period of time 14–24 h, a comparative nephrotoxicity study was performed regarding the effects of CCA and its compounds *per se*. Histopathological and histochemical features were correlated with the concentration of the total arsenic and chromium in mice kidney.

Animals were subcutaneously injected with CCA (7.2 mg/kg arsenic and 10.2 mg/kg chromium *per body weight*), CrO₃ (10.2 mg/kg), As₂O₅ (7.2 mg/kg) and NaCl (0.9%) *per se*.

The histopathological examination of the renal sections evidenced acute tubular necrosis in the groups of animals exposed to CCA (in both periods of time).

Although the same contents of pentavalent arsenic and hexavalent chromium were injected in treated animals with CCA and with the prepared solutions of As₂O₅ and CrO₃, the arsenic concentration on kidneys of CCA-exposed animals was much higher than those in animals exposed to As₂O₅ (32- and 28-fold higher at 14 and 24 h, respectively). However, the elimination of chromium seems to occur similarly in the kidneys of animals treated with CCA and CrO₃ *per se*. Interactions among the components of CCA result in a marked decrease of the ability of kidney to eliminate simultaneously both analytes. The nephrotoxicity of CCA was higher than its components *per se*, evidencing a possible synergetic effect.

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Introduction

Chromium copper arsenate (CCA) was broadly used as a wood preservative for more than 60 years (e.g. decks, playground facilities) [1,2]. CCA type C was the most applied formulation (47.5% CrO₃, 18.5% CuO and 34% As₂O₅, by weight) [3,4]. Exposure to CCA

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occurs during the managing of treated wood and related equipment [5]. Workers directly involved in CCA-treated material may be exposed to high levels of CCA through direct dermal contact, inhalation of aerosols or particulates and inadvertent ingestion [6]. Few studies have been conducted on the health effects of CCA. CCA is corrosive to the skin, eyes and digestive tract. People exposed to very high levels of CCA, from sawing wood that still had liquid CCA in it or from living in a home contaminated with ash containing high levels of chromium(VI), arsenic and copper, experienced serious health effects including nosebleeds, digestive system pain and bleeding, itching skin, darkened urine, nervous system effects such as tingling or numbness of the hands and feet and confusion, and rashes or thickening and peeling of the skin [6]. Consequently, concerns have been raised owing to the high levels of arsenic and chromium concentrations in treated wood, due to the potential human contact in occupational environments and to the ecological exposure [6–9].

The nephrotoxicity of metals and metalloids is well described, chromate and arsenate being examples of chemicals that adversely affect kidneys [10–13].

Mason and Edwards [14] assessed the nephrotoxicity of $\text{Na}_2\text{Cr}_2\text{O}_7$, Na_3AsO_4 and CuSO_4 , on rats, concluding that altogether these compounds may show a greater acute toxicity risk than its components *per se*. More recently, after summarizing different studies involving toxicity of CCA-treated wood, Katz and Salem [15] concluded that more research is needed in order to characterize the chemistry and the toxicology of these treated building timbers.

Owing to the possible deleterious effects of CCA-treated wood on public health, it was freely phased-out from most residential applications [16]. However, due to its durability and leach resistance, CCA will remain in previously constructed structures and surrounding environments [4,17].

To clarify the potential hazard of CCA-treated wood building products, the nephrotoxicity of CCA and its constituents *per se* was evaluated using mice. To our knowledge no toxicity studies involving the administration of CCA in animal models are available.

Materials and methods

Chemicals and reagents

A CCA commercial solution was purchased in a local wood industry. A 200-fold dilution was then prepared and its pH was adjusted to 7. The content of this final solution was 1034 $\mu\text{g/L}$ of total chromium, 721 $\mu\text{g/L}$ of total arsenic and 3 $\mu\text{g/L}$ of total copper, as determined and confirmed by two independent techniques, namely

inductively coupled plasma mass spectroscopy (ICP-MS) and flame atomic absorption spectrometry (AAS). Two other solutions of As_2O_5 and CrO_3 (analytical reagent grade; Merck, Darmstadt, Germany) containing 721 $\mu\text{g/L}$ of arsenic and 1034 $\mu\text{g/L}$ of chromium were also prepared.

A Milli-Q water purification system (Millipore, Molsheim, France) was used to get ultrapure water (18.2 M Ω resistivity) for quantitative analysis.

China National Analysis Center for Iron and Steel (Beijing, China) supplied the standard reference material NCSZC 71001 beef liver.

For preparation of standards and digestion of kidneys, suprapur 65% concentrated nitric acid was used (Merck, Darmstadt, Germany).

Two standard solutions of arsenic and chromium (1000 mg/L each) (Riedel-de Haën, Seelze, Germany) were used to prepare daily solutions for analysis by graphite furnace atomic absorption spectrometry (GFAAS).

Animal treatment

Two-months-old ICR-CD1 male mice (26–40 g) purchased from Harlan Interfauna Ibérica S.A. (Barcelona, Spain), and set aside under controlled conditions (temperature at $22 \pm 2^\circ\text{C}$, relative humidity 40–60%, 12 h light–dark cycle), were housed (five *per* group) in stainless steel cages. Food and water were supplied *ad libitum*. Before experimental use, animals were allowed to acclimatize for one week. Mice were separated into eight groups corresponding to different times of exposure until sacrifice. Control animals were subcutaneously administrated with the vehicle (0.3 mL of 0.9% NaCl). Exposed groups received a single subcutaneous injection (0.3 mL) of As_2O_5 , CrO_3 or CCA *per se*. Considering the very low level of total copper in the pH-adjusted CCA solution (3 $\mu\text{g/L}$; less than 0.4% and 0.3% of the arsenic and chromium concentrations, respectively) and its toxicological and carcinogenic properties, no group was exposed to CuO.

After periods of 14 and 24 h, animals were sacrificed for kidney removal and subsequent analysis.

Body and kidney weight

Subsequent to 14 and 24 h of exposure, body and kidney weights were recorded. Relative organ weight ratio (organ weight per body weight $\times 100$) of each animal was calculated.

Histopathological examination

The right kidney from all groups of animals was fixed in Bouin's solution, dehydrated and embedded in

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