Published in final edited form as:

Chemosphere. 2013 May ; 91(8): 1082–1087. doi:10.1016/j.chemosphere.2013.01.005.

Arsenic toxicity in the human nerve cell line SK-N-SH in the presence of chromium and copper

LIGANG HU^a, JUSTIN B. GREER^b, HELENA SOLO-GABRIELE^c, LYNNE A. FIEBER^{b,*}, and YONG CAI^{a,d,*}

LIGANG HU: liganghu@hku.hk; JUSTIN B. GREER: jgreer@rsmas.miami.edu; HELENA SOLO-GABRIELE: hmsolo@miami.edu

^aDepartment of Chemistry & Biochemistry, Florida International University, 11200 SW 8th St, Miami, Florida, 33199, USA

^bDivision of Marine Biology and Fisheries, Rosenstiel School of Marine & Atmospheric Science, University of Miami, Florida, 33149, USA

^cDepartment of Civil, Architectural and Environmental Engineering, University of Miami, Coral Gable, Florida, 33124, USA

^dSoutheast Environmental Research Center, Florida International University, Miami, Florida, 33199, USA

Abstract

As, Cr, and Cu represent one potential combination of multiple metals/metalloids exposures since these three elements are simultaneously leached from chromated copper arsenate (CCA)-treated wood, a common product used for building construction, at levels that can be potentially harmful. This study investigated the neurotoxicity of As associated with CCA-treated wood when accompanied by Cr and Cu. The toxicity was evaluated on basis of a cytotoxicity model using human neuroblastoma cell line SK-N-SH. The cells were cultured with CCA-treated wood leachates or with solutions containing arsenate [As(V)], divalent copper [Cu(II)], trivalent chromium [Cr(III)] alone or in different combinations of the three elements. The toxicity was evaluated using variations in cell replication compared to controls after 96 hrs exposure. Among the three elements present in wood leachates, As played the primary role in the observed toxic effects, which exerted through multiple pathways, including the generation of oxidative stress. DOM affected the absorption of metals/metalloids into the test cells, which however did not obviously appear to impact toxicity. As toxicity was enhanced by Cu(II) and inhibited by Cr(III) at concentrations below U.S. EPA's allowable maximum contaminant levels in drinking waters. Thus assessing As toxicity in real environments is not sufficient if based solely on the result from As.

Keywords

Arsenic; copper; chromium; neurotoxicity; combined toxic effect

© 2013 Elsevier Ltd. All rights reserved.

CORRESPONDING AUTHOR: LYNNE A. FIEBER: Tel: 1-305-421-4906, Fax: 1-305-421-4600, lfieber@rsmas.miami.edu. YONG CAI: Tel: 1-305-348-6210, Fax: 1-305-348-3772, cai@fiu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Health problems associated with arsenic (As) exposure are currently of great concern worldwide. In addition to acute toxicity, impact of long-term and low dose As exposure through drinking water has received much attention. Skin, bladder, and lung cancers have been observed in populations ingesting As-contaminated drinking water (WHO, 2003). Both the International Agency for Research on Cancer (IARC) and the U.S. Environmental Protection Agency (U.S. EPA) have categorized As as a class one human carcinogen (IARC, 2007; U.S.EPA, 2009a). In addition to its carcinogenic effects, As exposure can also have neurological consequences for humans (Blom et al., 1985; Gerr et al., 2000; Hafeman et al., 2005), with peripheral neuropathy the most frequent consequence of As exposure (Rahman et al., 2001; Rodriguez et al., 2003). Affected patients show significantly lower nerve conduction velocities, often resulting in irreversible nerve damage. Central nervous system effects of As toxicity have been suggested on the basis of results obtained from both epidemiological and animal model studies (Wright and Baccarelli, 2007). Depression, irritability, anxiety, sleeping difficulty, memory loss, headache and inability to concentrate were common complaints observed in arsenicosis patients (Rahman et al., 2001). Long-term consequences, including mental retardation have been reported (Dakeishi et al., 2006). Furthermore, evidence from animal studies showed that As can transport through the placenta from mother mice to their newborn pups, and resulted in learning and behavioral deficits (Jin et al., 2006; Xi et al., 2009).

Neuropsychological effects of As exposure in combination with other metals have been suggested. In a study of association between the hair levels of As, Mn, and Cd and neuropsychological function and behavior in school-aged children in U.S.A., it was observed that the low intelligence scores was most prominent among children in whom hair levels of both Mn and As were greater than the median values in the sample (Wright et al., 2006). Results based on animal models also showed the combined neurotoxicity of As/Cd (Rodriguez et al., 1998) and As/Pd (Mejia et al., 1997). In addition, nutritional factors have been reported to affect the neurotoxic effects of As in humans (Calderon et al., 2001; Kapaj et al., 2006).

Release of As from chromated copper arsenate (CCA) -treated wood is one of the major anthropogenic sources of As contamination in the US (USGS, 2005). The potential risks of exposure to As associated with CCA-treated wood commanded great attention over the last decade. The wood treatment industry voluntarily withdrew the treated products for most residential settings effective from January 1, 2004, resulting in a substitution with copper-only wood preservatives, in particular alkaline copper quat (ACQ). However, the problem will exist for a long time for its continuous utilization in several areas, such as industrial applications, structures in marine environments and load bearing components of structures in terrestrial environments, and its long service life even for the wood sold for residential and industrial uses prior to 2004 (Hasan et al., 2010). In addition to As, Cr and Cu are also simultaneously leached from CCA-treated wood at levels potentially harmful to aquatic organisms (Weis and Weis, 2006). Therefore, exposure associated with CCA-treated wood should in reality be a coexposure of As, Cr, and Cu as opposed to As alone model. Our understanding of the toxicity of these elements in natural leachate associated with CCA-treated wood is limited. Previous results from animal models have shown that co-administration of the chemicals in the CCA formula (sodium dichromate, sodium arsenate, and copper sulphate) resulted in enhanced toxicity in comparison to any of these chemicals alone (Mason and Edwards, 1989). Assessment of the toxicity of CCA-treated wood in humans is limited by the lack of understanding of the toxic effects of combined and individual components of such leachates, yet differences in toxicity between exposure to all three elements in wood leachates together and to As alone can be expected. Unfortunately,

previous studies evaluating impact of CCA-treated wood focused mainly on As (U.S.CPSC, 2003) because of its highest toxicity to humans among the three elements. U.S. EPA has recently issued a statement on Cr(VI) in drinking water, requiring water systems to test for it, in response to a study released on December 20, 2010 by an Environmental Working Group (U.S.EPA, 2010). This action reflects the significance of assessing the health effects of Cr. Excluding Cr and Cu in the toxicity studies could result in inaccurate estimation of the potential risks associated with CCA-treated wood.

The purpose of this study was to investigate As neurotoxicity with the coexistence of Cr and Cu based on a cytotoxicity model using the human neuroblastoma cell line SK-N-SH used for cell mediated cytotoxicity assays and understood to be a reasonable proxy for a neural clonal cell line due to the high expression of neural markers (Biedler et al., 1973). As(V), Cr(III), and Cu(II) were selected for this study since they are the major elemental species present in the CCA-treated wood leachates (Khan et al., 2006b; Song et al., 2006). The cell line SK-N-SH grown in the presence of wood leachates or of synthetic mixtures containing each element alone or different combinations of the three elements. The toxicity was evaluated by observing the effects on proliferation and cell survival after 96 hrs exposure. Since dissolved organic matter (DOM) present in the wood leachates may significantly affect As bioavailability and species transformation of As, the effects of DOM on uptake and toxicity was investigated. Dimethyl sulfoxide (DMSO) was used to verify if the toxic effects of As associated with CCA-treated wood is related to cellular redox potential homeostasis.

2. Materials and Methods

2.1 Cell lines and Chemicals

Sodium arsenate heptahydrate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$, 99%), DMSO, poly-l-lysine, trypsin and ethylenediaminetetraacetic disodium dehydrate (EDTA, 99%) were purchased from Sigma-Aldrich. Chromium chloride hexahydrate ($\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$, 98%), cupric sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), hydrogen peroxide (H_2O_2 , 30%) and tetrabutylammonium hydroxide (TBAH, 1 M solution in methanol) were purchased from Fisher Scientific. SK-N-SH human neuroblastoma cells were obtained from ATCC (Manassas, VA) and were grown to confluence in complete medium consisting of high glucose (4.5 g/L) Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (Atlanta Biologicals, Atlanta, GA) with 2 mM l-glutamine, 100 U/mL penicillin, and 100 g/mL streptomycin at 37°C in a 5% CO_2 atmosphere. Test solutions of the wood leachates were the run-off rainwater from CCA-treated woods. The details of the experimental wood decks and sample preparation can be found in Supplementary Materials.

2.2 Cell Culture

SK-N-SH was plated on poly-l-lysine-coated cell culture vessels in complete medium. For evaluating the effects of different concentrations of evaporated leachates and standard solutions of As, Cr, Cu and their mixture, triplicate cell cultures were plated at a concentration of $\sim 5 \times 10^5$ cells, in 25 cm^2 vented-cap flasks (T25) in 5 ml of complete medium. Initial cell counts, T_0 , were measured 24 hours after plating. The cells in 3 or 5, 1.08 mm^2 fields were counted under phase contrast and averaged to obtain the average number of cells in a dish. All cells in an experiment came from the same tissue culture passage. Cells were then exposed to test solutions in triplicate cultures. Other chemicals, including metal/metalloid standards, DMSO, and DOM, were added after the T_0 cell counting. At the conclusion of the 96 hour exposure, final cell counts, T_f were made. In experiments in which cell concentrations of metals/metalloids were assessed, cells were harvested by trypsinization, washed three times with cold PBS, and then refrigerated in PBS.

at 4 °C before further processing. Aliquots of culture medium (T_0 and spent media, T_f) were analyzed for metals to confirm all exposure levels. Normalized cell proliferation, calculated as $(T_f/T_0)/(T_{fc}/T_{0c})$, where T_f and T_0 are the final and initial cell counts in test media, and T_{fc} and T_{0c} are the final and initial cell counts in controls, respectively.

3. Results

The concentrations of As, Cr and Cu in evaporated, reduced volume rainwater leachates from July-October 2007, for example plotted in Fig. S1, varied together for all decks except the ACQ deck. The ACQ deck was treated only with Cu and thus the As and Cr concentrations in leachates from this deck were nominal. Leachates containing sub- μM total As, Cr or Cu in complete media had positive or no growth effects on SK-N-SH cells. Increase in concentrations of these three metals/metalloids reduced the capability of this cell line to proliferate, and at very high concentrations, killed all of the cells (data not shown). Leachates from the ACQ deck did not kill cells in the range of Cu concentrations found in leachate from the other decks (Fig S2).

3.1 Effects of As on cell proliferation

Cell proliferation was inhibited in As alone and also in leachates containing As with Cr and Cu (Fig. 1). The effect on normalized cell proliferation $(T_f/T_0)/(T_{fc}/T_{0c})$ could be fitted by Boltzmann distributions with similar slopes but different EC_{50} 's. In As standard solution, proliferation was inhibited at $3 \mu\text{M}$, with 100% cell death observed by $100 \mu\text{M}$. The calculated EC_{50} values for As in wood leachates and in As standard solution, 10.7 ± 2.0 and $21.1 \pm 1.1 \mu\text{M}$, respectively, were significantly different. This result suggested that the inhibiting effect of As on cell proliferation and survival was greater in wood leachates containing Cr and Cu. It should be pointed out that the cell numbers still increased and cells appeared healthy even though cell proliferation was obviously inhibited compared with the controls in both wood leachates and As standard solution when As concentrations were less than 24 and $30 \mu\text{M}$ in wood leachates and As(V) standard, respectively (roughly estimated from Fig. 1).

The total concentration of As in cells increased with the concentrations of As in both wood leachate and in As standard solutions (Fig. S3). The logarithmic forms of the As concentration in cells against that in media fitted well to linear regressions (straight lines A and B in Fig. S3), with $Y = 1.37 \cdot X - 2.47$ (for As alone, $R^2 = 0.91$) and $Y = 0.88 \cdot X - 6.03$ (for wood leachates, $R^2 = 0.40$). The differences in slopes of the two regression lines were not significant, whereas the difference in intercepts was statistically significant.

3.2 Effects of Cu(II) on cell proliferation

Analysis of the effects of Cu standard on cell proliferation and death (Fig. 2) demonstrated that 0.01 – $100 \mu\text{M}$ Cu had a positive effect on cell proliferation in the SK-N-SH cell line. Suppression of cell proliferation was observed only at $\text{Cu} = 433 \mu\text{M}$, with the EC_{50} at $428 \mu\text{M}$. A significantly different, left-shifted dose response plot was obtained for wood leachate toxicity as a function of Cu concentration (apparent EC_{50} at $1.5 \mu\text{M}$, $p < 0.05$), suggesting that leachate Cu is not driving the toxicity of wood leachates.

Intracellular Cu concentrations increased with the concentration of Cu in both wood leachate and in Cu(II) standard solution (Fig. S4). Similar to As, log cell Cu against log media Cu relationships were fitted to linear regressions whose slopes and intercepts were not significantly different. (A: for Cu alone, $Y = 0.75 \cdot X - 6.20$, $R^2 = 0.72$; B: for wood leachate, $Y = 1.06 \cdot X - 4.74$, $R^2 = 0.60$)

3.3 Effects of Cr(III) on cell proliferation

Cr(III) present in standard solution had positive effects on cell proliferation (Fig. 3) at Cr concentration less than 1.2 mM. Complete cell loss occurred at 21.4 mM, with the EC_{50} at 10 mM. Cr(III) toxicity may not be solely due to the presence of the metal, but to low pH of the culture solution containing Cr that dropped from 8.4 to 6.4 after addition of 21.4 mM Cr(III). Changes in pH values occurred only for trials with 21.4 mM Cr. When cell proliferation was plotted against the Cr concentration in wood leachate a sharp leftward shift of the dose response was noted, similar to the case when plotting the effect of leachate as a function of Cu concentration. Cell proliferation in experimental flasks began to reduce sharply at 1.0 μ M and cells were killed at tens of μ M Cr(III) (EC_{50} 1.1 μ M).

In order to evaluate the effects of Cr-containing wood leachate without the confounding effects of high As and Cu, experiments were conducted in which Cr(III) was spiked into the reference wood leachate collected from an untreated wood deck in the field. The wood leachate contained 22.3 mg/L TOC (similar to the CCA-treated leachate), 0.007 μ M As, 0.008 μ M Cr, and 0.071 μ M Cu. Compared with the result of CCA-treated wood leachates, cell proliferation was not clearly affected by Cr present in the reference wood leachate at Cr less than 1000 μ M (Fig. 3, star symbols and dotted line), suggesting that cell toxicity of CCA-treated wood leachates was not due to Cr.

Intracellular Cr concentrations increased with Cr concentration in Cr(III) standard solution, wood leachates, and Cr(III) spiked in the reference wood leachate. Cr uptake in cells was significantly faster in wood leachates and Cr(III) spiked in the reference wood leachate than in Cr(III) standard solution. Regression lines for wood leachate and Cr(III) spiked in the reference wood leachate were not significantly different in either slope and intercept, while both lines were significantly different from the slope of the Cr(III) standard solution. These results indicate that the matrix generated from wood leachate significantly enhanced Cr(III) uptake [Fig. S5, A: Cr(III) standard solution, $Y = 0.17 \cdot X - 8.84$, $R^2 = 0.45$; B: wood leachate, $Y = 0.70 \cdot X - 5.88$, $R^2 = 0.51$; and C: Cr(III) spiked in the reference wood leachate, $Y = 0.78 \cdot X - 5.43$, $R^2 = 0.80$].

Matrix experiments, where the leachate was replaced by different sources of DOM, showed similar effects between the reference wood leachates collected from the field and wood extract prepared in the laboratory or commercially available humic acid (Data not shown). Spiking the reference wood leachate greatly enhanced Cr uptake in cell (Fig. S5). However, the resulting inhibition of cell proliferation was minor in comparison with wood leachates (Fig. 3).

3.4 Combined effect of As(V), Cr(III), and Cu(II) on cell proliferation

Experiments employing different combinations of As(V), Cr(III), Cu(II) in standard solutions utilized an As(V) concentration of 15 μ M, which fell in the most sensitive range of As concentration affecting cell proliferation (Fig. 1). Added Cr(III) and Cu(II) were 30 and 15 μ M, respectively, corresponding to the average concentrations of Cr and Cu in all wood leachates collected from the field experiment. As shown in Fig. 4, the presence of As in solutions of any combinations synergistically hindered the cell proliferation compared to those without As, i.e. the controls and the combinations of Cu(II) + Cr(III). Combining Cr(III) with Cu(II) did not produce significant harmful effects on the cells. The presence of Cu(II) in the solutions of As(V) or As(V) + Cr(III) was found to significantly decrease the cell proliferation compared with As(V) alone or As(V) + Cr(III) (Fig. 4A). In contrast, the presence of Cr(III) significantly reduced the toxic effects of As(V) on the cells (Fig. 4B). Although the effect of Cr(III) on the toxicity of As(V) + Cu(II) combination was not

significantly different, the average cell proliferation when Cr(III) was absent was nearly half of that when Cr(III) was present [As(V) + Cu(II), 27%; As(V) + Cu(II) + Cr(III), 49%].

DMSO, as a widely used reactive oxygen species (ROS) scavenger, can eliminate oxidative stress effect on cell proliferation. Experiments with DMSO, designed to help in elucidating the mechanism of As toxicity and the combined toxic effects of As and Cu, showed that DMSO at 100 mM levels did not affect cell proliferation (Fig. 5A). The effect of As (15 μ M) on cell proliferation was significantly less with the addition of DMSO. DMSO also significantly reduced the deleterious effects of the As/Cu combination and of wood leachates (Fig. 5B).

4. Discussion

At the range of concentrations of As, Cr and Cu in wood deck leachates, the main effect on the tumorigenic cell line SK-N-SH was to slow the rate of proliferation in cultures over 96 hr rather than acute toxic effects which would lead to outright cell death. These chronic effects were dramatic with proliferation reduced to around 20% of controls when As concentration was less than 30 μ M in wood leachates. As is likely the main toxic component of leachates due to the close agreement in slope and EC₅₀ between the dose-response fits of leachate and standard As solutions (EC₅₀ of 10.7 and 21.1 μ M, respectively) on cell proliferation. EC₅₀ for Cr at (10 mM) and for Cu at (428 μ M) in standard solutions, were far outside the range found in CCA leachates (0.5–8.0 μ M and 0.5–10.0 μ M, respectively (Hasan et al., 2010)), suggesting these metals may not exert noteworthy individual toxic effects on human cells at the concentrations encountered in leachates. The typical reverse “S” shape curve of toxic effect when cell proliferation was plotted against Cu or Cr in wood leachates could be explained by that concentrations of Cu and Cr in wood leachates were positively correlated with concentration of As in wood leachates as indicated in the field wood deck leaching experiment (Hasan et al., 2010). In other words, the reverse “S” shape proliferation curve actually reflected the toxicity of As other than Cu or Cr.

Previous studies (Khan et al., 2006a; Khan et al., 2006b) on the leachability of As associated with the CCA-treated wood showed that the concentration of As in the wood leachates were mostly below 65 μ M, suggesting that the release of As from CCA-treated wood could pose subtoxic and chronic effects. Several mechanisms of chronic toxicity related to As exposure, including generation of oxidative stress, interference with DNA repair, and perturbation of signal transduction pathways, have been suggested (Schoen et al., 2004). As shown in Fig. 5, toxicity of As(V) could be partially reduced with the presence of DMSO, which could eliminate the intracellular reactive oxidative species. These results imply that As(V) could possibly exert toxicity on SK-N-SH partially through increasing oxidative stress. Noticeably, the presence of DMSO could not totally eliminate the toxicity of As(V), suggesting that pathways other than the increase in oxidative stress by As may also be involved.

The uptake rate of As in wood leachates was actually lower than As alone. As shown in Fig. S3, the two lines have similar slopes but different intercepts, suggesting that the bioavailability of the As in wood leachates could be lower than that for As standard. Since As could significantly bind to DOM (Chen et al., 2006) and wood leachates contained quite high concentrations of DOM, it is plausible that the lower bioavailability of As in wood leachates might be a result of the reduction of As free ions due to binding with DOM. However, higher toxicities were observed when exposed to wood leachates than when exposed to As standards, implying that additional factors other than As could contribute to the observed toxicity. Although the uptake rate of Cr was significantly increased by the presence of wood leachates (Fig S5.), no dramatically harmful effects on cell proliferation

was observed with the increased intracellular Cr concentration. Similarly, Cu present in the wood leachates did not show harmful effects on cell proliferation. The results from the experiments with different combinations of the three elements clearly demonstrated that the synergistic effects among the different metals/metalloids. In the presence of Cu(II), the toxic effect of As(V) on cell proliferation was enhanced. Although an essential trace element for most living organisms, Cu, when present in excess level, can mediate the production of oxidative stress and direct oxidation of lipids and proteins, and result in DNA damage. (Rana, 2008) DNA damage normally might be repaired by cellular defense system even after taking up exogenous Cu. (Noussipiel, 2009) However, As could inhibit DNA repair, (Rossman et al., 2002) when cells are exposed to As and Cu simultaneously. The experiment with DMSO seems to support the above hypothesis. The presence of DMSO significantly reduced the toxic effect of the mixture of As(V) and Cu(II) in standard solutions, as well as the toxic effect of wood leachates (Fig. 5B).

The concentration of Cu(II) in the As and Cu mixed standard for combined effect experiments was 15 μ M, which was below U.S. EPA' maximum contamination level of Cu in drinking water (MCL = 1300 ng/mL or 20.5 μ M). (U.S.EPA, 2009b) In other words, Cu, at levels that is considered safe, could enhance the toxicity of As, suggesting that assessing As toxicity in real environments is not sufficient if based solely on the result from As. Contrary to Cu(II), Cr(III) provides protection from As toxicity. It has been reported in previous studies that incubating cells with Cr(III) can prevent intracellular glutathione depletion. (Jain and Rogier, 2003) This is beneficial in maintaining oxidative stress homeostasis and therefore could reduce the risk of As induced oxidative stress. In addition, since glutathione could affect As intracellular metabolism, effect of Cr(III) on intracellular glutathione may subsequently alter As toxicity. (Vahidnia et al., 2007) Similar to Cu(II), the effect of Cr(III) on the toxicity of As could also be observed at concentrations of Cr(III) below the MCL. DOM in wood leachates increased cell uptake of Cr(III), as evidenced for the test of combination of Cr(III) and reference wood leachates (Fig. S5). The differences in slopes of the uptake curves for Cr(III) with and without DOM might imply the existence of different uptake pathways. This is different than for As where differences in intercepts were observed (Fig. S3), suggesting bioavailability was likely altered by the presence of DOM. While Cr(III) has been accepted as a nutrient and widely used in human health supplements for a long time, the biochemical roles in the cell are still under debate. (Nielsen, 2007) Cr(III) has shown the potential to react with DNA in vitro especially at high concentrations. (Eastmond et al., 2008) The enhancing effect of DOM on Cr(III) uptake suggests an increase in harmful potential; however, no obvious harmful effects were observed in our experiments.

5. Conclusions

Human nerve cell line SK-N-SH cell proliferation in wood leachates was inhibited due principally to As, among the three elements present in the CCA-treated wood leachates. The generation of oxidative stress is likely one of the pathways for exertion of toxic effects by As. Cu or Cr at the concentration levels observed in environmental samples had no effect on cell health and cell proliferation; however, As toxicity was enhanced by Cu and inhibited by Cr at concentration below U.S. EPA' maximum contamination level in drinking water. When these elements coexist in the environment, such as the case in CCA-treated wood leachates, As toxicity could be over or under estimated when based solely on the results of As.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was partially supported by NIEHS ARCH (S11 ES11181) and NIH-MBRS (3 S06 GM008205-20S1) programs. This is contribution # XXX of Southeast Environmental Research Center at FIU.

References

- Biedler JL, Helson L, Spengler BA. Morphology and growth, tumorigenicity, and cytogenetics of human neuroblastoma cells in continuous culture. *Cancer Res.* 1973; 33:2643–2652. [PubMed: 4748425]
- Blom S, Lagerkvist B, Linderholm H. Arsenic Exposure to Smelter Workers - Clinical and Neuropsychological Studies. *Scand J Work Env Hea.* 1985; 11:265–269.
- Calderon J, Navarro ME, Jimenez-Capdeville ME, Santos-Diaz MA, Golden A, Rodriguez-Levy A, Borja-Aburto V, Diaz-Barriga F. Exposure to arsenic and lead and neuropsychological development in Mexican children. *Environ Res.* 2001; 85:69–76. [PubMed: 11161656]
- Chen ZR, Cai Y, Solo-Gabriele H, Snyder GH, Cisar JL. Interactions of arsenic and the dissolved substances derived from turf soils. *Environ Sci Technol.* 2006; 40:4659–4665. [PubMed: 16913121]
- Dakeishi M, Murata K, Grandjean P. Long-term consequences of arsenic poisoning during infancy due to contaminated milk powder. *Environ Health.* 2006; 5:1–7. [PubMed: 16451722]
- Eastmond DA, MacGregor JT, Slesinski RS. Trivalent chromium: assessing the genotoxic risk of an essential trace element and widely used human and animal nutritional supplement. *Crit Rev Toxicol.* 2008; 38:173–190. [PubMed: 18324515]
- Gerr F, Letz R, Ryan PB, Green RC. Neurological effects of environmental exposure to arsenic in dust and soil among humans. *Neurotoxicology.* 2000; 21:475–487. [PubMed: 11022857]
- Hafeman DM, Ahsan H, Louis ED, Siddique AB, Slavkovich V, Cheng ZQ, van Geen A, Graziano JH. Association between arsenic exposure and a measure of subclinical sensory neuropathy in Bangladesh. *J Occup Environ Med.* 2005; 47:778–784. [PubMed: 16093927]
- Hasan AR, Hu LG, Solo-Gabriele H, Fieber L, Cai Y, Townsend T. Field-scale leaching of arsenic, chromium and copper from weathered treated wood. *Environ Pollut.* 2010; 158:1479–1488. [PubMed: 20053493]
- IARC. Monographs on the evaluation of carcinogenic risks to humans, Volume 100: a review of human carcinogens. 2007. <http://www.iarc.fr/>
- Jain S, Rogier K. Chromium (Cr3+) prevents glutathione (GSH) depletion and activates glutathione reductase (GR) in acetoacetate (AA) and tertiary-butylhydroperoxide (TBH)- treated human red blood cells (RBC). *Free Radical Bio Med.* 2003; 35:S132–S133.
- Jin YP, Xi SH, Li X, Lu CN, Li GX, Xu YY, Qu CN, Niu YH, Sun GF. Arsenic speciation transported through the placenta from mother mice to their newborn pups. *Environ Res.* 2006; 101:349–355. [PubMed: 16458287]
- Kapaj S, Peterson H, Liber K, Bhattacharya P. Human health effects from chronic arsenic poisoning- A review. *J Environ Sci Heal A.* 2006; 41:2399–2428.
- Khan BI, Jambeck J, Solo-Gabriele HM, Townsend TG, Cai Y. Release of arsenic to the environment from CCA-treated wood. 2. Leaching and speciation during disposal. *Environ Sci Technol.* 2006a; 40:994–999. [PubMed: 16509348]
- Khan BI, Solo-Gabriele HM, Townsend TG, Cai Y. Release of arsenic to the environment from CCA-treated wood. 1. Leaching and speciation during service. *Environ Sci Technol.* 2006b; 40:988–993. [PubMed: 16509347]
- Mason RW, Edwards IR. Acute Toxicity of Combinations of Sodium Dichromate, Sodium Arsenate and Copper-Sulfate in the Rat. *Comp Biochem Phys C.* 1989; 93:121–125.
- Mejia JJ, Diaz-Barriga F, Calderon J, Rios C, Jimenez-Capdeville ME. Effects of lead-arsenic combined exposure on central monoaminergic systems. *Neurotoxicol Teratol.* 1997; 19:489–497. [PubMed: 9392784]
- Nielsen, FH. Summary: The clinical and nutritional importance of chromium – Still debated after 50 years of research. In: Vincent, JB., editor. *The Nutritional Biochemistry of Chromium(III)*. Elsevier; Amsterdam, The Netherlands: 2007. p. 265–276.

- Nouspikel T. DNA Repair in Mammalian Cells. *Cell Mol Life Sci.* 2009; 66:965–967. [PubMed: 19153660]
- Rahman MM, Chowdhury UK, Mukherjee SC, Mondal BK, Paul K, Lodh D, Biswas BK, Chanda CR, Basu GK, Saha KC, Roy S, Das R, Palit SK, Quamruzzaman Q, Chakraborti D. Chronic arsenic toxicity in Bangladesh and West Bengal, India - A review and commentary. *J Toxicol-Clin Toxic.* 2001; 39:683–700.
- Rana SVS. Metals and apoptosis: Recent developments. *Journal of Trace Elements in Medicine and Biology.* 2008; 22:262–284. [PubMed: 19013355]
- Rodriguez VM, Dufour L, Carrizales L, Diaz-Barriga F, Jimenez-Capdeville ME. Effects of oral exposure to mining waste on in vivo dopamine release from rat striatum. *Environ Health Persp.* 1998; 106:487–491.
- Rodriguez VM, Jimenez-Capdeville ME, Giordano M. The effects of arsenic exposure on the nervous system. *Toxicol Lett.* 2003; 145:1–18. [PubMed: 12962969]
- Rossman TG, Uddin AN, Burns FJ, Bosland MC. Arsenite cocarcinogenesis: An animal model derived from genetic toxicology studies. *Environ Health Persp.* 2002; 110:749–752.
- Schoen A, Beck B, Sharma R, Dube E. Arsenic toxicity at low doses: epidemiological and mode of action considerations. *Toxicol Appl Pharm.* 2004; 198:253–267.
- Song JK, Dubey B, Jang YC, Townsend T, Solo-Gabriele H. Implication of chromium speciation on disposal of discarded CCA-treated wood. *J Hazard Mater.* 2006; 128:280–288. [PubMed: 16165268]
- U.S.CPSC. Briefing package, petition to ban chromated copper arsenate (CCA)-treated wood in playground equipment (petition HP01-3). Washington DC: 2003.
- U.S.EPA. Integrated Risk Information System (IRIS). 2009a. <http://www.epa.gov/>
- U.S.EPA. National Primary Drinking Water Regulations (EPA 816-F-09-004). 2009b. <http://www.epa.gov/>
- U.S.EPA. Chromium in drinking water. 2010. <http://www.epa.gov/>
- USGS. Arsenic Statistics and Information. 2005. <http://www.usgs.gov/>
- Vahidnia A, Van der Voet GB, de Wolf FA. Arsenic neurotoxicity - A review. *Hum Exp Toxicol.* 2007; 26:823–832. [PubMed: 18025055]
- Weis, JS.; Weis, P. Effects of wood treated with chromated copper arsenate on aquatic biota. In: Townsend, TG.; Solo-Gabriele, H., editors. *Environmental Impacts of Treated Wood*. Taylor & Francis; Boca Raton: 2006. p. 173-189.
- WHO. Arsenic in Drinking-water, Background document for development of WHO Guidelines for Drinking-water Quality. 2003. <http://www.who.int/>
- Wright RO, Amarasinghwardena C, Woolf AD, Jim R, Bellinger DC. Neuropsychological correlates of hair arsenic, manganese, and cadmium levels in school-age children residing near a hazardous waste site. *Neurotoxicology.* 2006; 27:210–216. [PubMed: 16310252]
- Wright RO, Baccarelli A. Metals and neurotoxicology. *J Nutr.* 2007; 137:2809–2813. [PubMed: 18029504]
- Xi SH, Sun WJ, Wang FZ, Jin YP, Sun GF. Transplacental and early life exposure to inorganic arsenic affected development and behavior in offspring rats. *Archives of Toxicology.* 2009; 83:549–556. [PubMed: 19212760]

HIGHLIGHTS

1. As was the main factor determining the toxic effects of the leachates from CCA-treated wood
2. As (V) toxicity was enhanced when accompanied by Cu(II) and reduced when accompanied by Cr(III)
3. Generation of oxidative stress is likely one of the pathways for exertion of toxic effects by As.
4. As toxicity in wood leachates could be over or under estimated when based solely on the results of As

Figure 1.

Comparisons of As(V) toxicity in wood leachates and in As(V) standard solution. The solid line is the fitted curve of As(V) in standard solution, and the dash line is the fitted curve of As in wood leachates. The calculated EC_{50} were $10.7 \mu\text{M}$ for As in wood leachates and $21.1 \mu\text{M}$ for As standard solution.

Figure 2.

The effects of Cu(II) on SK-N-SH cell proliferation in wood leachates and in Cu standard solution. The solid line is the fitted curve of Cu in standard solution, and the dash line is the fitted curve of Cu in wood leachates. The Boltzmann curves and their slopes were significantly different ($p < 0.05$; one-way ANOVA with Tukey's multiple comparisons test).

Figure 3.

The effects of Cr(III) on SK-N-SH cell proliferation in Cr standard solution, wood leachates, and Cr spiked in a reference wood leachate. The solid, dash and dotted lines are the fitted curves of Cr in standard solution, in wood leachate, and in the reference wood leachate, respectively.

Figure 4. Combined effects on cell proliferation between As(V) and Cu(II) (A), and As(V) and Cr(III) (B). The cell proliferation were significantly reduced by the presence of Cu(II) when exposed to As(V) or combination of As(V) with Cr(III). The cell proliferation were significantly enhanced by the presence of Cr(III) when exposed to As(V) or combination of As(V) with Cr(III) ($p < 0.05$; one-way ANOVA with Tukey's multiple comparisons test).

Figure 5. The effects of As(V) (A) and combination of As/Cu, and wood leachates (WL) (B) on SK-N-SH cell proliferation with or without the presence of DMSO.