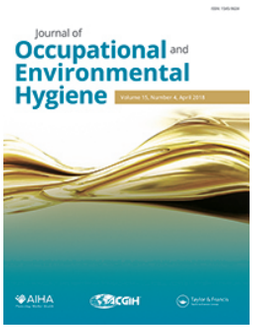

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Physical chemical properties and cell toxicity of sanding copper-treated lumber

Jennifer D. Sisler[a], Chaolong Qi[b], Walter McKinney[a], Justine Shaffer[a], Michael Andrew[a], Taekhee Lee[a], Treye Thomas[c], Vincent Castranova[d], Robert R. Mercer[a], and Yong Qian[a]

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ABSTRACT

To protect against decay and fungal invasion into the wood, the micronized copper, copper carbonate particles, has been applied in the wood treatment in recent years; however, there is little information on the health risk associated with sanding micronized copper-treated lumber. In this study, wood dust from the sanding of micronized copper azole-treated lumber (MCA) was compared to sanding dust from solubilized copper azole-treated wood (CA-C) and untreated yellow pine (UYP). The test found that sanding MCA released a much higher concentration of nanoparticles than sanding CA-C and UYP, and the particles between about 0.4–2 μm from sanding MCA had the highest percentage of copper. The percentage of copper in the airborne dust from sanding CA-C had a weak dependency on particle size and was lower than that from sanding MCA. Nanoparticles were seen in the MCA $PM_{2.5}$ particles, while none were detected in the UYP or CA-C. Inductively coupled plasma mass spectrometry (ICP-MS) analysis found that the bulk lumber for MCA and CA-C had relatively equal copper content; however, the $PM_{2.5}$ particles from sanding the MCA had a higher copper concentration when compared to the $PM_{2.5}$ particles from sanding UYP or CA-C. The cellular toxicity assays show that exposure of RAW 264.7 macrophages (RAW) to MCA and CA-C wood dust suspensions did not induce cellular toxicity even at the concentration of 200 μg $PM_{2.5}$ wood dust/mL. Since the copper from the treated wood dust can leach into the wood dust supernatant, the supernatants of MCA, CA-C and UYP wood dusts were subjected to the cellular toxicity assays. The data showed that at the higher concentrations of copper (≥5 μg/ml), both MCA and CA-C supernatants induced cellular toxicity. This study suggests that sanding MCA-treated lumber releases copper nanoparticles and both the MCA and CA-C-treated lumber can release copper, which are potentially related to the observed in vitro toxicity.

KEYWORDS
Lumber; micronized copper; physical chemical properties; sanding

Introduction

The use of copper in wood treatments has been popular since the 1700s when the first wood preservative was invented. The use of copper in the cupric or copper (II) oxidation state as a wood preservative is inexpensive and effective for protecting wood against a majority of wood-destroying fungi and insects. However, there are copper-tolerate fungi that copper does not protect against; therefore, any copper-based treatment also has an organic co-biocide.[1] There are two major water soluble copper-based wood treatments: alkaline copper quaternary (ACQ) and copper azole (CA).[2]

In 2006, a new preservative method of micronized copper came onto the market. In this new technique, copper is no longer solubilized in water. Instead, the copper carbonate is milled into fine particles and is used along with a dispersant to keep the copper from clumping and to maintain the dispersion when diluted in water for pressure treatment.[1,4] The average diameter of the copper carbonate particles after the milling process is approximately 300–500 nm. This process is advantageous over solubilizing copper because the concentration reached with micronized copper is higher than that of solubilized copper, leading to reduced production costs. The micronized copper azole (MCA) preservative formulation has 96.1% copper with either 3.9% tebuconazole or a 1:1 ratio of tebuconazole:propiconazole.[4] The MCA has begun to grow in popularity, and with that comes a growing concern for its environmental and human risks.

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Up to this point, it is controversial whether the copper carbonate used in the MCA formula is an engineered nanoparticle (ENP) or a microparticle. Furthermore, while sanding MCA-treated woods, copper carbonate may be released with the airborne wood dust. It is unknown what amount of the micronized copper preservative in the wood dust and what kind of size ranges they would form in the airborne wood dust. Nanoparticles are a class of particles that have at least one dimension in the range of 1–100 nm. One key characteristic of nanoparticles is their high surface area per mass as compared to the corresponding large particles.[5] The high surface area of nanoparticles could potentially induce electronic disruption and structural defect to form the surface oxidant reactive sites, leading to an increase in biological reactivity of the material.[5] Multiple in vivo and in vitro studies have shown that nanoparticles are able to cross the cell membrane, leading to alterations of cell physiology and ultimately cytotoxicity.[5–7] Human exposure to ENP not only limits to pristine forms, but also includes nanoparticles across life cycle.[8] It has been found that life cycle particle matter (LCPM) may differ from their corresponding pristine nanoparticles in physio-chemical properties and toxicological profiles.[9] Therefore, the incorporation of ENP into manufacturing products has become a large concern over the past decade and it is of interest to characterize the nature of MCA-treated wood dust from sanding and to determine their potential toxic effects.

The present studies sought to determine (1) the size distributions of sanding dusts of MCA-treated wood vs solubilized copper-treated wood (CA-C) and untreated yellow pine (UYP), (2) if free copper or copper nanoparticles are released from the sanding dust, and (3) if the sanding dust from treated lumbers is more toxic than that from UYP *in vitro*.

Materials and methods

Three types of lumber were used within this study: untreated yellow pine (UYP) manufactured by EACOM Timber Corporation (Montreal, Quebec, Canada), and two types of ground contact treated lumbers (micronized copper azole type C (MCA)), manufactured by Madison Wood Preservers, Inc. (Madison, VA) with a preservative content of 0.14 pound per cubic foot (PCF, equivalent to 2.2 kg/m^3) and copper azole type C (CA-C), manufactured by CN Tucker Lumber Company (Pageland, SC) with a preservative content of 0.15 PCF (2.4 kg/m^3).

Characterization of sanding dust in real time

Laboratory testing system

Figure 1 is a diagram of the laboratory testing system used in this study to collect data to characterize sanding dust. The system was designed and constructed in accordance with European Standard EN 1093-3.[10] A dust collection air handling unit (PSKB-1440, ProVent LLC, Harbor Springs, MI) was used as an air mover for the system. The air handling unit was connected to an automatic tool testing chamber through a 0.3 m diameter duct approximately 6.4 m long. A funnel section connected the duct to the automatic tool testing chamber, which had a square cross section with sides 1.2 m long. A blast gate installed on a wye branch duct fitting upstream of the air handling unit was used to adjust the air flow rate passing through

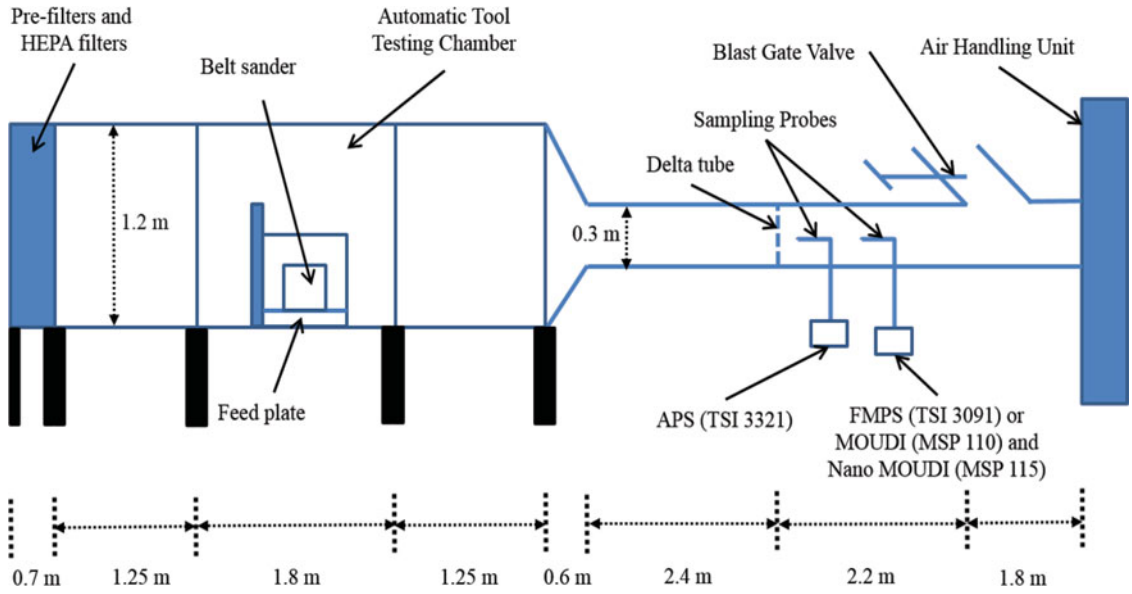

Figure 1. Engineered laboratory testing chamber.

the testing system by allowing bypass air to enter the air handling unit through the gated wye fitting. Once turned on, the air handling unit was set to draw room air into the testing system at a flow rate of 0.64 m^3/s (equivalent to 1,350 cubic feet per minute (CFM)). This flow rate was set by manually adjusting the blast gate valve and was monitored by a micromanometer (PVM100, Airflow Developments Ltd., UK) connected to a delta tube (306AM-11-AO, Midwest Instrument, Sterling, MI). The delta tube functioned as an averaging pitot tube with 4 pressure-averaging ports on the front and back of a tear-shaped or circular cylinder.[14] The delta tube was mounted on the duct about 2.4 m downstream (8 duct diameters) from the funnel section. The accuracy of the flow rate measured by the delta tube was verified by comparing the flow rate obtained from its manufacturer's calibration equation[15] to that measured by using a 10-point pitot tube traverse of the duct performed in the horizontal and vertical planes (the result was about 0.8% difference).[12] Two aerosol sampling ports on the duct were used to mount the sampling probes of all the sampling instruments used in this study. These two ports and the delta tube formed the sampling section of the system. The location of this sampling section on the duct was designed to meet the requirement of European Standard EN 1093-3[10] for taking representative samples, which was verified experimentally in a previous study using the same system.[16] The testing system provides automatic sanding of the test substrates in an automatic tool testing chamber, and a systematic characterization of emissions including the emission rates and the chemical composition of the emissions.

Belt sander and belts used in the real-time test

A 3 in x 21 in (76 mm x 533 mm), 8 ampere, variable speed belt sander (model 352VS, Porter-Cable, Jackson, TN) was used in this study. The sander was used with 240 grit sanding belts (Metalite R228 Abrasive Belt, Aluminum Oxide, Cotton Backing, Norton Saint-Gobain Abrasives, Worcester, MA). Belts were changed when the type of lumber sanded was changed.

Direct reading instruments

An Aerodynamic Particle Sizer Spectrometer (APS, model 3321, TSI Inc., Shoreview, MN) provided real-time direct reading measurement of the size distribution of the generated dusts with aerodynamic diameters between 0.542–20 µm. In addition, a Fast Mobility Particle Sizer Spectrometer (FMPS Model 3091, TSI, Inc., Shoreview, MN) was used to measure aerosol particles in the range of mobility diameter from 5.6–560 nanometers (nm), with 32 channels of resolution. Both the APS and the FMPS take representative samples from the emission and provide real-time size distribution measurement with a 1 sec time resolution. The 1 sec time resolution allowed both instruments to capture the entire dust cloud profile for each individual sanding pass and avoided overlaps of measurement between 2 adjacent passes. The details on the operations of the APS and FMPS are described in the supplemental information.

Filter samplers

A Micro-Orifice Uniform Deposition Impactor (MOUDI, Model 110, MSP Corp, Shoreview, MN) sampler was used to collect size-selective filter samples for copper content analysis according to NIOSH Method 7303, using inductively coupled argon plasma, atomic emission spectroscopy (ICP). A Nano-MOUDI 3-stage cascade impactor stack (Model 115, MSP Corp, Shoreview, MN) was connected via a coupling tube to the 10-stage MOUDI. Since no copper was found in the UYP (see the Result session below), this test was done for MCA and CA-C only. The details on sample collection and analyses using MOUDI and Nano-MOUDI are described in the supplemental information.

Operating procedure for a sanding test

The automatic tool testing chamber was used to make a fixed number of repeated passes with the belt sander across the width of the board as the wood was advanced along its length using the automatic feeding mechanism. The height of the sander was adjusted to maintain contact with the board surface. Before conducting a sanding test, the automatic tool testing chamber was programmed to perform a pre-determined number of passes with the belt sander. Each pass included the following steps: (1) the feed plate fed the lumber board; (2) the 2D actuator lowered the belt sander to the pre-determined height to remove about 0.03–0.05 in of the wood surface; (3) power was supplied to the belt sander; (4) the 2D actuator moved the belt sander across the width of the board; (5) the 2D actuator moved the belt sander back across the width of the board to its original horizontal position; (6) the belt sander was turned off; and (7) the 2D actuator raised the belt sander back to its original vertical position. A delay of about 5 sec was programmed between steps (3) and (4) to ensure the belt reached its designed rotating speed before making a sanding pass. Sanding was accomplished by moving the belt sander across the width of a board. The sanding speed, referred to as the sanding feed rate, can also be controlled by the PLC and was set at 2.54 cm/s in this study.

When the APS and FMPS were the only instruments used, three to six tests were conducted for each type of lumber. In addition, a baseline test was conducted when

the sander was running but not sanding any wood. Ten sanding passes were performed in each test.

In contrast to the APS and FMPS, which are direct-reading instruments, the MOUDI and Nano-MOUDI required the collection of a detectable mass of material for subsequent analysis. Thus, when the MOUDI and Nano-MOUDI were used, about 200 passes were conducted to avoid overloading the filters on the first few stages, while attempting to collect sufficient sample masses on the remaining stages. Supplemental information provides additional details on the testing procedure.

Production and collection of bulk wood dust for toxicology studies

We conducted the toxicology studies at a different location from the aforementioned laboratory sanding test. To avoid cross contamination during wood dust storage and shipment, we made extra efforts on the production and collection of wood dust for toxicology studies at the toxicology laboratory. Dust from sanding the 3 types of lumber was generated using a Belt Sander (Skil, model 3376-01, 4 in x 36 in, Robert Bosch Tool Corp., Mt. Prospect, IL) with 240 grit aluminum oxide sanding belt (Powertec, part no. 110200) and collected for toxicology studies within a glove box (Cleatech LLC, 2100-2-B, 35 in W x 24 in D x 25 in H). Lumber was cut into 2-ft sections and sanded to a depth of 1 in. Sanding dust was allowed to settle and was then collected.

Re-aerosolization and analysis of wood dust particles

The collected bulk wood dust was re-aerosolized using an acoustic generator inhalation system as previously described.[17,18] Details on the analysis are described in the supplemental information.

The particle size distributions (mass distributions and number distributions) of the re-aerosolized wood dust were determined using a MOUDI and an ELPI (Electrical Low Pressure Impactor, Dekati Ltd.). Greased foils were used for the MOUDI stages. A scanning electron microscope (Hitachi S-4800, Hitachi High Technologies America Inc., Pleasanton, CA) was used to analyze particle physical morphology by drawing aerosol samples at a flow rate of 1 L/min from the sample chamber onto 25 mm diameter 0.2 µm pore sized polycarbonate filters (Whatman, GE Healthcare Bio-Sciences, Pittsburgh, PA) for approximately 5 sec.

Collection of $PM_{2.5}$ wood dust for in vitro assays

Sanding wood generally produces a significant amount of particles that are too large (greater than 10 µm) to be inhaled and deposited into the parenchyma of the lungs. A method was developed to separate and collect $PM_{2.5}$ fraction of particles from the rest of the dust so that it could be used for both *in vitro* assays and pharyngeal aspiration exposure in mice. For *in vitro* assays, a bank of 5 sample filters (Whatman 25 mm, 0.2 µm pore sized polycarbonate filters), each with sample flow rates of 1 L/min, was used to collect $PM_{2.5}$ particles from the sample chamber. The cyclone was removed and cleaned after 20 min of use. This was done to keep the larger particles that coat the sides of the cyclone from building up to the point that they could break lose and exit the cyclone. After 5 rounds of 20 min sample runs, the filters were removed. The filter cassettes were carefully opened and a stainless steel spatula was used to gently knock the wood dust into a glass test tube. This process was repeated several times for each type of wood dust until the test tubes had a sufficient amount of material for *in vitro* assays.

Toxicity analysis of wood dust in vitro

Cell culture

Mouse RAW 264.7 macrophages cell lines (RAW) were purchased from ATCC (Manassas, VA) and were maintained per ATCC guidelines (www.atcc.org). For each experiment, RAW cells were plated at the appropriate density in 96 well cell culture plates. After 24 hr, the cells were treated with supernatant media and assayed 24 hr later.

Cytotoxicity of wood dust in RAW cells

To measure the cytotoxicity of wood dust, a colorimetric method for sensitive quantification of viable cells in proliferation and cytotoxicity assay was applied. RAW were plated at 20,000 cells per well in a 96 well plate; then 24 hr later, RAW were dosed with 0, 0.75, 1.56, 3.125, 6.25, 12.5, 25, 50, 100, and 200 µg/mL of UYP, MCA and CA-C $PM_{2.5}$ for 24 hr. Cellular proliferation was measured using a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay (CellTiter 96 Aqueous One Solution Cell Proliferation Assay kit (Promega, Madison, WI)) following the manufacturer's guidelines.

Suspension of wood dust $PM_{2.5}$ particles

To measure the amount of copper leaching from the wood dusts and test their toxicity in vitro, the wood dust suspensions were produced in the cell culture medium. $PM_{2.5}$ sanding dust particles were suspended in Dulbecco's Modified Eagle's medium (DMEM) to make cell culture suspension at a concentration of 1 mg/mL and then sonicated for 1–2 min. Samples were vortexed immediately before use.

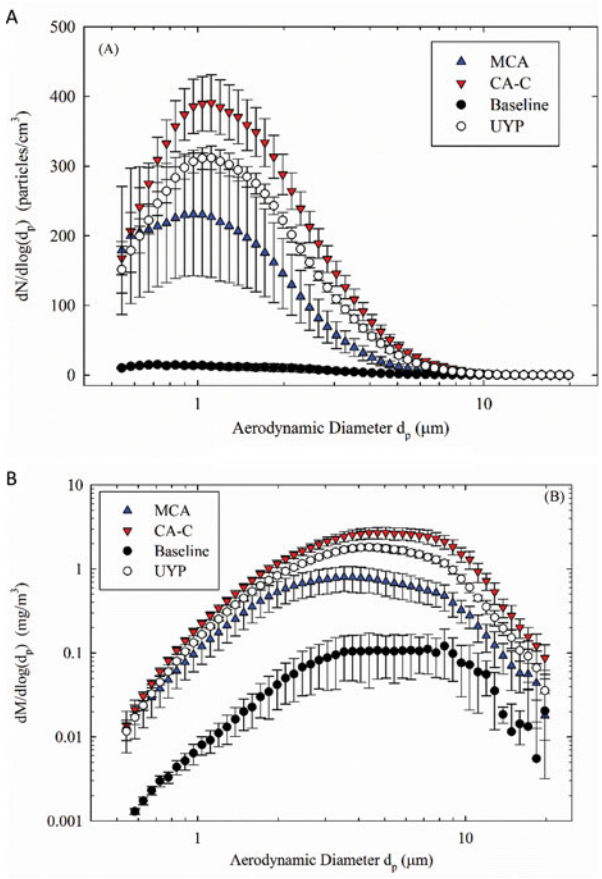

Figure 2. Size distribution obtained from the APS for sanding dust from different lumbers (a) number-based and (b) mass-based. MCA: micronized copper azole–treated wood sanding dust. CA-C: copper azole-treated wood sanding dust. UYP: untreated yellow pine sanding dust.

Copper in media

To measure the amount of copper leaching from the wood dust, wood dust (1 mg/mL) was mixed with media for 24 hr at 4˚C with constant shaking. After the 24 hr, the media was centrifuged at 500 g for 5 min to remove the larger wood dust particle and the supernatant was used to treat RAW for assays. The procedure can be found in Supplemental Figure 2. The total amount of copper in the supernatant was measured using inductively coupled plasma-mass spectrometry (ICPMS). To analyze the amount of free copper nanoparticles, the supernatant was also analyzed using enhanced darkfield microscopy.

Cytotoxicity of wood dust-exposed media

RAWs were plated in a 96 well plate (BD Biosciences, Franklin Lakes, NJ) at a density of 20,000 cells per well. Once the cells settled for 24 hr, they were treated with the supernatant media for an additional 24 hr before assaying for toxicity. The amount of copper per sample was calculated based upon the ICM-PS data seen in Figure 5.

To measure the changes in cellular proliferation, the Cell Titer 96® Aqueous One Solution Cell Proliferation Assay kit (Promega, Madison, WI) was used following manufacturer's guidelines.

Enhanced-darkfield light microscopy imaging of nanoparticles

The PM2.5 wood dust was mounted onto a slide and coverslipped. It was analyzed for nanoparticles within the lumber. The wood dust media was also analyzed for free nanoparticles. Details on the analysis are described in the supplemental information.

Statistical analysis

The statistical analysis for comparing cytotoxicity levels between UYP, MCA, and CA-C consisted of analysis of variance (ANOVA) with pre-planned pairwise comparisons at each concentration. This was performed using SAS PROC MIXED.[19] All statistical tests were two-tailed with significance level equal to 0.05.

Results

Real time characterization of sanding dust particles

Size distribution of the airborne dust from sanding different woods

The data obtained from the APS for the airborne dust generated from sanding different woods are shown in Figure 2. A baseline test with the belt sander running without sanding wood was conducted to investigate particles released from the running sander alone. Overall, these size distributions have similar shapes with the number-based geometric mean diameter (d_{pg}) around 1 μm (Figure 2A) and the mass-based d_{pg} around 4–5 μm (Figure 2B) with CA-C producing more dust than MCA.

The particles observed from the baseline test with a total number concentration of 10.2 ± 3.4 particles/cm^3 (mean ± standard deviation) may be mainly attributed to the sander's motor running in the no-load mode. The total concentration (both number and mass), d_{pg}, and geometric standard deviation (σ_g) measured by both the APS and FMPS for all the tests are listed in Table 1. The data of total concentration were obtained from the instruments' readings; and the data of d_{pg} and σ_g were obtained from lognormal fitting of the size distributions.

As shown in Figure 2, the error bars for the size distribution from sanding MCA are larger than those from sanding CA-C, which are also larger than those from sanding UYP. It is unknown how much the treatments

Table 1. Characterization of aerosolized sanding wood dust.

		Number Based			Massed Based		
		N_{tot} (particles cm^{-3})	d_{pg}	σ_g	M_{tot} (μg^{m-3})	d_{pg}	σ_g
Baseline	APS	10.2 ± 3.4	0.91 μm	2.43	81.8 ± 45.0	5.12 μm	1.93
	FMPS	$8.3 \times 10^3 \pm 8.3 \times 10^2$	9.9 nm	1.34	0.6 ± 0.3	261.7 nm	1.85
UYP	APS	204.7 ± 11.4	1.15 μm	1.95	1213.6 ± 82.3	4.34 μm	N/A
	FMPS	$9.7 \times 10^4 \pm 5.1 \times 10^4$	25.0 nm	1.92	4.1 ± 0.5	N/A	2.58
MCA	APS	150.7 ± 54.5	0.95 μm	2.05	586.7 ± 191.2	3.80 μm	1.97
	FMPS	$1.8 \times 10^5 \pm 1.2 \times 10^5$	31.8 nm	1.73	7.9 ± 7.6	133.3 nm	2.58
CA-C	APS	260.0 ± 27.8	1.18 μm	1.95	1888.1 ± 278.1	4.69 μm	1.87
	FMPS	$1.1 \times 10^5 \pm 1.0 \times 10^5$	21.0 nm	1.97	4.9 ± 1.0	N/A	N/A

The total particle concentration (N_{TOT}/M_{TOT}), geometric mean diameter (D_{PG}) and geometric standard deviation (σ_G) for each mode of the APS and FMPS, data mean ± standard deviation; N/A means not available as lognormal fitting was not obtainable for these two cases. MCA: micronized copper azole-treated wood sanding dust. CA-C: copper azole-treated wood sanding dust. UYP: untreated yellow pine sanding dust.

contributed to these observed discrepancies as unevenness on the wood surfaces may also affect these observations. The particle concentrations from sanding different woods are higher than those observed during the baseline test. Comparing the means of three to six tests of each wood type, the total particle mass concentration based on the APS data for the CA-C was significantly higher than that for the UYP ($p = 0.003$ for number and $p = 0.001$ for mass), which was significantly higher than that for the MCA in terms of mass ($p = 0.003$). The higher total particle number concentration from sanding UYP than that from sanding MCA was not significant ($p > 0.146$).

The total concentration, geometric mean diameter and geometric standard deviation measured by the FMPS are listed in Table 1. The APS data did not show a large number of airborne wood dust particles (peak of 400 particles/cm^3) in the size range of 0.542–20 μm. The concurrent FMPS data (see Figure 3) revealed that there was a large amount of nanoparticles (smaller than 100 nm) generated during the sanding tests. Compared to UYP, the treated woods released a larger amount of nanoparticles. The total number concentration from sanding MCA is consistently higher than CA-C or UYP.

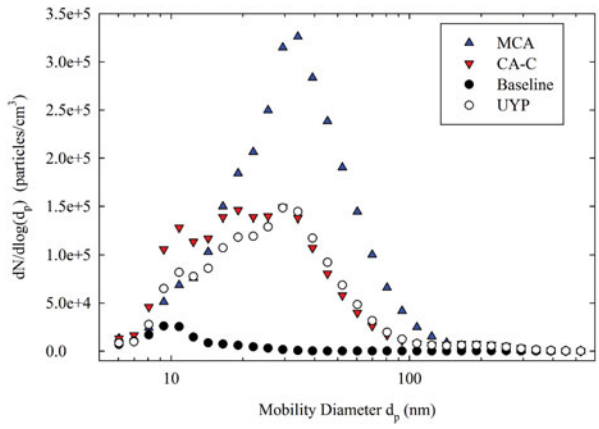

Figure 3. Number-based particle size distribution of sanding dust from all lumber samples analyzed by FMPS (Fast Mobility Particle Sizer Spectrometer). MCA: micronized copper azole–treated wood sanding dust. CA-C: copper azole-treated wood sanding dust. UYP: untreated yellow pine sanding dust.

Copper content in the sanding wood dusts

The filter samples collected from the MOUDI and the Nano-MOUDI were analyzed for copper content. Figure 4A shows the normalized mass-based copper size

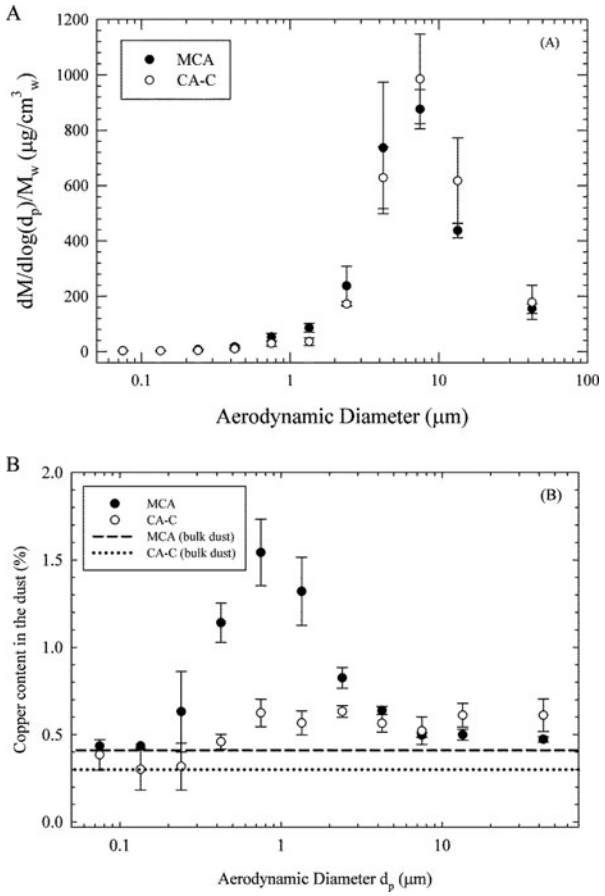

Figure 4. Copper content in MCA and CA-C bulk and sanding dust measured with NIOSH Method 7303, using inductively coupled argon plasma, atomic emission spectroscopy (ICP). (A) Normalized mass-based copper distribution of sanding MCA and CA-C wood dust. (B) percentage of copper content in airborne sanding dust from MCA and CA-C.

distribution from these samples. The procedure for processing the data from MOUDI and Nano-MOUDI is described in details in the supplemental information. The limit of detection (LOD) for copper was 0.2 µg. It was found that all the Nano-MOUDI samples had copper content below the LOD, so they were not included in Figure 4. A new piece of wood was used for each replicate test and its mass was measured before and after the test. The volume of the wood removed by sanding during the MOUDI sampling, i.e., V_w, was obtained by dividing the mass difference by the wood density. The normalized mass-based copper size distributions from sanding MCA and CA-C were similar as shown in Figure 4, with the d_{pg} around 7.5 µm. Most MOUDI samples for MCA and CA-C with aerodynamic diameters below 0.1 µm had a copper content below the copper LOD. Larger amounts of copper were found in the dust of larger sizes.

For each MOUDI sample, the copper content expressed as a percentage was calculated by dividing the copper mass by the dust mass, and plotted in Figure 4B. The copper percentages in the dusts larger than 10 µm and smaller than 0.3 µm approached the levels found in their corresponding bulk dust samples. The particles between about 0.4–2.4 µm from sanding MCA had the highest percentage of copper. The percentage of copper in the airborne dust from sanding CA-C had a weak dependency on particle size, and it was mostly lower than that from sanding MCA.

Metal content in lumber and $PM_{2.5}$ fraction of sanding wood dust

UYP, MCA, and CA-C lumber samples were analyzed for metals and the list of the metal content of the lumber can be found in Supplemental Table 1. The copper content within the lumber is shown in Figure 5A. It can be seen that the copper content between MCA and CA-C is relatively similar, but was undetectable in UYP. Once the dust of $PM_{2.5}$ fraction was isolated, it was also analyzed for the total metal contents (Supplemental Table 2) and the amount of copper (Figure 5B). The $PM_{2.5}$ fraction of MCA dust contained a significantly higher amount of copper when compared to CA-C, while UYP did not have any detectable copper. These data are consistent with the copper contents observed in the MOUDI samples (Figure 4B), and they suggest that both MCA and CA-C treated lumber contained equal amounts of copper; however, the $PM_{2.5}$ fraction of MCA had a significantly higher concentration of copper than CA-C $PM_{2.5}$. ICPMS was performed to determine the leaching of the metal content into the cell culture media, DMEM (Supplemental Table 3). The data showed that MCA leached significantly more copper into the DMEM than CA-C (Figure 5C). Copper was not detected in the media control or UYP samples, suggesting the copper found in MCA and CA-C samples was due to leaching of the pressurized treatment. These data suggest that copper is released from the pressurized treated lumbers of both MCA and CA-C.

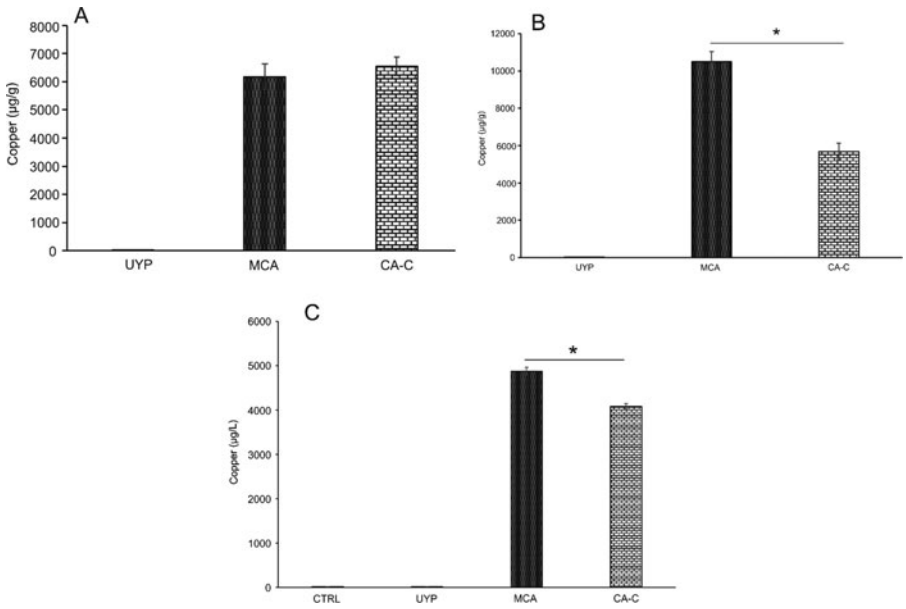

Figure 5. Copper content measured by ICPMS. Inductively coupled plasma mass spectrometry (ICPMS) analysis of UYP, MCA, and CA-C in (A) lumber, (B), $PM_{2.5}$, and (C) DMEM. n = 3 experiments ± standard deviation. * represents $p < 0.05$ significance between MCA and CA-C. MCA: micronized copper azole–treated wood sanding dust. CA-C: copper azole-treated wood sanding dust. UYP: untreated yellow pine sanding dust.

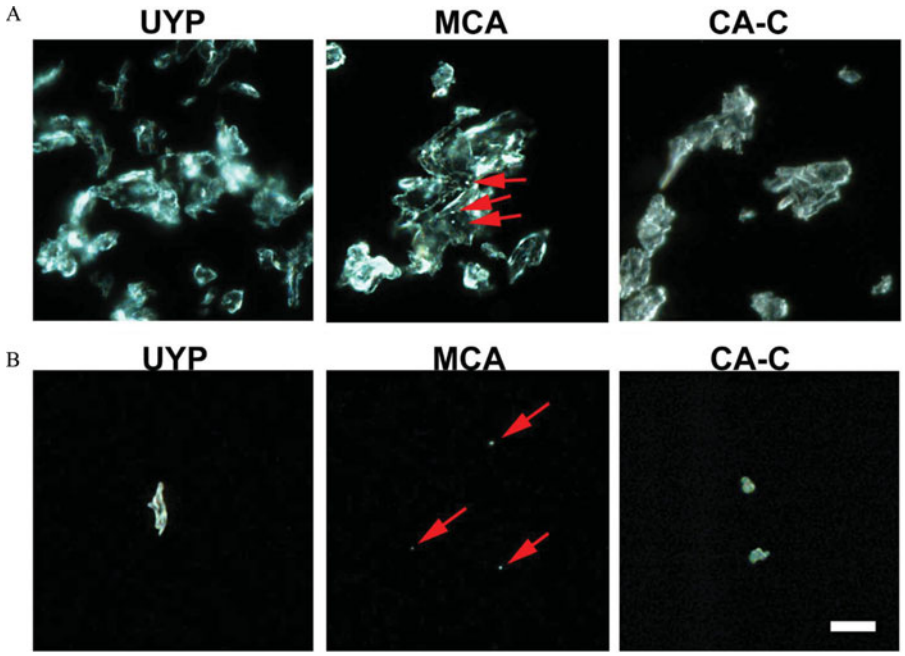

Figure 6. Enhanced darkfield imaging of UYP, MCA, and CA-C $PM_{2.5}$ particles. (A) Images are a representation of dry UYP, MCA, and CA-C $PM_{2.5}$. Red arrows show the nanoparticles embedded in the wood dust. (B) Representative images of sanding dust supernatant after mixing UYP, MCA, and CA-C $PM_{2.5}$ for 24 hr. Red arrows indicate free nanoparticles. MCA: micronized copper azole-treated wood sanding dust. CA-C: copper azole-treated wood sanding dust. UYP: untreated yellow pine sanding dust.

To determine if the copper released into the DMEM was soluble copper or free copper nanoparticles, samples were analyzed using enhanced darkfield imaging. Results of $PM_{2.5}$ wood dust analysis suggest that copper was found with the MCA dust sample as nanoparticles embedded into the wood dust while both CA-C and UYP dust did not contain cooper nanoparticles (Figure 6A). To further determine if these nanoparticles were released into the solution upon mixing sanding dust with DMEM, enhanced darkfield images were taken of the wood dust supernatant. Figure 6B illustrates that MCA samples did have free nanoparticles in the media while CA-C and UYP did not, suggesting that the free nanoparticles are due to the micronized copper azole treatment specifically and not the CA-C treatment. Quantification and the identification of the free nanoparticles were attempted; however, within a 24-hr period, the free nanoparticles were no longer detectable and were not quantifiable because of the low concentration and instability of the nanoparticles. This would suggest that the copper that is released into the DMEM could be copper nanoparticles; however, further testing would need to be done to verify this finding.

Analysis of wood dust toxicity in vitro

Toxicity of the three types of lumber was determined using RAW by measuring cellular proliferation using a MTS assay. $PM_{2.5}$ was added directly to the cells; however, there were no detectable differences in the toxicity between UYP, MCA, or CA-C (Figure 7). Since the copper from the treated wood dust can leach into the media, the sanding dust supernatant was added to the RAW for the toxicity measurements using a MTS assay. As the concentration of the copper increased within the supernatant media, the cellular toxicity increased only in the MCA and CA-C leachates (≥5 µg/mL). There was little to no toxicity seen in the RAW treated with

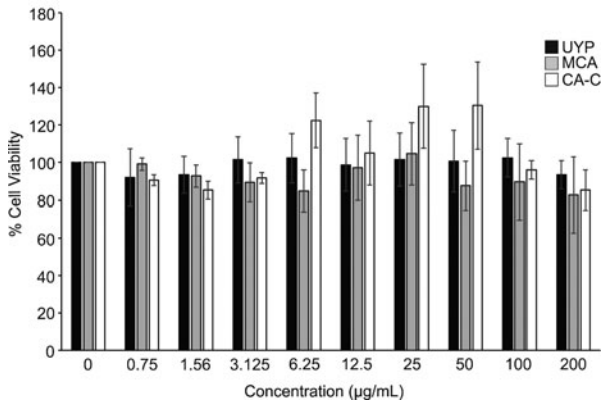

Figure 7. Sanding wood dust induced cytotoxicity of RAW. Mouse macrophage cell line RAW 264.7 were treated with varying concentrations of UYP, MCA, or CA-C $PM_{2.5}$ sanding dust particles for 24 h. Cells were then assayed using a MTS assay. Values represent the average percent cell viability of n = 3 independent experiments ± standard error. MCA: micronized copper azole-treated wood sanding dust. CA-C: copper azole-treated wood sanding dust. UYP: untreated yellow pine sanding dust.

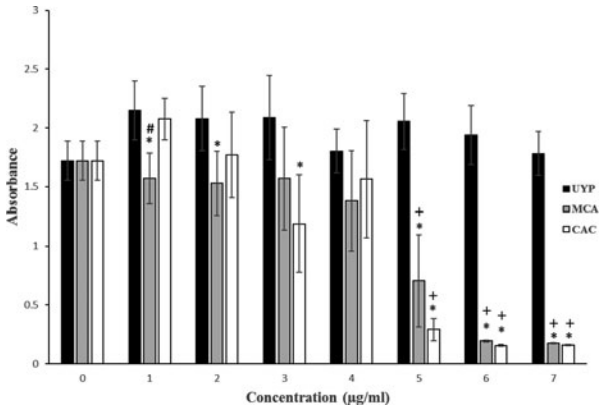

Figure 8. Cytotoxicity induced by sanding dust supernatant of UYP, MCA, and CA-C $PM_{2.5}$ Particles in RAW. Supernatant from UYP, MCA and CA-C $PM_{2.5}$ particles were used to dose RAW with the varying copper concentrations. The Y-axis represents MTS absorbance ($\lambda = 595$ nm). Values are representative of the means of MTS assay absorbance's ± standard error. $n = 3$ independent experiments. * represents $p < 0.05$ significance compared to UYP. # represents $p < 0.05$ significance between MCA and CA-C. + represents $p < 0.05$ significance compared control. MCA: micronized copper azole-treated wood sanding dust. CA-C: copper azole-treated wood sanding dust. UYP: untreated yellow pine sanding dust.

UYP supernatant (Figure 8). At the low concentrations of copper in the standing dust supernatant of MCA and CA-C, no cellular toxicity was observed; however, both supernatants from MCA and CA-C were toxic at the concentration of above 5 µg/mL copper (Figure 8).

Discussion

The mass-based size distribution of the sanding dust shown in Figure 2B illustrates noticeable differences among the three types of lumbers. It is unknown how much the treatments contributed to these observed discrepancies as unevenness on the wood surfaces and other wood characteristics may also affect these observations.

The data shown in Figure 3 and listed in Table 1 from the FMPS measurement indicates that MCA treatments leads to a considerably larger number of airborne nanoparticles than UYP and CA-C. Because of their smaller sizes, these nanoparticles did not lead to a quantifiable mass by either gravimetric measurement for the overall particles or ICPMS for metal elements in this study.

For the MOUDI samples with quantifiable mass, Figure 4B clearly illustrates the difference in copper content of the wood dust from sanding MCA and CA-C. For CA-C, the copper content had a weak dependency on particle size and it did not deviate from the level found in the corresponding bulk dust. However, for MCA, the copper content of the MCA sanding dust exhibited a clear peak between about 0.4–2 µm, which was considerably higher than the level in its corresponding bulk dust.

The difference observed in Figure 4B between MCA and CA-C may be attributed to the different treatments. In the CA-C treatment, copper may be more uniformly solubilized into the wood matrix, resulting in its more uniform distribution in the wood dusts of varying sizes. This may also explain why no copper particle was observed in the wood dust from sanding CA-C (Figure 6). While in the MCA treatment, copper carbonate particles with an average diameter about 300–500 nm were pressed into the wood matrix. These copper particles may be liberated in the forms of free particles and their aggregates during sanding, which may explain the peak of copper content in the wood dusts between about 0.4–2 µm (Figure 4B) and the observation of copper particles in the samples from sanding MCA (Figure 6).

The additional copper content in the wood dust between about 0.4–2 µm from sanding MCA may cause additional concern for an occupational risk when sanding MCA as this size range falls into the respirable particle size for human. In addition, the larger number of nanoparticles observed from sanding MCA deserves further investigation of their compositions to determine whether they pose additional occupational risk.

To analyze the different content in the bulk material compared to the $PM_{2.5}$, the samples were analyzed using ICPMS. It was determined that the bulk material MCA and CA-C had equal amounts of copper content; however, no detectable levels of copper were observed in the bulk UYP (Figure 5A). Analyzing the copper content of the $PM_{2.5}$, the MCA had significantly higher amounts of copper compared to the CA-C and again there was no detectable amounts of copper in the UYP (Figure 5B), demonstrating that the MCA sanding dust releases more copper than the CA-C sanding dust. These results may explain why the lower concentrations (1–2 µg/mL) of the sanding dust supernatant of MCA induced more toxic effects than that of CA-C (Figure 8), suggesting that MCA treatment might be more toxicity than CA-C. Given the nature of MCA treatment and the higher concentration of copper in the MCA sanding dust, it is likely that the cellular toxicity may be due to the effects of copper or a combination of the copper and the co-biocide azole. The difference in the cellular toxicity between MCA and CA-C supernatants could also be due to the different forms of copper in MCA and CA-C treated lumber. Copper is in a solubilized form in CA-C whereas it exists as a particulate form in MCA. The average size of the copper in MCA sanding dust is approximately 300 nm, which may be considered nanosized.[20] Enhanced darkfield analysis showed the $PM_{2.5}$ fraction of MCA sanding dust had embedded nanoparticles. Some of these nanoparticles

also appear in sanding dust supernatant collected from the MCA sanding suspension. However, to determine if these are indeed engineered copper nanoparticles requires future studies. Moreover, since the form of copper in MCA treatment is copper carbonate and there is no available toxicity data for copper carbonate nanoparticles *in vivo* and *in vitro*, more studies are needed to determine why MCA treatment is more toxic than CA-C treatment in RAW cells.

Our results showed that exposure of RAW cells to MCA and CA-C wood sanding dust supernatants induced cellular toxicity (Figure 8); however, MCA and CA-C sanding dust suspensions did not induce cellular toxicity even at the concentration of 200 μg $PM_{2.5}$ wood dust/mL (Figure 7), which is consistent with the results of a recently published article.[20,21] It is possible that 2 factors may contribute to these findings. First, the wood dusts in suspension may cover the RAW cells to block the cells' access the leached copper. Second, for the cells treated with the wood dust suspensions, the average concentration of copper in the culture media could be much lower than that of the sanding dust supernatant. Even at 200 μg wood dust/ mL, the total content of copper is approximately 2 μg (10492 μg copper/1 g MCA $PM_{2.5}$). The leached rate by the end of 24 hr incubation time is 46% (4,875 μg/10,492 μg) based upon data collected from ICPMS.

In conclusion, this study demonstrates that sanding MCA-treated lumber released nanoparticles and the MCA sanding dust had a higher concentration of copper at the $PM_{2.5}$ fraction compared to CA-C. Although exposure of RAW to MCA and CA-C wood dust suspensions did not induce cellular toxicity even at the concentration of 200 μg $PM_{2.5}$ wood dust/mL, both supernatants from MCA and CA-C were toxic at the concentration of above 5 μg/mL copper. In the future, we will analyze the effects of the PM2.5 fraction of sanding dust *in vivo*.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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