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Heavy Metal Toxicity of Pressure Treated Wood Leachates with MetPLATE™

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Chemical preservatives are added to manufactured wood products to prevent biological decay. Preservative-treated dimensional lumber, poles and plywood are used to construct fences, decks, docks, utility poles and other all-weather structures. Building contractors also utilize small amounts of treated wood products in the construction of residential and commercial structures. Over the years, several different preservative formulations have been developed by the wood preservative industry, including the oil-borne preservatives creosote and pentachlorophenol and the water-borne preservative chromated copper arsenate (CCA). In recent decades, chromated copper arsenate has been the most prominent wood preservative. The American Wood Preserving Institute (AWPI) reported that in 1997, approximately 144 million pounds of CCA were utilized in the US for the production of 450 million cubic feet of treated wood (AWPI, 1997). Nearly 15% of the CCA-treated wood products in the US are produced in Florida (Solo-Gabriele et al., 1997). Concerns over the impact of the heavy metals used in CCA on human health and the environment prompted the industry to stop the manufacture of CCA-treated wood products for most residential uses at the end of 2003 (USEPA, 2002). The new generation of wood preservative solutions standardized by the American Wood Preservers' Association (AWPA, 1996) and commercially available in the US in recent years include a variety of copper-based chemicals, such as alkaline copper quaternary (ACQ), copper boron azole (CBA), copper citrate (CC) and copper dimethyldithiocarbamate (CDDC). The potential impact on human health and the environment from wood treated with these copper-based preservatives has not been researched to the same extent as those treated with CCA. While the absence of arsenic is favorable from a human health perspective, the additional copper raises concerns with respect to impact on aquatic ecosystems (Flemming et al., 1989).

One method of assessing the relative impact of different wood preservatives on aquatic environments is to leach the wood samples in the laboratory and to measure the chemical concentrations in the leachates. The measurement of chemical leaching alone, however, provides only part of the information needed. Chemical measurements do not indicate whether the leached chemical is bioavailable to the species of concern. Toxicity testing is needed for such an evaluation. In recent years, toxicity test kits have increased in popularity as tools

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for the rapid evaluation of adverse environmental effects on aquatic organisms (Petanen et al., 2003; Gutierrez et al., 2002). In most cases, these kits detect general toxicity. The rapid enzymatic assay MetPLATE™, however, is specific for the detection of heavy metal toxicity (Bitton et al., 1994). The assay is highly sensitive to many metals (Nelson and Roline, 1998) and has been used to detect metal toxicity in various environmental samples (Bitton and Morel, 1998).

This paper examines the utility of the MetPLATE™ assay for assessing the toxicity of leachates produced from wood products treated with heavy metal-containing wood preservatives. Since the toxicity resulting from a heavy metal depends on the bio-available portion of the concentration of metal in solution, there are situations where the preservatives leached from the treated wood products will be bound by naturally occurring chemicals, reducing the environmental risk posed. Humic and fulvic acids, for example, may complex with copper reducing its bioavailability. Since Met-PLATE™ is specific to heavy metal toxicity and because it is a rapid assay (typically 3-h per test), it has the potential to be a useful screening tool. The experiments presented in this paper were conducted as part of a larger study (Townsend et al., 2003) that evaluated the leachability of wood treated with CCA and Cu^{+2}-based preservatives. The general aquatic toxicity of these leachates was compared using an algal, an invertebrate and a bacterial assay. Heavy metal-specific toxicity was assessed using MetPLATE™. The MetPLATE™ results from these experiments, as well as several additional experiments, are presented herein.

MATERIALS AND METHODS

A uniform stock of untreated southern yellow pine was purchased and sent for preservative treatment. The details concerning the wood selection and preservation procedure are available (Townsend et al., 2003). Four bundles, each comprised of eight wood pieces, were sent to chemical manufacturing facilities for treatment (CCA-1, CBA, CC and ACQ). A fifth bundle was sent to a separate wood treatment facility for CCA-treatment (Sample CCA-2). Additional southern yellow pine was set aside as an untreated control. The only treated wood sample not obtained in this fashion was the CDDC-treated wood. Shortly after the commencement of the research, the CDDC manufacturing facility closed. Samples of CDDC-treated wood previously produced by the manufacturer were utilized in the study.

Treated wood samples were individually leached with four leaching fluids: the Toxicity Characteristic Leaching Procedure (TCLP) fluid, the Synthetic Precipitation Leaching Procedure (SPLP) fluid, deionized (DI) water, and synthetic seawater (SW). Different extraction fluids were utilized to determine how the chemical constituents of treated wood might behave under different environmental conditions. The TCLP fluid (a buffered acetic acid solution; EPA Method 1311) was designed to simulate leaching conditions in solid waste landfills whereas the SPLP fluid (a diluted mixture of sulfuric and nitric acid;

EPA Method 1312) simulates the leaching of solid waste exposed to acidic rainfall. The other leaching fluids used (DI and SW) were not from any standardized leaching test protocol, but represent fluids often used in previous studies on treated wood. The synthetic seawater was prepared by mixing Instant Ocean™ with distilled water according to the manufacturer's instructions.

The treated wood samples utilized in the leaching tests were cut into small blocks (2.5 by 5 by 10 cm) using a mill saw. The blocks were then ground to less than 3 mm using a Fritsch Pulverisette® 19-mill grinder. The objective of the size reduction was to meet the requirements in standard waste leaching procedures (<0.95 cm in its shortest dimension), and to create a homogeneous sample for comparison of relative leaching. The leaching solutions were prepared according to US EPA SW 846 Methods (1996). Two liters of leaching fluid were combined with 100 g of ground wood (yielding a 1:20 solid to liquid ratio) in a glass container which was capped with a Teflon-lined lid and placed on a rotary extractor for 18 ± 2 hours. The recovered leachates were filtered using a pressurized filtration apparatus with a 0.7-µm borosilicate glass fiber filter (Environmental Express TCLP filters). Separate aliquots were removed for metal analysis and toxicity testing. The samples for metal analysis were preserved (pH <2) using nitric acid, while those for the toxicity assays were analyzed immediately following filtration or were stored in a freezer until analysis.

As a follow up to the standardized batch tests with ground wood, a second set of experiments was conducted using whole wood blocks. Wood blocks of approximately 80 grams were cut from the same source of treated wood lumber as discussed above using a 25-cm power miter saw. The wood blocks were prepared in triplicate and were placed in glass jars containing 1600 mL of DI water for 24 hours. This again resulted in a 1:20 solid to liquid ratio. The samples for metal analysis were collected using the pressure filtration procedure previously described and were preserved accordingly. Samples for toxicity testing were collected prior to filtration and were stored in a freezer until analysis.

The preserved samples were acid digested (EPA 3010A) and analyzed (EPA Method 6010B) by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). The instrument detection limits were 0.03 mg/L, 0.04 mg/L and 0.05 mg/L for arsenic, chromium, and copper, respectively.

Leachate samples were tested for heavy metal toxicity using MetPLATE™, which is based on inhibition of ß-galactosidase activity in an *E. coli* strain (Bitton et al., 1994). The MetPLATE™ kit contains a lypophilic bacterial reagent, chlorophenol red galactopyranoside (CPRG) that serves as the substrate for *β*-galactosidase, and moderately hard water (MHW) as a diluent. The bacterial reagent was rehydrated with 5-mL of diluent and thoroughly mixed by vortexing. A 100-μL aliquot of the bacterial reagent was added to a 900-μL aliquot of the leachate or its appropriate dilution in a test tube and mixed by vortexing. Test tubes were incubated for 90 minutes at 35^0C. A 200-μL aliquot of the suspension (leachate +

bacteria) was transferred to a 96-well microplate to which 100 μL of CPRG (the enzyme substrate) was added, followed by shaking. The microplate was incubated at 35°C for color development. Conversion of the yellow substrate (CPRG) to the purple product (chlorophenol red) was quantified at 570 nm using a Multiskan microplate reader. Results for the toxicity tests were expressed as the percent of leachate diluted with moderately hard water that produced a 50% reduction in enzyme activity (EC_{50}). Positive copper controls (using a solution prepared from $CuSO_4$) were also assayed.

RESULTS AND DISCUSSION

Leachates from untreated southern yellow pine were found to be non-toxic to the MetPLATE™ test bacteria (As, Cr and Cu were not detected in the untreated wood leachates). The treated wood leachates all showed some degree of toxicity using MetPLATE™. The results of all six treated wood samples for both experiments are summarized in Table 1. The results include both EC_{50} values and the corresponding concentrations of copper, chromium and arsenic (in mg/L).

The EC_{50} values obtained in experiment 1 (the batch tests) ranged from 0.46 to 2.26% for CCA and from 0.12 to 1.52% for the copper-based preservatives. As a whole, the toxicity was greatest (i.e. lower EC_{50} values) in leachates from the wood samples treated with the copper-based preservatives relative to leachates from CCA-treated wood. This follows the same trend as found using algal, invertebrate and MicroTox™ assays (Townsend et al, 2003). The wood products treated with the copper-based preservatives leached less arsenic and chromium (in this case below detection), but leached greater concentrations of copper (up to 117 mg/L in the case of copper citrate leached with TCLP). While the test kit is highly sensitive to copper (EC_{50} of 0.11 mg-Cu^{+2}/L), it is less sensitive to chromium (EC_{50} of 6.9 mg-Cr^{+3}/L; Bitton et al., 1994) and not sensitive to arsenic. As a note, while the chromium in CCA treating solution does occur in the hexavalent form (Cr^{+6}), it is reduced to the trivalent form (Cr^{+3}) in the wood.

The leachates from experiment 2 were found to have lower concentrations of heavy metals relative to experiment 1, and the EC_{50} values were correspondingly higher (they were less toxic). The reason for the lower concentrations was the greater particle size; experiment 2 leached 80-g wood blocks while experiment 1 leached wood with the consistency of sawdust. The blocks in experiment 2 were also leached under quiescent conditions, where the experiment 1 samples were rotated end over end. Again, the CCA-treated wood leachates (EC_{50} values of 38 and 39.6%) were less toxic than any of the copper-based treated wood leachates tested (EC_{50} values ranging from 0.7 to 6.9%).

Since copper was suspected to be the primary toxicant to the MetPLATE™ bacteria, the EC_{50} values measured for all leachate samples were plotted as a function of their corresponding copper concentrations (see Figure 1) for each treated wood sample. For the purpose of comparison, the same scale was used for each axis in each plot. The figure shows the lower toxicity of the less-

Table 1. Metal content and toxicity of chemically treated wood leachates.

Sample[a]	EC_{50} (%)	Cu (mg/L)	Cr (mg/L)	As (mg/L)	Type of Sample
CCA-1 DI	1.74 (0.96)[b]	3.32 (0.1)	1.95 (0.06)	6.19 (0.17)	Sawdust
CCA-1 TCLP	0.46 (0.21)	8.70 (0.06)	3.33 (0.15)	7.88 (0.31)	Sawdust
CCA-1 SPLP	1.68 (0.35)	4.13 (0.12)	2.47 (0.07)	8.90 (0.21)	Sawdust
CCA-1 SW	1.15 (0.42)	10 (0.52)	1.84 (0.08)	3.78 (0.32)	Sawdust
CCA-1 DI	39.6 (17.4)	0.39 (0.16)	0.31 (0.12)	1.8 (0.8)	Blocks
CCA-2 DI	1.96 (0.12)	4.03 (0.15)	2.11 (0.07)	6.38 (0.14)	Sawdust
CCA-2 TCLP	1.0 (0.08)	8.67 (0.39)	3.36 (0.15)	7.87 (0.26)	Sawdust
CCA-2 SPLP	2.26 (0.3)	3.97 (0.20)	2.08 (0.06)	6.44 (0.08)	Sawdust
CCA-2 SW	0.89 (1.13)	10.0 (0.46)	1.81 (0.04)	3.64 (0.08)	Sawdust
CCA-2 DI	38.0 (7.35)	0.65 (0.17)	0.36 (0.14)	0.61 (0.23)	Blocks
ACQ DI	0.42 (0.04)	28.8 (1.02)	<0.04	<0.03	Sawdust
ACQ TCLP	0.2 (0.03)	79.2 (1.97)	<0.04	<0.03	Sawdust
ACQ SPLP	0.8 (0.09)	29.04 (1.03)	<0.04	<0.03	Sawdust
ACQ SW	0.22 (0.03)	42.1 (1.6)	<0.04	<0.03	Sawdust
ACQ DI	5.94 (0.43)	8.94 (3.66)	<0.04	<0.03	Blocks
CBA DI	0.32 (0.06)	27.4 (0.52)	<0.04	<0.03	Sawdust
CBA TCLP	0.18 (0.03)	54.4 (1.74)	<0.04	<0.03	Sawdust
CBA SPLP	0.24 (0.03)	26.7 (0.23)	<0.04	<0.03	Sawdust
CBA SW	0.41 (0.02)	43.4 (0.85)	<0.04	<0.03	Sawdust
CBA DI	4.97 (0.15)	11.1 (1.3)	<0.04	<0.03	Blocks
CC DI	0.14 (0.05)	63.6 (2.4)	<0.04	<0.03	Sawdust
CC TCLP	0.15 (0.03)	116.5 (0.8)	<0.04	<0.03	Sawdust
CC SPLP	0.12 (0.03)	61.8 (2.74)	<0.04	<0.03	Sawdust
CC SW	0.16 (0.04)	55.12 (1.75)	<0.04	<0.03	Sawdust
CC DI	0.7 (0.06)	24.08 (4.08)	<0.04	<0.03	Blocks
CDDC DI	1.52 (0.3)	6.76 (0.28)	<0.04	<0.03	Sawdust
CDDC TCLP	0.97 (0.08)	10.45 (0.38)	<0.04	<0.03	Sawdust
CDDC SPLP	1.32 (0.01)	7.08 (0.05)	<0.04	<0.03	Sawdust
CDDC SW	0.66 (0.09)	10.54 (0.07)	<0.04	<0.03	Sawdust
CDDC DI	6.93 (1.04)	1.28 (0.15)	<0.04	<0.03	Blocks

[a] See introduction for definition of acronyms, [b]Arithmetic mean (standard deviation) from three replicates

concentrated leachates from experiment 2, and illustrates the pattern of increased copper concentrations exhibiting more toxicity. Lines representing the toxicity expected to occur solely as a result of dissolved free copper (Cu^{+2}) are plotted as dashed lines. These lines were created using the EC_{50} values obtained for Cu^{+2} from a copper sulfate solution (0.11 mg/L). The EC_{50} in % (the % of leachate diluted in moderately hard water) that would result in a copper concentration equal to 0.11 mg/L was calculated for a series of leachate copper concentrations. A majority of the EC_{50} (%) and copper (mg/L) measurements fell within the range that would be expected from Cu^{+2} toxicity alone. This indicates that copper was the primary element causing toxicity. This is expected since MetPLATE™ is heavy metal specific, with copper being the most sensitive of the three metals. The organic co-biocides added to the copper-based preservatives should not impact toxicity. The CCA-treated wood samples tested leached the lowest copper concentrations and showed the least toxicity. Next lowest was the CDDC-treated wood. The ACQ- and CBA-treated wood leachates were of similar magnitude. The CC-treated wood samples leached the most copper and were found to have the highest MetPLATE™ toxicity (the lowest EC50 values). It is noted that the wood preservatives most likely to replace CCA in the residential sector are ACQ and CBA.

The results presented above demonstrated that MetPLATE™ was a sensitive tool for comparing aquatic toxicity of the different treated wood products. When compared to results from algal, invertebrate, and bacterial general toxicity assays (Townsend et al. 2003), MetPLATE™ was found to predict the same degree of relative toxicity among treated wood samples. MetPLATE™ was found to be less sensitive than the algal and invertebrate assays, but more sensitive than MicroTox™.

The needed sensitivity of a toxicity test will depend on the leachate concentrations encountered. As seen by the differences between experiments 1 and 2, the type of leaching procedure performed will impact the metal concentrations and thus the measured toxicity. MetPLATE™ was adequately sensitive even in the case when treated wood blocks were leached under quiescent conditions. Certainly one must use caution when applying the results of laboratory leaching tests (and subsequent toxicity assays) to predict risk in the aquatic environment (where factors such as dilution must be considered), but the use of a rapid test on leachates created in the laboratory can be a good screening tool for relative toxicity.

The applicability of MetPLATE™ for assessing the toxicity of future wood preservative compounds will depend on the type of preservative. Many of the newer proposed preservatives do not utilize heavy metals. Some are based on inorganic chemicals such as boron, while others rely solely on organic preservatives.

Considering the fact that ACQ and CBA are the most likely replacements for CCA (at least in the near term), perhaps the greatest utility for a rapid heavy-metal specific assay such as MetPLATE™ is to assess the impacts of natural water bodies on reduction in toxicity. When faced with the decision of whether to use wood preserved with ACQ or CBA for an application such as a dock or pier, or switching to an alternative such as plastic lumber or concrete, potential aquatic toxicity will likely be one of the factors considered. As described earlier, however, many natural waters have chemicals present that will reduce the bioavailability of copper. For example, Ma et al. (1999) showed that the toxicity of copper to *Ceriodaphnia dubia* decreased with increasing copper complexation with dissolved organic carbon (DOC). Some water bodies contain large concentrations of DOC which may act to limit the impact of copper leached from. a preserved wood structure. MetPLATE™ may be valuable tool for assessing the ability of a natural water body to bind copper from CCA-, ACQ-, and CBA-treated wood when used in aquatic applications. This warrants further exploration, and work would be needed to develop protocols to translate results

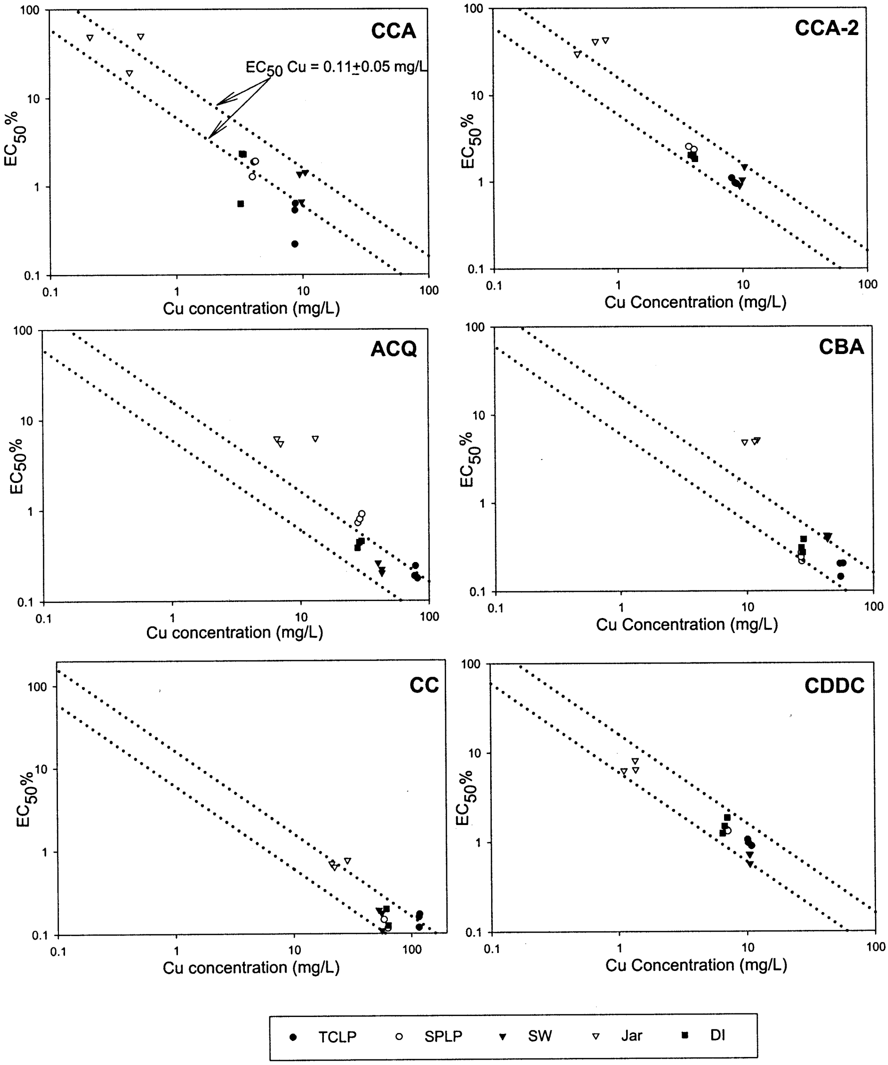

Figure 1 Copper concentration (mg/L) vs. toxicity EC_{50}
(The dotted lines indicate the range of toxicity measured from Cu^{+2} alone)

from tests on laboratory leachates to real world environments.

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