In vitro Bioaccessibility of Copper Azole Following Simulated Dermal Transfer from Pressure-Treated Wood

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Abstract:

Micronized copper azole (MCA) and micronized copper quaternary are the latest wood preservatives to replace the liquid alkaline copper and chromated copper arsenate preservatives due to concerns over the toxicity or lack of effectiveness of the earlier formulations. Today, the use of MCA has become abundant in the wood preservative industry with approximately 38 million pounds of copper carbonate being used to treat lumber each year. Despite this widespread usage, little information is available on the bioaccessibility of this preservative upon gastrointestinal exposure. Using a simulated hand-to-mouth/gastric system exposure study we investigated several types of commercially available copper-treated lumber products as-purchased and after exposure to outdoor weathering conditions. Soluble and particulate fractions of copper were measured after transfer to and release from surface wipes passed along copper-treated lumber and exposed to synthetic stomach fluid (SSF, pH 1.5) or DI water. Wipes passed along new boards contained greater amounts of copper than wipes from weathered boards. The total copper recovered from the wipes after microwave extraction varied among the different wood types. For all wood types the copper released into SSF was more soluble than what was soluble in DI water. The data suggest that copper from treated wood is highly bioaccessible in SSF regardless of wood type and weathering condition.

Keywords: micronized copper, wood preservative, bioaccessibility, exposure, stomach fluid

Introduction:

For the past 30 years, copper has been used to treat and protect pressurized lumber against bacteria, insects, mold, fungi, and decay (Clausen, 2003). The earliest and most predominant wood preservative formulation used for buildings, decks, utility poles, furniture and other outdoor applications was comprised of chromium, copper oxide, and arsenate (CCA). This mixture was highly effective in controlling pests over long periods of time due to a combination of active ingredients and persistence in the environment. However in 2003, the U.S. EPA restricted the residential use of CCA-treated wood due to concerns over the toxicity of the arsenate and chromium constituents (USEPA, 2003). This restriction led to the replacement of CCA with liquid ammoniacal and amine –based formulations. The liquid alkali copper preservatives also had drawbacks in that LAC’s high water solubility resulted in high rates of leaching and corrosion of metal hardware (Dhyani and Kamdem, 2012; Freeman and McIntyre, 2008).

Ideal wood preservative formulas intended for use in outdoor construction and applications have high retention of the active ingredients in the wood with slow release over time, are highly effective in different types of wood, have low metal corrosion potential, and the ability to withstand extreme weather conditions. Micronized copper quaternary (MCQ) and azole (MCA) formulations are the most recent replacements developed to resolve the inherent issues of previous formulations while displaying the desirable properties ideal for outdoor use. Micronized copper also has the ability to enter wood microstructures (e.g. lumens and pits) thus providing a persistent source of copper to guard against the damage caused by fungi and decay (Matsunaga et al., 2009; Stirling et al., 2008; Xue et al., 2012). Such beneficial features have made this new class of copper-based wood preservatives a popular alternative to liquid alkali copper preservatives as evident with over 5 billion board feet of micronized copper-treated boards being sold since its introduction into the marketplace in 2006 through 2011 (Griffin, 2011).

With such widespread micronized copper usage, concern has surfaced regarding the risk of human exposure to the newest copper-based preservative formulations. Although copper is an essential element in the human body and is required for metabolic function, gastrointestinal

59 exposure to copper concentrations exceeding 4-6 ppm can cause vomiting, nausea, and/or
60 abdominal pain (ATSDR, 2013). Measuring the *in vivo* bioavailability (“the fraction of an
61 ingested dose that crosses the gastrointestinal epithelium and becomes available for distribution
62 to internal target tissues and organs”) after oral ingestion (USEPA, 2007a) is necessary when
63 assessing the risk and potential effects of human exposure. As an alternative to *in vivo*
64 bioavailability testing, *in vitro* measures of bioaccessibility have been shown to complement
65 supporting data in risk assessments (Bradham et al., 2015). Bioaccessibility is defined as “the
66 total amount of a chemical present in ingested food, water, or ingested soil and sediment
67 particles that at maximum can be released during digestion” (Peijnenburg and Jager, 2003) and is
68 also an indicator for potential risk of exposure. To our knowledge, no research has simulated
69 hand-to-mouth/oral ingestion of micronized copper preservatives using surface wipe sampling
70 and subsequent gastrointestinal exposure as a surrogate for hand to mouth/stomach exposure. To
71 date, studies have investigated the chemistry of micronized copper in wood (Xue et al., 2012),
72 simulated dermal transfer of micronized copper from treated wood (Platten et al., 2016) and the
73 *in vitro* bioaccessibility of micronized copper-treated wood dust (Santiago-Rodríguez et al.,
74 2015).

75 Platten et al. (2016) characterized the micronized copper from copper-treated wood and
76 investigated the simulated dermal transfer of micronized copper from lumber to surface wipes
77 using newly-purchased wood boards and boards that were weathered over the course of 399
78 days. The researchers found that wipe samples from wood treated with aqueous copper azole
79 contained 90% copper bound to organics (i.e. lignin and cellulose) while wipes from one form
80 of MCA-treated wood (MCA-1) contained approximately 50% copper carbonate and 50% Cu
81 bound to organics. An alternative MCA formula (MCA-2) had average copper particle sizes
82 smaller than MCA-1; and MCA-2-treated lumber contained 88% copper carbonate and 12%
83 copper complexed with organics. The percentages of copper bound to the organics were too large
84 to pass through 10 kDa pore size filters which are used to isolate the soluble copper fractions
85 from the total copper detected in the wood.

86 In a recent study we investigated the *in vitro* bioaccessibility of micronized copper from
87 micronized copper-treated wood dust exposed to synthetic stomach fluid (SSF) or deionized (DI)
88 water (Santiago-Rodríguez et al., 2015). Only 14-25% of the total copper measured in alkali
89 copper-treated and micronized copper-treated wood dusts were soluble in water. In contrast to DI

water, micronized copper-treated wood dust was over 90% soluble in synthetic stomach fluid which suggested an elevated level of human bioaccessibility upon oral exposure for this material. The differences in copper solubility were attributed to differences in the pH of water (pH 7.0) and SSF (pH 1.5) extractants. Micronized copper exposed to a low pH (1.5) was highly soluble while copper exposed to DI water poorly dissolved. The enhanced dissolution in SSF may also have resulted from ionic copper binding with the glycine in SSF (Santiago-Rodríguez et al., 2015). Although strides have been made in understanding these wood products, bioaccessibility information was still lacking in the amounts and types of micronized copper (soluble vs. insoluble) from copper-treated wood residue that is transferred from surface-to-hand-to-mouth in a gastrointestinal system. In this study, we simulated this common route of gastrointestinal exposure and investigated the concentrations and types of micronized copper azole that are transferred from the surfaces of copper-treated boards to polyester surface wipes and subsequently released upon exposure to synthetic stomach fluid. A study by Thomas et al. (2004) has shown that surface wipe sampling is an effective surrogate for children's hands, because there is no person-to-person variability and the transfer efficiency of arsenic-wood residue from the surface of treated wood onto polyester wipes strongly correlates with the transfer efficiency of arsenic-wood residue onto human hands (0.83 correlation coefficient). We presumed that micronized copper would have a similar transfer efficiency correlation and the total copper adhered to the wipes would reach the gastric system. We also examined whether copper released during the wiping process exhibited the same dissolution properties for newly purchased and weathered lumber. Investigating the release and dissolution processes allows better prediction of long-term behavior of metal-based wood preservatives and how the physicochemical and reactive properties of these preservatives may influence long term exposure risk under real-world scenarios.

2.0 Materials and Methods:

2.1 Surface Wipe Sampling

Four wood types (one liquid copper-treated [ACA], two micronized copper-treated [MCA], and one untreated [southern yellow pine]) intended for above-ground use were purchased from a national chain home improvement store. Boards belonging to each wood type

were weathered as previously described for 399 days (Platten et al., 2016). Surface wipe sampling of the as-purchased and weathered boards for each wood type were performed according to protocols developed by the Consumer Product Safety Commission (CPSC) (Thomas et al., 2004). Individual polyester cloth wipes were dampened to two times their original weight with 0.9% saline solution. The wipe was attached to a 1.1 kg weight and pulled back and forth along a 450 cm² area on the board 5 times (10 passes). Each cloth wipe was passed over a different location on a single board. The area sampled on each board was 450 cm² and the sampling surface area on each wipe was 50 cm². After sampling, each wipe was stored in an individual 50 ml centrifuge tube.

2.2 Bioaccessibility Assay

All glassware and vessels were acid-washed in 20% nitric acid for 24 hours and rinsed three times with deionized water (DI, 18 MΩ·cm, ASTM Type I trace element quality, Millipore, Bedford, MA). DI water was used to prepare all solutions. Synthetic stomach fluid (SSF) with a pH of 1.5 to mimic the highly acidic environment in the stomach under normal fasting conditions was prepared as previously described (Bradham et al., 2011) using 0.42 M HCl (32-35% analytical grade), 0.40 M glycine (certified ACS grade) obtained from Fisher Scientific, Inc. (Pittsburgh, PA) and DI water. Thirty ml of SSF or DI water was added to each tube containing a wipe sample or control. After attaching screw caps, the tops of the tubes were wrapped with parafilm to prevent leakage and the tubes were shaken continuously at room temperature for 1 hour at 140 rpm on an orbital shaker.

Micronized copper azole (34.54% copper, 0.62% azole) served as a positive control and was used to identify whether surface wipes affected the release of copper into solution. 300 µL of micronized copper azole (350 ppm) was applied to individual wipes. Wipes were suspended during copper azole application to ensure no loss of copper azole sample. The surface wipes were allowed to dry overnight. Next, micronized copper azole-treated wipes or 300 µL of copper azole were transferred to 50 ml centrifuge tubes containing 30 ml of SSF or DI water and allowed to shake at 140 rpm for 1 hour at room temperature.

To collect the total copper (soluble + insoluble) fraction released from the wipes, a Buchner funnel system attached to a vacuum was set up and a 50 ml centrifuge tube was placed into the receiving flask to collect the solution. The wipe and solution were transferred to the

funnel and vacuum was applied to drain the solution from the wipes. An aliquot of the solution was collected and designated as the whole fraction. A separate aliquot of the solution was transferred to a 10 kDa centrifuge filter unit (Amicon Ultra-15, 10K, Millipore, Bedford, MA) and centrifuged at 5000 x g for 10 minutes. An aliquot of the filtrate was designated as the soluble fraction. The two collected fractions (solution and filtrate) were acidified in 2% nitric acid (67-70% Optima™, Fisher Scientific, Inc., Pittsburgh, PA) and stored at 4°C until further analysis via Inductively Coupled Plasma - Mass Spectrometry (ICP-MS). The Buchner funnel system was washed with 20 ml of 20% nitric acid and thoroughly rinsed with DI water between each sample (Fig. 1). Matrix (SSF only) and matrix spiked with 30 parts per billion (ppb) of copper standard were included in the bioaccessibility assay. Extractions for all wipes passed along wood boards were performed in duplicate for water and triplicate for SSF. Extractions for wipes treated with copper azole technical material were performed in duplicate for water and SSF. Extractions for copper azole technical material alone were performed in duplicate for water and SSF.

2.3 Total copper extraction from surface wipes

An additional set of wipes (three wipes per copper-treated wood type and two wipes per untreated wood type) were placed onto a cutting mat using acid-washed plastic forceps. An acrylic ruler and a rotary cutter were used to measure and cut, respectively, a 3 in.² square from the sample area of each wipe. The square was cut into 9-1 inch squares and each piece was weighed and the masses recorded. The average mass for all wipes was 1.9 ± 0.1 mg for wipes sampled from new boards and 1.81 ± 0.09 mg for wipes sampled from weathered boards. Each 1 inch square was transferred to a Teflon™ digestion vessel containing 10 ml of concentrated nitric acid (67-70% Optima™, Fisher Scientific, Inc., Pittsburgh, PA) and allowed to pre-digest for 15 minutes under a fume hood. After 15 minutes, the vessels were properly sealed and the samples digested using MARS-5 or MARS-6 microwave systems (CEM Corporation, Matthews, NC). All samples underwent microwave-assisted digestion for 15 minutes at an operating temperature of 200°C at 1200 W (100% power) with a maximum pressure of 800 psi under standard control settings. The method concluded with a 5 minute cooling period in the oven and an additional 1 hour cooling period in the fume hood. The samples were diluted to 2% nitric acid concentration prior to analysis by ICP-MS (Fig. 1). After ICP-MS analysis, the measured copper

concentrations for all 9 squares for each wipe were compiled to obtain total copper concentration per wipe. For each digestion set, reagent blanks and 30 ppm copper-spiked reagent blanks were also prepared and analyzed.

2.4 Instrumental Analysis

Backscatter electron - scanning electron microscopy (BSE-SEM) imaging (JEOL JSM-7600F, Tokyo, Japan) was used to confirm the presence of copper (Cu) particles on wipes passed along 1 month old MCA-1 and MCA-2-treated woods. Elemental analysis was performed using energy dispersive X-ray spectroscopy (EDS) mapping (Fig. 2). The quantification of Cu concentrations from surface wipe samples were performed according to USEPA Method 6020A using inductively coupled plasma-mass spectrometry (ICP-MS) (USEPA, 2007b) or USEPA Method 6010D using inductively coupled plasma-optical emission spectrometry (ICP-OES, Thermo Scientific, Waltham, MA, USA) (USEPA, 2014). Use of ICP-MS or ICP-OES was dependent on availability of the instrument and detection limits. Procedures for calibration, calibration verification, quality assurance and quality control as detailed in Methods 6010D or 6020A were also performed. Commercially available reference standards (VHG Labs, Manchester, NH) were used for analysis. Scandium was used as an internal standard (spectral line 335.5 nm) for ICP-OES and 45Sc and 89Y for ICP-MS. The method reporting limit for copper was 1.0 ppb (ICP-MS) or 2.5 ppb (ICP-OES). The linear dynamic range of 0.001 (ICP-MS) or 0.0025 (ICP-OES), to 2.0 ppm was verified based on successful recoveries of low and high level calibration verification solutions. Any solutions with Cu concentrations below the method reporting limit were reported as “below detectable reporting limits” and designated as “ND” for not-detectable. Duplicates, matrix spikes, and blanks were included in the analysis. The range of blanks were below the 1.0 ppb or 2.5 ppb lowest level calibration verification (LLCV) recovery limit. Duplicates were within 75-125% of the expected value. Matrix spiked samples were within 80-120% of the expected value. Dilution check samples were within 90-110% of the expected values (Supplementary 1).

To obtain the corrected copper concentration, the measured copper concentration ($\mu\text{g/L}$) was multiplied by the sample solution volume (L). Next, this value was divided by the average

mass of the wipe (g.). The total insoluble and soluble fractions that were released were expressed as percent *in vitro* bioaccessibility (IVBA) and were calculated using the formula:

$$\% \text{ IVBA} = \frac{\left(\frac{\text{in vitro extractable } \mu\text{g Cu}}{\text{wipe}} \right)}{\left(\frac{\text{Total } \mu\text{g Cu}}{\text{wipe}} \right)} \times 100\%$$

2.5 Statistical Analysis

Two-way ANOVA with significance levels at 0.05 or lower was used to examine the relationship between wood type and *in vitro* bioaccessibility. In cases where significance levels were below 0.05, post hoc Tukey's tests were performed to identify significant differences between each treatment. For any data that failed tests for normality and variance, square root transformations were undertaken to achieve normality and equal variance prior to analysis. All analyses were performed using SigmaPlot 13.0 (Systat Software Inc., San Jose, CA).

3.0 Results and Discussion

3.1 Total copper in surface wipes passed along the surfaces of ACA, MCA-1, and MCA-2 wood products

One purpose of this study was to ascertain the percent *in vitro* bioaccessibility (IVBA) of Cu released from surface wipes during exposure to simulated stomach fluid after the wipes were passed along the surfaces of three as-purchased or weathered copper-treated (ACA, MCA-1, MCA-2) and untreated boards. To calculate IVBA, total Cu concentrations present in the wipes and the relative percent of particulate Cu in soluble form released into SSF was determined. The concentrations of Cu transferred onto surface wipes during passage along untreated wood was significantly lower ($p < 0.001$, $0.01 \pm 0.03 \mu\text{g/wipe}$) than the Cu transferred to wipes passed along Cu-treated wood (Table 1). The amount of Cu released into SSF from wipes passed along untreated boards was unquantifiable for new and weathered boards. Among the as-purchased boards, the highest total Cu concentrations were released from MCA-1 wipes followed by ACA and MCA-2 (Table 1a). These results are consistent with a study conducted by Platten et al. (2016) in which the researchers investigated the simulated dermal transfer of micronized and aqueous copper azoles from boards to polyester wipes collected in a similar fashion to those used

for this study. Wipes sampled from as-purchased boards in the Platten study yielded Cu concentrations of approximately 1, 7.5, and 2 mg/m² for wipes passed along ACA, MCA-1, and MCA-2, respectively, for the first wipe event. In our study, wipe samples yielded Cu concentrations of 3 ± 2 , 15 ± 9 , and 2.7 ± 0.6 mg/m² for ACA, MCA-1, MCA-2-treated wipes. Platten et al. found that the amount of Cu transferred from ACA and two different MCA- wood types to polyester wipes decreased then stabilized during the course of a 399-day weathering process. In Platten et al.'s study as well as our study, the weathering process likely resulted in the leaching and release of Cu from all treated lumber types over time which decreased the total amount of Cu available to wipes passed along weathered ACA and MCA-treated boards.

3.2 Copper extracted from ACA, MCA-1, and MCA-2- treated surface wipes in DI water or SSF.

The amount of Cu extracted from surface wipes passed along Cu-treated lumber and exposed to either DI water (water-soluble fraction) or SSF (bioaccessible fractions) are reported as percentages of the total Cu measured in wipes. The total copper measured in the wipes are designated as "total Cu". The portion of copper released from the wipes into water or SSF, relative to the total, is designated as "released"; and the portion of soluble copper that was isolated from the released fraction using a 10 kDa filter is designated as "soluble" or "10 kDa fraction".

Values for the percent Cu released into water relative to the total Cu measured in the wipes for ACA, MCA-1, and MCA-2 wood-exposed wipes were $39.20 \pm 0.03\%$, $21 \pm 10\%$, and $27 \pm 4\%$, respectively. Soluble Cu (ionic) and organic complexes released from the wipes were separated from particulate Cu (complexed to larger wood fragments or present as particulate copper carbonate) by passage through a 10 kDa filter. The percentages of soluble Cu recovered from water extractions for new ACA, MCA-1 and MCA-2 boards were $33 \pm 3\%$, $6 \pm 1\%$, and $20 \pm 2\%$.

The recovery of released Cu from surface wipes passed along as-purchased ACA, MCA-1 and MCA-2 boards and subsequently exposed to SSF were $43 \pm 8\%$, $44 \pm 6\%$, and $100 \pm 24\%$, respectively. The soluble fractions of Cu detected in the 10 kDa filtrate of ACA, MCA-1, and MCA-2 wipe solutions after SSF exposure were $44 \pm 8\%$ for ACA, $45 \pm 6\%$ for MCA-1, and $100 \pm 9\%$ for MCA-2 (Table 1a). Wipes passed over weathered ACA, MCA-1, and MCA-2 wood released $100 \pm 44\%$, $100 \pm 22\%$, and $100 \pm 13\%$ Cu, respectively, after exposure to SSF. The

soluble Cu (10 kDa filtrate) in the wipe solution for weathered ACA was $100 \pm 66\%$, $100 \pm 15\%$ for MCA-1, and $100 \pm 12\%$ for MCA-2 (Table 1b). Individual values in excess of 100% can be attributed to variability in Cu concentrations along the sampling area of the boards. The source of this variability may stem from alterations in the uniformity of Cu on the boards surface during periods of wood transport and storage from manufacturer to study site.

The concentrations of Cu measured in surface wipes passed along as-purchased Cu-treated (ACA, MCA-1 and MCA-2) wood boards and exposed to DI water for 1 hour are shown in Table 1a and Fig. 3. The amount of soluble Cu which passed through the 10 kDa membrane from the water extract was significantly lower than the total copper measured in the wipes for the MCA-1 wipe sample suggesting the presence of Cu complexed to wood fragments or larger copper carbonate particles. This was not observed for the wipes passed over ACA or MCA-2 wood types. However, when the wipes from all wood types were treated with SSF, essentially all of the released Cu was in the soluble form. This was also observed for the weathered wood (Figure 4) where there was no significant difference between the total percent Cu released and the percent Cu that was recovered in the 10 kDa filtrate ($p = 0.938$). These results indicate that nearly all of the released copper was solubilized among all wood types and treatment conditions when treated with SSF. The high level of IVBA may be attributed to the low pH of the SSF (as compared to low Cu solubility in the neutral pH of water) (Dhyani and Kamdem, 2012; Santiago-Rodríguez et al., 2015). In a recent study in which we investigated IVBA of micronized Cu quaternary leachate from wood dust exposed to water and SSF, Cu solubility was greatly enhanced at pH 1.5 in SSF while little Cu dissolved at pH 7.0 in deionized water (Santiago-Rodríguez et al., 2015). Increased Cu dissolution in SSF may have also been facilitated by the binding of Cu to the carboxyl group in glycine ($\text{H}_2\text{NCH}_2\text{COO}$) included in the SSF solution. Kim et al. (2011) have shown that in the presence of glycine, Cu dissolution likely occurs due to the availability of more glycine carboxyl groups to which Cu can bind and then undergo conversion to soluble species, especially at acidic pHs. Based on our previous conclusions and Kim et al.'s findings we suggest that the low pH of SSF (pH 1.5) and the presence of glycine enhanced Cu bioaccessibility in all wipes containing Cu residues from Cu-treated wood samples. In contrast to high Cu dissolution in SSF, low solubility was found in wipes exposed to DI water. In addition to absence of glycine in the extractant, and the neutral pH of water, binding affinity may have also hindered Cu dissolution in water. In a study by Rafatullah et al. (2009), Cu ions adsorbed

and bound more tightly to cellulose and lignin in meranti sawdust at pH 6 and greater than at pH's 1-5. The authors attributed this behavior to the loss of positive charge and an increase in lignin and cellulose's electrostatic attraction to Cu ions at higher pH. In the case of our study, it is likely that the Cu ion and copper particulates which were already bound to the wood remained tightly bound to the organic wood components upon exposure to DI water.

In our previous study (Santiago-Rodríguez et al., 2015), we recorded 83-90% Cu dissolution from Cu-treated wood dust in SSF. In contrast to SSF, only 14-25% of the Cu released from Cu-treated wood dust was extractable in water. In this study, we expected nearly all of the total Cu measured in the surface wipes to be released in SSF for as-purchased and weathered woods. Although 100% of the Cu was released from wipes passed along weathered wood, only 40% of the total Cu was released from surface wipes passed along the as-purchased boards. This relatively low recovery of total Cu from the polyester wipes even at low pH may have resulted from copper complexing with glycine bound to the wipes, incomplete wetting by the extraction solutions, and/or physical adherence of wood fragments of lignin and cellulose to the polyester fibers. The strength of the bond between Cu and glycine bound to the wipes may have prevented copper from being released into and subsequently extracted from SSF. Platten et al. (2016) also found that 90% of the Cu in ACA and 50% of the Cu in MCA-1 were bound to lignin and cellulose in as-purchased boards. This complex may reduce the wettable surface area thereby making lignin-bound Cu less accessible to the dissolving properties of the surrounding acidic environment. As for weathered wood, in which there was 100% copper bioaccessibility, the Cu and lignin/cellulose complexes may have become unstable over time due to weathering and Cu particle and wood aging resulting in the dissociation of Cu from organic wood components and the conversion of copper carbonate to ionic Cu. Moreover, Cu that was impregnated in the wood may have migrated to the surface of the lumber when wood swelled and shrank during periods of simulated rain and heat (Siau, 2012). The migration of this Cu may have affected the release of Cu and the variability between replicates during this process.

Regarding differences in wood treatment type, Platten et al. (2016) noted that MCA-2 had smaller nanoparticle sizes than MCA-1. As particle size decreases, surface area increases (Borm et al., 2006). The higher surface area in MCA-2 relative to MCA-1 likely made MCA-2 more bioaccessible to the acidic environment resulting in enhanced dissolution in this study. The strength of Cu's bond to lignin and cellulose is also an additional factor that may be responsible

for incomplete release and recovery of MCA-2. Cu undergoes cation exchange reactions with the carboxylic groups in lignin and phenolic hydrolysis in cellulose resulting in the formation of stable complexes (Xie et al., 1995; Zhang and Kamdem, 2000) reducing the amount available for complexation with glycine.

For SSF, there were no significant differences between the Cu released from the wipes and 10 kDa filtrate fractions among the different wood types ($p=0.911$). Overall, the relative amounts of Cu released from the wipes into SSF for both soluble and insoluble Cu were highest for MCA-1 followed by MCA-2 and ACA for both as-purchased and weathered boards. The differences between wood conditions (as-purchased versus weathered) across all treatments were significant ($p<0.001$), with the new wood releasing more Cu than the weathered wood. It is likely that the bulk of the Cu originally present in the outermost layers of the new lumber leached out of the wood during the 399-day weathering process and prior to wipe sampling. Freeman et al. (2008) have shown that ACA has high water solubility and leaching rates. So enhanced leachability as a result of weathering was expected.

3.3 Bioaccessibility of copper azole and copper azole-treated wipes in SSF and water

The behavior of technical micronized copper azole (similar to that used for pressure-treated lumber) was investigated in solution (DI water, SSF) and after application to the polyester surface wipes. The total concentration of micronized copper azole technical material (MCA-tech) measured after dilution in water from a concentrated stock solution was 315 ± 0.849 mg/L. The soluble fraction (the amount filtered through 10 kDa filters) was 0.12 ± 0.02 mg/L or 0.04% of the total Cu. Of the total amount of MCA-tech added to SSF, 97% was soluble passing through the 10 kDa filter (Figure 5). The total amount of Cu released from wipes directly treated with MCA-tech, dried, and subsequently exposed to water was $47 \pm 8\%$. This result suggests that the particulate copper carbonate bound to the polyester wipe material was not efficiently extracted in water. This result partly accounts for the poor extraction efficiency for the wipes passed over the various wood types. Only 0.1% of the total Cu was soluble (detected in the 10 kDa filtrate). In contrast to water, the total percent Cu released from wipes upon exposure to SSF was $110 \pm 9\%$. The soluble Cu released (detected in the 10 kDa filtrate) was $105 \pm 3\%$ (Figure 5). The 97% release of Cu from micronized copper azole incubated in SSF is similar to the results observed in our previous study in which the release of Cu from wood dust exposed to SSF

was approximately 90%. These data suggest that Cu from micronized copper azole in suspension dissolves to a significant degree in SSF. Further, upon application of MCA-tech to surface wipes followed by incubation in SSF, the percent bioaccessibility remained close to 100%.

4.0 Conclusions

In this study we used simulated surface transfer and *in vitro* bioaccessibility methods to investigate hand-to-mouth transfer and subsequent gastric exposure to Cu originating from as-purchased and weathered Cu-treated lumber. When compared to weathered boards, our data indicates greater concentrations of Cu were transferred from newly purchased boards to surface wipes and the bulk of the transferred and released Cu was bioaccessible for all wipes passed along Cu –treated woods prior to SSF exposure. Surface wipes had negligible influence on Cu bioaccessibility and copper azole alone completely solubilized in SSF. This provided further confirmation that the copper azole compound is highly bioaccessible in SSF while Cu remains only slightly soluble in water. The high level of GI bioaccessibility implies a potential for an elevated short term risk of copper ion exposure during use of this popular class of treated wood. Also, the results of this study and previously published literature suggest that children may be exposed to ionic copper through interaction with structures constructed from micronized-copper treated wood.

Young children (from birth to age 10) often engage in frequent hand-to-mouth behaviors (Moya et al., 2004) (with an average of 9.5 and 20 hand-to-mouth events per hour for intermediate and short term exposure, respectively) (Tulve et al., 2002) thus putting children at risk of Cu exposure. The Institute of Medicine's Food and Nutrition Board set a Tolerable Upper Intake Limit (TUIL) for Cu at 1000 µg/day for children 1-3 years old and 3000 µg/day for children 4-8 years old (Trumbo et al., 2001). Platten found that levels as high as 2580 µg of Cu may be transferred to hands based on simulated exposure scenarios. We concluded that 44% (MCA-1) and 100% (MCA-2) of Cu is released from micronized copper-exposed wipes and nearly all of the released Cu is bioaccessible in SSF. Our findings coupled with Platten's findings suggest that about 1032 µg of Cu for MCA-1 and about 2580 µg of Cu for MCA-2 may be released from surface wipes and would be solubilized upon oral ingestion. For children between 1 and 8 years old, rubbing micronized copper-treated wood boards and subsequently putting contaminated hands in their mouths may expose children to Cu amounts that meet or exceed the

daily TUIL, exclusive of the Cu typically ingested from their normal diet and habits (e.g. taking vitamins). While this study does not examine the toxicological implications of children's exposure to levels of Cu above the TUIL, future studies should be conducted to address the negative effects of short and intermediate exposures to these environmentally relevant Cu levels.

Disclaimer

The United States Environmental Protection Agency (U.S. EPA), through its Office of Research and Development (ORD), has funded and managed the research described here. It has been subjected to the Agency's administrative review and has been approved for publication. Certain trade names and company products are mentioned in the text or identified in illustration in order to specify adequately the experimental procedures and equipments used. Such identification does not imply endorsement or recommendation for use by the U.S. EPA.

Acknowledgements

This project was supported in part by J. L. Griggs's appointment to the Research Participation Program at the Office of Research and Development, U.S. Environmental Protection Agency, administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and U.S. EPA. We would like to thank Dr. Lenibel Santiago-Rodriguez for her technical support in developing the Cu fractionation method and John Misenheimer for his technical support with the ICP-OES measurements.

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Figure 1. Pictorial diagram of extraction method

Figure 2: (a) BSE-SEM micrograph of MCA-1- treated wood fibers recovered from a cloth wipe and deposited onto a 0.45 mm membrane filter. (b) BSE-SEM micrograph of Cu found in MCA-2-treated wood fibers recovered from a cloth wipe and deposited onto a 0.45 mm membrane filter. The red arrow and circle identify crystalline forms of Cu present. (c) EDS chemical map of Cu within the solid materials identified in the BSE for MCA-2.

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552 Table 1: Total Cu concentrations in wipes passed along weathered or as- purchased wood,
 553 copper concentrations released into water or SSF, and soluble copper concentrations in the 10
 554 kDa filtrate after exposure to water or SSF. (a) The concentrations of copper detected and
 555 released from surface wipes passed along as- purchased lumber and exposed to water (N=2) or
 556 SSF (N=3) for 1 hour. (b) The concentrations of copper detected and released from surface
 557 wipes passed along weathered lumber and exposed to SSF for 1 hour (N=3).

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559 a. As-Purchased

Wood Type	Total copper on wipes in $\mu\text{g/wipe}$ (whole wipe microwave digests)	WATER		SSF	
		Copper extracted in water in $\mu\text{g/wipe}$ (Percent)	Copper solubilized in water in $\mu\text{g/wipe}$ (Percent) (10 kDa centrifugation)	Copper extracted in SSF in $\mu\text{g/wipe}$ (Percent)	Total copper solubilized in SSF in $\mu\text{g/wipe}$ (Percent) (10 kDa centrifugation)
Untreated	3.0	3.0 ± 2.0 (100)	1.7 ± 0.6	2.6 ± 1.8 (90 $\pm$ 60)	2.6 ± 1.8 (90 $\pm$ 60)
ACA	130 ± 90	52.28 ± 0.05 (39.20 $\pm$ 0.03)	44 ± 4 (33 $\pm$ 3)	57 ± 11 (43 $\pm$ 8)	58 ± 11 (43 $\pm$ 8)
MCA-1	672 ± 387	142 ± 75 (21 $\pm$ 10)	42 ± 8 (6 $\pm$ 1)	300 ± 41 (44 $\pm$ 6)	304 ± 43 (45 $\pm$ 6)
MCA-2	119 ± 25	32 ± 5 (27 $\pm$ 4)	24 ± 3 (20 $\pm$ 2)	159 ± 29 (100 $\pm$ 24)	158 ± 11 (100 $\pm$ 9)

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561 b. Weathered

Wood Type	Total copper on wipes in $\mu\text{g/wipe}$ (whole wipe microwave digests)	Copper extracted in SSF in $\mu\text{g/wipe}$ (Percent)	Copper solubilized in SSF in $\mu\text{g/wipe}$ (Percent) (10 kDa centrifugation)
UNTREATED	0.03	0.03 ± 0.2 (4.0 $\pm$ 2.0)	0.004 ± 0.002 (0.4 $\pm$ 0.2)
ACA	0.023 ± 0.003	0.03 ± 0.01 (100 $\pm$ 44)	0.04 ± 0.02 (100 $\pm$ 66)
MCA-1	0.8 ± 0.3	0.9 ± 0.2 (100 $\pm$ 22)	0.9 ± 0.1 (100 $\pm$ 15))
MCA-2	0.10 ± 0.01	0.10 ± 0.01 (100 $\pm$ 13)	0.10 ± 0.01 (100 $\pm$ 12)

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Figure 3. Total Cu released from surface wipes and soluble Cu recovered in the filtrate after wipes were passed along as-purchased ACA, MCA-1, or MCA-2 lumber, exposed to water or SSF for 1 hour and, for soluble fractions, passed through filters with 10 kDa pore size. Cu concentrations are expressed as $\mu\text{g}/\text{wipe}$.

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Figure 4. Total Cu released from surface wipes and soluble Cu recovered in the filtrate after wipes were passed along weathered ACA, MCA-1, or MCA-2 lumber, exposed to water or SSF for 1 hour and, for soluble fractions, passed through filters with 10 kDa pore size. Cu concentrations are expressed as $\mu\text{g}/\text{wipe}$.

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598 Figure 5. Release of micronized copper azole from surface wipes treated with technical copper
599 azole and the dissolution of copper azole after exposure to water or SSF. Total Micronized
600 copper azole technical material (MCA-tech) alone and as released from surface wipes and
601 soluble Cu recovered in the filtrate after MCA-tech or surface wipes treated with MCA-tech were
602 exposed to water or SSF for 1 hour and, to obtain soluble fractions, passed through filters with
603 10 kDa pore size. Cu concentrations are expressed as mg/L.

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