# Tissue Distribution of Copper in Mice Following Exposure to Arsenic in Drinking Water

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***Abstract*—Arsenic (As) is a common and conspicuous toxicant. The tissue distribution and correlation between tissues for copper was assessed in male C57Bl/6 mice exposed to 0, 50, 500, or 5000 ppb arsenic in their drinking water for 3 weeks. The copper concentration in blood increased linearly with increasing arsenic doses, whereas the copper concentration in liver or kidney decreased linearly with respect to the dose applied. These results indicate that there may be tissue difference in the disturbed levels of copper in the mice blood, liver, and kidney by subchronic exposure to arsenic in drinking water.**

***Index Terms*—arsenic, copper, drinking water, mice**

## I. Introduction

Arsenic (As) is a ubiquitous metalloid found mainly in food, soil, and ground water. Because As in soil or rock is dissolved easily into surrounding water, inorganic As is frequently present at elevated concentrations in ground water [1]-[3]. Consequently, more than one hundred million individuals who drink ground water are at risk of elevated As exposure via drinking water [4].

Inorganic As in drinking water is known to be a potent human carcinogen, a causal factor of cancers in skin, urinary bladder, kidney, lung, and liver [1]. In addition, chronic exposure to As through drinking water is associated with an increased risk of several non-cancer diseases that affect many organs, including the blood circulatory system, liver, and kidney [2], [5].

The mechanisms underlying As toxicity and/or carcinogenicity remain unclear. However, one study has demonstrated that the relationship between toxic metal exposure and tissue concentrations of essential metals over prolonged exposures could be an important factor for chronic toxicities, which were mediated by a disturbance in the distribution of essential metals, such as copper (Cu), rather than the exposed metal alone [6].

Cu is an essential nutrient required for the activities of important enzymes such as cytochrome c oxidase and lysyl oxidase, whereas the reactive nature of Cu ions can also cause cellular damage as a result of free radical generation [7]. Therefore, tissue Cu concentrations and distribution in the body are strictly regulated to ensure an adequate supply of Cu to cuproenzymes and the removal of excess Cu. The importance of Cu homeostasis is demonstrated by the severity of genetic disorders that affect Cu metabolism, such as Menkes disease and Wilson disease [8].

To explore the mechanism of As toxicity concerning Cu homeostasis, we investigated whether subacute exposure to As in drinking water may change the concentrations of Cu in blood, liver and kidney, as well as the correlation of Cu concentrations among these organs.

## II. Materials and Methods

BALB/c mice (n = 40, 7-week-old males weighing 24.3 ± 1.0 g) were divided into four groups: saline-treated control and 50, 500, and 5000 ppb As-treated groups. Mice (n = 10 per group) groups received 0, 50, 500 or 5000 ppb As in the form of $NaAsO_2$ in their drinking water for 3 weeks. Control animals were given distilled deionized water only. Water consumption was measured throughout the study period, and the mice were weighed weekly. Mice and $NaAsO_2$ were purchased from Samtako Laboratories (Osan, Korea) and Wako Chemical Co. (Osaka, Japan), respectively. Other reagents were of the highest quality available and were obtained from commercial sources. Experiments were approved by the Institutional Animal Care and Use Committee of Keimyung University, Korea. Experiments were conducted according to the NIH guidelines for the care and use of laboratory animals. Animals were housed in a specific pathogen-free (SPF) facility, with free access to food and water. At the end of the treatment regimen, mice were sacrificed by $CO_2$ asphyxiation, and blood, liver, and kidney were harvested immediately. Tissue samples were placed immediately into cryovials and stored at –70 ℃ until use.

Once ready for analysis, the tissue samples were thawed, and about 0.5 ml of blood or 50 mg of dry tissue was digested with 10 ml $HNO_3$ (70%) in a microwave digestion system. The Cu content was determined by inductively coupled plasma-mass spectrometry (Elan 900;

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Perkin-Elmer Co., Norwalk, USA). The analytical performance is assessed periodically through participation in the Korea Food and Drug Administration's proficiency testing program for trace elements in whole blood and tissue. Internal quality control (IQC) materials covering the expected range of concentrations were analyzed at the beginning and end of each batch of specimens and throughout each analytical run (Table I).

TABLE I. ANALYSIS CONDITION OF ICP-MS

| Description | Value | Step value | Setting time(sec) |
|---|---|---|---|
| Nebulizer gas flow | 0.85 | 0.01 | 10 |
| Lens voltage | 5.75 | 0.25 | 0 |
| ICP rF power | 1300 | 25 | 15 |
| Analog stage voltage | -2200 | -100 | 2 |
| Pulse stage voltage | 1150 | 50 | 2 |
| Discriminator threshold | 70 | 5 | 2 |
| AC rod offset | -2 | 0.5 | 0 |

The Cu concentrations in the tissues of As-treated animals were compared to those of the controls using a one-way analysis of variance followed by a post hoc Duncan test. Statistical significance was defined as $P < 0.05$ or $P < 0.01$. Mean values were calculated, along with the standard error (SE) for each mean value. All statistical analyses were conducted using SAS 9.1 software (SAS Institute Inc., Cary, USA).

## III. RESULTS

No signs of overt toxicity were observed in the mice throughout the duration of the study. Although the body weights were slightly lower at the time of terminal necropsy due to overnight fasting, body weight gain was not significantly affected by As exposure (Fig. 1). The water intake did not differ significantly among the treatment groups. The calculated average daily As intakes determined weekly and averaged over the entire study period were 10.5 ± 0.9, 89.2 ± 13.9, and 1039.4 ± 171.6 mg sodium arsenite/kg body weight/day for the 50, 500, and 5000 ppb groups, respectively.

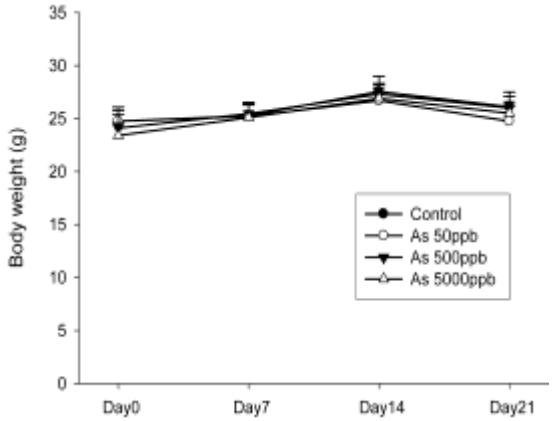


Figure 1. Average weekly body weight during 3 weeks of exposure to As in drinking water. No significant differences were observed in body weight gain among exposure groups. Values are the mean ± SD in each group (n = 10).

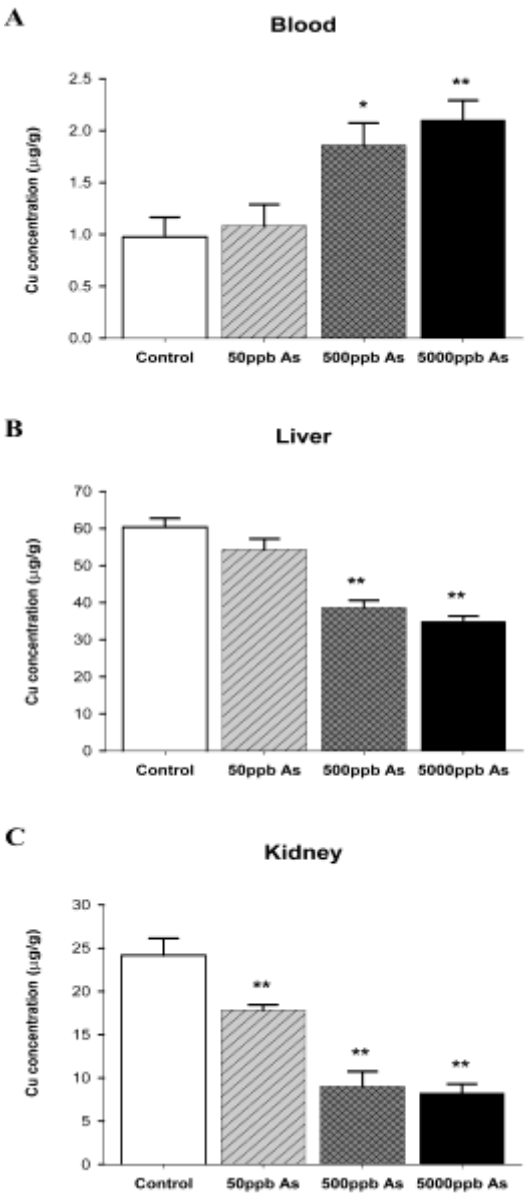


Figure 2. Concentration of Cu in blood (A), liver (B), and kidney (C) after 3 weeks of exposure to 50, 500, and 5000 ppb As in drinking water. Values are the mean ± SE in each group (n = 10). *P < 0.05, **P < 0.01, compared to the control group.

Concentration data for the Cu detected in whole blood, liver, and kidney at the end of 3 weeks of drinking water exposure to As were above the detection limits and are presented in Fig. 2. Cu concentrations were the lowest in blood compared to liver or kidney (Fig. 2A). In blood, the Cu concentration increased dose-dependently with increasing As treatment concentration and was significantly elevated in the 500 ppb ($P < 0.05$) and 5000 ppb ($P < 0.01$) groups compared to the control. The mean blood Cu concentrations at terminal sacrifice in the control, 50, 500, and 5000 ppb As group were 0.98, 1.08, 1.86, and 2.10 μg/g, respectively. Contrary to the Cu level in blood, the Cu levels in liver and kidney indicated a dose-dependent decrease with increasing As treatment concentrations. Especially, Cu levels in liver of the 500 and 5000 ppb As-treated groups, and those in kidney of the 50, 500, and 5000 ppb As-treated groups showed

statistically significant decreases compared to the control ($P < 0.01$). The mean liver Cu concentrations at terminal sacrifice in the control, 50, 500, and 5000 ppb As group were 60.37, 54.31, 38.63, and 34.90 μg/g, respectively, and, in kidney, those groups were 24.17, 17.83, 9.00, and 8.24 μg/g respectively.

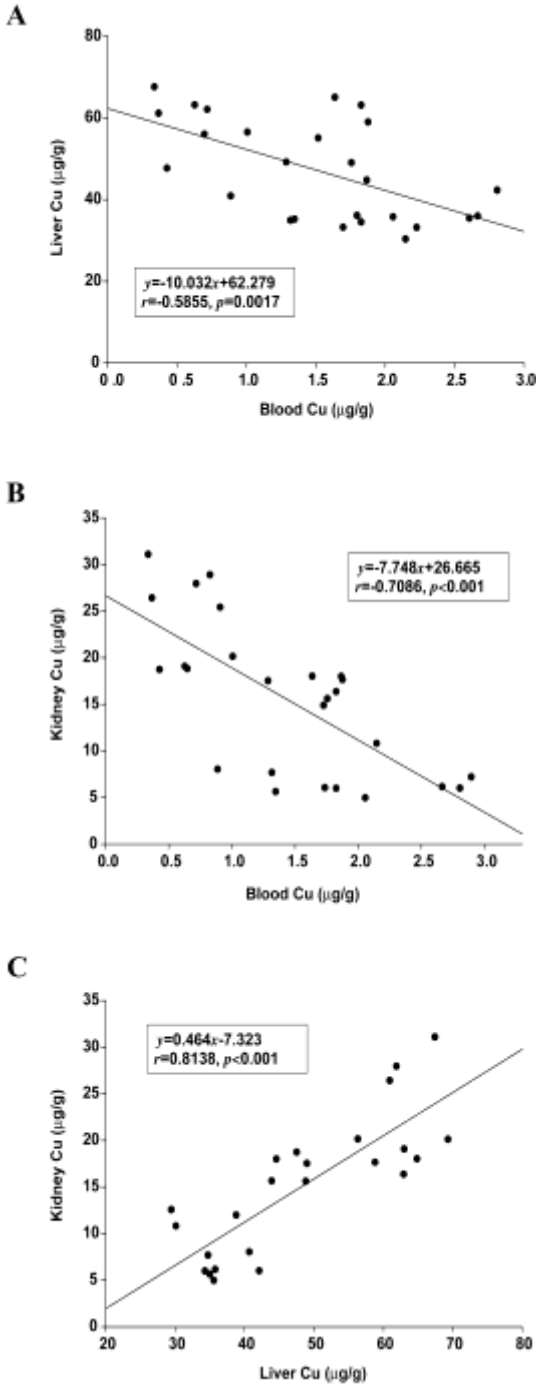


Figure 3. Correlations between liver Cu and blood Cu (A), between kidney Cu and blood Cu (B), and between kidney Cu and liver Cu (C). Data were analyzed by linear correlation.

A linear regression analysis was used to examine whether the Cu concentrations were correlated among blood, liver, and kidney. When blood concentrations of Cu were plotted against liver or kidney concentrations of Cu, significant inverse correlations were observed between blood and liver (correlation coefficient (r) = –0.5855, P = 0.0017) and between blood and kidney (r = –0.7086, P < 0.001) (Fig. 3A and Fig. 3B). However, the Cu concentrations between liver and kidney exhibited a significant positive association (r = 0.8138, P < 0.001) (Fig. 3C).

## IV. Discussion

This study reports Cu accumulation in blood, liver, and kidney across a wide dose range under conditions of subchronic exposure to As in drinking water. Our results clearly demonstrate that the distribution and accumulation of Cu is both tissue-specific and dose-dependent, and it is correlated among tissues. It is known that the toxic chemicals can interfere with essential trace elements and the interactions between toxic metals and essential trace elements may result in disturbances in the homeostasis of essential trace elements, such as Cu [9].

Physiological balance among trace elements is essential for human health and is controlled by metal-binding proteins, which comprise one third of all mammalian proteins [10]. Metallothioneins (MTs) are one of the major inducible metal-binding proteins involved in homeostasis and the detoxification of metals [11]. MT binds preferentially to cadmium and zinc, but it also has the capacity to bind As and Cu [12]-[14]. Moreover, As is known to be an inducer of MTs [15]. Consequently, excess levels of As can compete or interfere with essential elements, including Cu [16].

Cu is an integral component of various cuproenzymes, including cytochrome C oxidase, lysyl oxidase, superoxide dismutase (SOD), dopamine β-oxidase, tyrosinase and ceruloplasmin [17]. The biological functions of Cu include electrontransfer catalysis by means of its two accessible oxidation states [18]. Likewise, Cu plays a key role in a number of essential reactions in human.

Therefore, these results suggest that As-induced toxicity may be induced in part by the disturbance of Cu homeostasis. However, further studies are needed to fully address the effects of As on the tissue distribution of Cu and whether changes in Cu concentration in tissues are a primary or secondary effect of As treatment. Moreover, it is also necessary to further understand mechanisms that As interacts with essential trace elements in specific tissue and induces a differential distribution among tissues. The present findings provide a foundation for the future study of trace elements interaction to enhance the understanding of metal toxicity.

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