A Preliminary Comparative Toxicity Assessment of Materials Used in Aquatic Construction

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This project was undertaken by Environment Canada in an effort to acquire sufficient toxicity data on materials that may be used for construction of projects in the aquatic environment, such as wharves, bridges, docks and boardwalks. Materials for toxicity testing were provided by members of the Canadian Institute of Treated Wood (CITW), Enviroage Plastics and Bois Ecotherm. Additional materials were purchased by Environment Canada. Employees of Environment Canada were responsible for collection of test materials and the toxicity testing was conducted at Environment Canada's Atlantic Region Toxicity Laboratory, Moncton, NB. The authors would like to thank the members of the CITW for their assistance, Bois Ecotherm and Enviroage Plastics for provision of their samples and the Environment Canada laboratory staff for their diligent testing.

ABSTRACT

Environment Canada undertook a preliminary comparative toxicity assessment of selected materials that may be utilized in construction projects located in the aquatic environment. That project involved introducing Fahlstrom sized test stakes (3.9x0.54x26.4 cm) to both fresh and salt water for toxicity testing. Rainbow Trout, *Daphnia magna* and Microtox testing was conducted in freshwater and Threespine Stickleback were utilized for the salt water testing. Test protocols followed the Environment Canada Acute Lethality Tests 1990-1992. This preliminary assessment utilized untreated Hemlock, Plastic Wood, Wood treated with Life Time Wood Treatment, Bois Ecotherm, and stakes treated with CCA, Creosote or ACQ. Prior to introduction of the test organisms, the test materials were submerged in tanks of various sizes, using glass as a counterweight for 24 hours. The toxicity results suggest that Plastic Wood, Untreated Hemlock, Ecotherm Wood and wood treated with Lifetime Wood Treatment are generally non-toxic to the test species in the freshwater or the marine environment. The other three treatments (i.e. CCA, Creosote, ACQ) were toxic in varying degrees, and the testing indicated that Creosote and CCA are less toxic than ACQ treated wood.

This report has been prepared to release the data in a timely fashion. The testing program is on-going, and the report will be released in a revised version as results of tests on additional treated and untreated wood samples and other construction materials are obtained.

1.0 INTRODUCTION

Locally sawn timber, for centuries, was the material of choice for many construction projects in the aquatic environment, particularly those projects that were in close proximity to forest lands, including the construction of bridges and wharves. It was noted that certain species of trees did not withstand the elements or biological attacks as well as had been anticipated in comparison to the effort of construction. Graham (1973) indicates in his History of Wood Preservation that in the Eastern Mediterranean there were efforts to protect wood from decay as early as 2000 B.C. Protection of wood structures had escalated over the centuries such that by 1838 a pressure treating vessel was utilized for the treatment of lumber, a process patented by John Bethel and this process remains as the basis for wood treating till the present day (Freeman et al, October 2003). Therefore from that timeframe forward many projects that were undertaken in water were constructed with treated lumber. Eventually concerns were expressed over the potential aquatic toxicity of structures built in and over the water, introducing chemical preservative to the receiving water. Specific treatment and use pattern information had not been determined and there were questions arising over the environmental safety of structures placed in the water.

In 1997 the Wood Treatment Industry released a document on the Best Management Practices for the Use of Treated Wood in Aquatic Environments. That document outlined the treatment rate for various species of wood and intended final use and also included the post treatment requirement for removal of residual deposits through additional vacuum or steaming in the treatment chamber. In the early 2000's, the Pest Management Regulatory Agency (PMRA) included in the label directions " Use Limitations " for each heavy duty wood preservative product, which further defined where and how the treated products may be used. Some treated wood products may be used in water, while others may only be used in the splash zone, interpreted to be normally out of the water.

The public, contractors and other regulators have been seeking advice from many federal (including Environment Canada), provincial and municipal authorities on the environmental safety of the use of construction materials (including wood treated with heavy duty wood preservatives) in aquatic environments and potential alternative products. Although a re-evaluation of the heavy duty wood preservatives has been undertaken in North America, there has never been a consolidated effort to compare the environmental acceptability of all construction materials that are in use in aquatic construction, and thus allow a comparison of their potential effects when alternative materials are available for use. In an attempt to address this use pattern and aquatic toxicity of treated wood products, commercial wood treating companies were approached to acquire treated wood samples that could be used in a comparative toxicity assessment of materials that may be used in aquatic construction projects within the region. Test stakes (Fahlstrom) were provided from commercial wood treatment facilities along with untreated stakes. Recycled plastic lumber was provided by a Plastic Wood Manufacturer and Lifetime Wood Treatment was purchased at a regional distributor.

Those initial materials were subjected to four different Environment Canada Acute Lethality Tests. The tests were conducted on samples of wood and plastic that were milled to dimensions (3.9 cm. wide, 0.54 cm. high and 26.4 cm. long) that are in use by the wood treating industry as standard size test stakes (Fahlstrom). Test materials for this study included the following materials: Bois Ecothermo Wood, Plastic Lumber, Untreated Wood Stakes, stakes Treated with Lifetime Wood Treatment and stakes treated with CCA, Creosote or ACQ.

“ Bois Ecothermo Wood “, (a registered trade name) is produced through a heating process which involves increasing oven temperatures to between 185 and 215 degrees celsius drying the wood to the point of cell damage. This process is conducted with kiln dried, rough sawn lumber. Material destined for the market place is re-sawn or planed as required.

Plastic Lumber is made from recyclable plastic material. Individual boards or planks are formed through an extrusion process following heating and blending. This material consisted largely of plastic wrap, from hay bale storage, plastic pellets and chipped plastic bottles; as much of the material had been used outdoors and stored outside, the final product contained significant grit or sand which increased the difficulty of dimensional cutting. This material was sawn to the dimensions of the Fahlstrom Stakes.

Hemlock lumber cut to the dimensions of Fahlstrom Stakes were provided by one of the regional wood treatment facilities. These stakes were utilized for control samples for the study.

Lifetime Wood Treatment granules were mixed according to instructions provided on the package and painted on wood stakes. This material was purchased from a local retailer. It was painted onto several of the untreated Hemlock lumber stakes as per the manufacturer’s instructions.

Fahlsrom Stakes treated with a number of the heavy duty wood preservatives were received from regional wood treating facilities. Samples were received that had been treated with CCA (Chromated Copper Arsenate) at 6.4 kg/m^3 and also 22.4 kg/m^3; Creosote stakes had been treated at 40.4 kg/m^3 and ACQ (Alkaline Copper Quaternary) treated at 4 kg/m^3 and 6.4 kg/m^3. This pressure treating process involves the creation of a vacuum in the treatment cylinder, prior to flooding of the cylinder with the preservative treatment chemical, creating pressure. Sufficient time is then allowed for penetration, and finally in an effort to reduce the potential for leaching, surface flushing of the treated wood occurs with the normal diluent for that preservative or steam treatment for the water based treatments,

2.0 Methods and Materials

Testing was conducted according to the Environment Canada Aquatic Toxicity Test Methods for Rainbow Trout, Microtox, *Daphnia magna* and Threespine Stickleback.(Environment Canada 1990a, 1990b, 1990c and 1992).

2.1 Rainbow Trout Test Protocol

Testing was conducted according to the Environment Canada Aquatic Toxicity Test Method for Rainbow Trout (Environment Canada 1990a). If more than one stake was used, they were separated by small pieces of glass tubing so that the test water contacted almost the entire surface of each stake (refer to photograph 1).

Photograph 1

For the fish tests, the stakes would fit in the test containers, so the stakes were submerged in the test solutions by attaching them to glass rods with nylon cable ties (refer to photograph 1). If the glass rods floated, they were weighted down (above the surface of the water) so that the stakes were totally submerged (refer to photograph 2).

Photograph 2

The test solutions were prepared for these samples in dechlorinated City of Moncton municipal water, the same water used for holding of the rainbow trout. Due to the fish size, the volume chosen was 20 to 40 litres. All test solutions were pre-aerated for 30 minutes, initial water quality measurements were performed. Five to ten (first tests 5 all the last were 10) fish were randomly introduced into each test concentration. The test solutions were checked for mortalities frequently throughout the first day, then once a day thereafter until 96 hour termination. Any dead fish were removed when observed. Water quality measurements were performed daily. After 96-hours any surviving fish were euthanized. The lengths (cm) and weights (g) of the control fish were measured and the loading density was calculated. The percent survival at each concentration was determined.

A reference toxicant test was conducted with phenol once a calendar month. Using the mortality data at each test concentration, the 96 hour LC50 (concentration calculated to cause 50% mortality after 96-h exposure) was calculated using the methods of Stephan (1977). These values were entered into the control chart to ensure normal operating conditions were maintained, and that the population of fish used in the test was of normal sensitivity.

2.2 *Daphnia magna* Test Procedure

48-hour static bioassays were started on the samples using *Daphnia magna* neonates that were less than 24 hours old. Testing was conducted according to the Environment Canada test method “Biological Test Method: Acute Lethality Test Using Daphnia spp.” (Environment Canada 1990b).

The wood stakes were soaked for 24 hours in the *Daphnia magna* control/dilution water. The *Daphnia magna* testing was performed on this soaking liquid. The stakes were soaked for 24 h in 450 ml. of test water in a glass measuring cylinder weighted down as per the fish tests (refer to photograph 3), the stakes were then removed, then this water was used in the toxicity tests. The control/dilution water is reconstituted distilled water with a hardness of approximately 120 mg/L as $CaCO_3$. A series of dilutions were prepared of this soaking liquid by combining with additional control/dilution water. Ten neonates were introduced into each test concentration. The tests were checked for observations and temperature at 24 hours and terminated at 48 hours. Water quality measurements were performed at the start and end of the test. At test termination the numbers of organisms alive, immobile or dead were recorded. Immobilization is defined as an inability to swim within 15 seconds after gentle agitation of the liquid and mortality is defined as lack of movement of the body, appendages and heart when observed under a dissecting microscope for 5 - 10 seconds. The LC50, the concentration estimated to cause mortality in half the organisms, was estimated and the 95% confidence limits determined from statistical analysis using the methods of Stephan (Stephan, 1977).

A reference toxicant test was conducted with sodium chloride within fourteen days of testing. Using the mortality data at each test concentration, the 48 hour LC50 (concentration calculated to cause 50% mortality after 48-h exposure) was calculated using the methods of Stephan (1977). These values were entered into the control chart to ensure normal operating conditions were maintained, and that the population of organisms used in the test was of normal sensitivity.

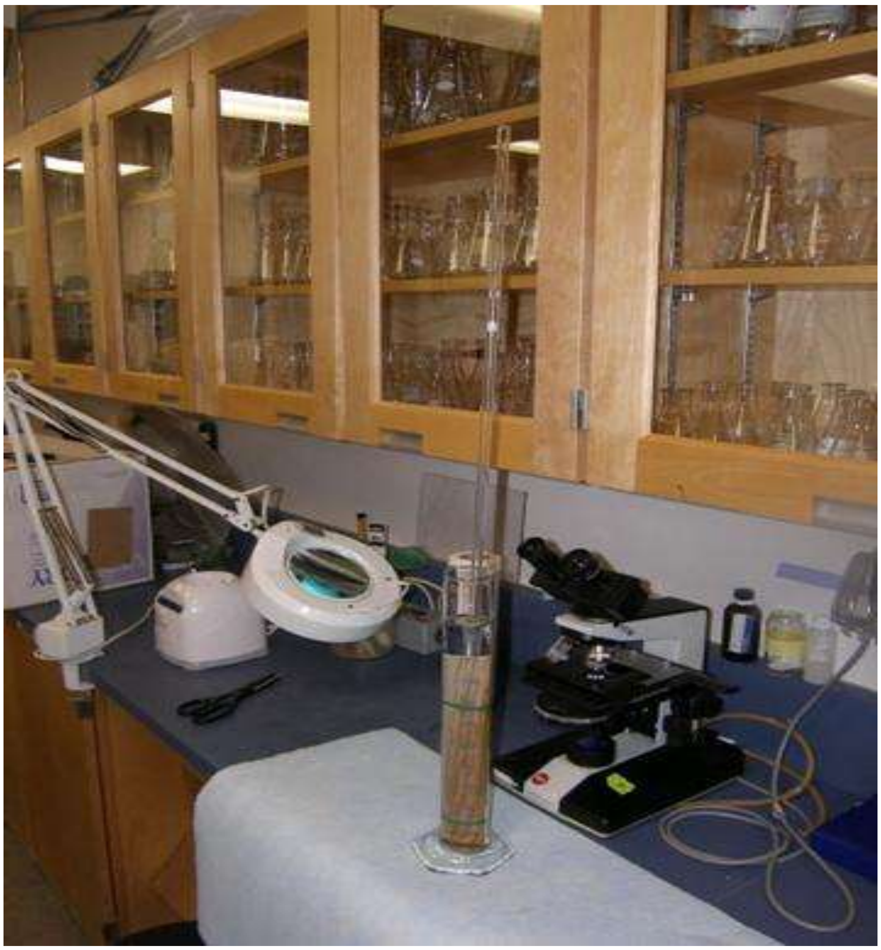
2.3 Microtox Test Procedure

15-minute static bioassays were started on the samples using the luminescent bacteria *Vibrio fischeri* (previously known as *Photobacterium phosphoreum*). Testing was conducted according to the Environment Canada test method “Biological Test Method: Toxicity Test Using Luminescent Bacteria, *Photobacterium phosphoreum*” (Environment Canada 1992).

This method exposes the bacterium to concentrations of the sample for 15 minutes, if toxic materials are present they interfere with the cellular respiration of the organism. This interference is measured as a decrease in light output by the bacterium, *Vibrio fischeri* (previously *Photobacterium phosphoreum*). The IC50, the concentration that causes inhibition of light by 50% is calculated using the Microtox Omni operating software.

Freeze dried bacterial reagent was reconstituted and held at 5 ± 0.5°C. A series of salinity adjusted concentrations of the test samples are prepared and acclimated to test temperature of 15 ± 0.5°C. The wood stakes were soaked for 24 h in 450 ml. of test water (distilled water) in a glass measuring cylinder weighted down as per the fish tests (refer to photograph 3).

Photograph 3



The stakes were removed, then this water was used in the toxicity tests. Dilutions of this solution were prepared in the test cuvettes as per the test procedure. While the stakes were soaked in freshwater as were the stakes for the Daphnia tests, the tests solutions are adjusted to salt water prior to testing as the bacterial test organism is a salt water organism. Bacterial reagent is added to dilution water and an initial light reading recorded. Aliquots of the test concentrations are then added to these samples of dilution water. After 15 minutes the light reading is again recorded. The IC50 is estimated from these light readings and 95% confidence limits are determined. The basic test format was used, which utilises a top concentration of 45%.

A reference toxicant test was conducted with zinc sulphate for each batch of tests. The IC50 calculated by the operating software was entered into the control chart to ensure normal operating conditions were maintained, and that the population of bacteria used in the test was of normal sensitivity.

2.4 Threespine Stickleback Test Protocol

96-hour static bioassays were started on the samples using threespine stickleback. Testing was conducted according to the Environment Canada test method "Biological Test Method: Acute Lethality Test Using Threespine Stickleback (*Gasterosteus aculeatus*)" (Environment Canada 1990c).

For the fish tests, the stakes would fit in the test container, so the stakes were submerged in the test solutions by attaching them to glass rods with nylon cable ties (refer to photograph 1). If the glass rods floated, they were weighted down (above the surface of the water) so that the stakes were totally submerged (refer to photograph 2). If more than one stake was used, they were separated by small pieces of glass tubing so that the test water contacted almost the entire surface of each stake (refer to photograph 1).

The test solutions were prepared for the samples in natural seawater, the same water used for holding of the threespine stickleback. The volume chosen was 25 litres. All test solutions were pre-aerated for 30 minutes, initial water quality measurements were performed. Five (five for all of the stickleback) fish were randomly introduced into each test concentration. The tests were checked for mortalities frequently throughout the first day, then once a day thereafter until 96 hour termination. Any dead fish were removed when observed. Water quality measurements were performed daily. After 96-hours any surviving fish were euthanized. The lengths (cm) and weights (g) of the control fish were measured and the loading density was calculated. The percent survival at each concentration was determined.

A reference toxicant test was conducted with phenol once a calendar month. Using the mortality data at each test concentration, the 96 hour LC50 (concentration calculated to cause 50% mortality after 96-h exposure) was calculated using the methods of Stephan (1977). These values were entered into the control chart to ensure normal operating conditions were maintained, and that the population of fish used in the test was of normal sensitivity.

3.0 RESULTS AND DISCUSSION

Toxicity results for all of the test stakes are summarized for all test species in Tables 1 to 4 for the four test organisms. Microtox tests were not conducted on the treated lumber stakes, hemlock, plastic lumber or Lifetime Wood Treatment. This test was added to the test battery after tests on these samples had been completed.

The reference toxicant test for rainbow trout using phenol had a calculated LC50 of 9.83 mg/L with 95% confidence limits of 8.29 and 11.7. The historical mean for this analysis is 10.4 mg/L with warning limits of 8.10 and 13.3. These results were within the warning limits and so the fish can be considered of normal sensitivity.

The reference toxicant test for *Daphnia magna* using sodium chloride had a calculated LC50 of 5500 mg/L with 95% confidence limits of 5120 and 5920. The historical mean for this analysis is 5540 mg/L with warning limits of 5050 and 6080. These results were within the warning limits and so the *Daphnia magna* can be considered of normal sensitivity.

The reference toxicant test for Microtox using zinc had a calculated LC50 of 1.34 mg Zn/L with 95% confidence limits of 1.22 and 1.44. The historical mean for this analysis is 1.05 mg/L with warning limits of 0.704 and 1.55. These results were within the warning limits and so the bacteria can be considered of normal sensitivity.

The reference toxicant test for threespine stickleback using phenol had a calculated LC50 of 12.5 mg/L with 95% confidence limits of 11.1 and 14.3. The historical mean for this analysis is 12.5 mg/L with warning limits of 8.72 and 17.9. These results were within the warning limits and so the fish can be considered of normal sensitivity.

The Fahlstrom stakes were not cut to fit the size of the test vessels, as this would have led to problems with freshly cut surfaces and possibly change in leaching rates of preserving chemicals. To normalize test exposures for the differing test volumes. (different solution volumes were used in the different tests ranging from 1.5 mL for Microtox test to 40 L for rainbow trout test), surface area of Fahlstrom stakes (cm^2) per volume of test solution (L) was chosen as our exposure units.

3.1 Toxicity to Rainbow Trout in Fresh Water

Table 1 lists the tested materials with the corresponding LC50 toxicity values. One to five stakes were placed in 20 to 40 litres of dechlorinated Moncton City water for 24 hours prior to addition of the Rainbow Trout. Results from this test are interpreted as follows: Untreated Wood, Plastic Wood, Ecotherm Wood and Wood Treated with Lifetime Wood Treatment are non-toxic to Rainbow Trout; CCA treated at 6.4 kg/m3 is less toxic than creosote treated at 40.04 kg/m3 which is less toxic than the other three treatments which are of similar high toxicity (CCA treated at 22.4 kg/m3, ACQ treated at 4 kg/m3, and ACQ treated at 6.4 kg/ m3).

Table 1
Rainbow Trout in Fresh Water

Material Tested	SA/vol (cm^2/L)	% Mortality	LC50 (cm^2/L)
ACQ 4 kg/m^3	29.6 17.7 5.92 0	100 100 100 0	< 5.92
ACQ 6.4 kg/m^3	29.6 17.7 5.92 0	100 100 100 0	< 5.92
Bois Ecotherm Wood	59.2 35.5 11.8 0	20 0 0 0	> 59.2
Untreated Hemlock	63.1 15.8	0 0	> 63.1
Lifetime® Treated Wood	39.4 23.7 7.89	0 0 0	> 39.4
Plastic Wood	78.9 23.7 0	0 0 0	> 78.9
CCA 6.4 kg/m^3	78.9 39.4 23.7 15.8 7.89	100 20 0 0 0	49.4 (42.5 - 57.6)
CCA 22.4 kg/ m^3	15.8 7.88	100 100	< 7.88
Creosote 40.04 kg/ m^3	23.7 15.8 7.89	100 100 0	11.2 (7.89 - 15.8)

3.2 Toxicity to *Daphnia magna* in Fresh Water

Table 2 lists the tested materials with the corresponding LC50 toxicity values. As a reduced water volume is required for Daphnia testing, the stakes were placed in 0.45 litres of water and allowed to sit for 24 hours. Test water was then removed and utilized as per Environment Canada test method (1990b); therefore with the reduced water volume only one stake was submerged in each vessel and dilutions of the resulting solution used in the tests. Results from this test indicate that Untreated Hemlock, Lifetime Treated Wood and Plastic Wood are non toxic to Daphnia. Ecotherm Wood exhibited 20% toxicity to Daphnia. CCA treated at 6.4 Kg/m^3 is less toxic to Daphnia than Creosote treated at 40.04 kg/m^3 which is less toxic than ACQ treated at 4 kg/m^3 and 6.4 kg/m^3 (Table 2).

Table 2
***Daphnia magna* in Fresh Water**

Material Tested	SA/vol of original soak solution (cm^2/L)	LC50 as % of soaked solution	LC50 (cm^2/L)
ACQ 4 kg/m^3	526	0.198 (0.0625 - 0.625)	1.04 (0.329 – 3.29)
ACQ 6.4 kg/m^3	526	0.130 (0.0617 – 0.260)	0.683 (0.324 – 1.37)
Bois Ecotherm Wood	526	>100	> 526
Untreated Hemlock	526	>100	> 526
Lifetime® Treated Wood	526	>100	> 526
Plastic Wood	526	>100	> 526
CCA 6.4 kg/m^3	526	32	168
CCA 22.4 kg/ m^3	526	< 1	< 5.26
Creosote 40.04 kg/ m^3	526	3.16 (1 - 10)_	16.6 (5.26 – 52.6)

3.3 Toxicity to Microtox in Fresh Water

Table 3 lists the test materials and provides the IC50 (50% Inhibiting Concentration) values in fresh water. The exposure protocol for this test was the same as in the Daphnia test procedure. This Luminescent Bacteria test was only applied to ACQ and Ecotherm wood. Bois Ecotherm was considerably less inhibiting to the bacteria than ACQ treated at 4kg/m^3 which was very slightly less inhibiting than ACQ treated at 6.4 kg/m^3 (Table 3).

Table 3
Microtox in Fresh Water

Material Tested	SA/vol of original soak solution (cm^2/L)	IC50 as % of soaked solution	IC50 (cm^2/L)
ACQ 4 kg/m^3	526	0.974 (0.846 – 1.12)	5.12 (4.45 – 5.89)
ACQ 6.4 kg/m^3	526	0.849 (0.695 – 1.04)	4.47 (3.66 – 5.47)
Bois Ecotherm Wood	526	15.4 (9.07 – 26.2)	81.0 (47.7 – 138)

3.4 Toxicity to Threespine Stickleback in Salt Water

Table 4 lists the materials which were tested with Threespine Stickleback and the corresponding LC50 toxicity values. As with the Rainbow Trout, 1 to 5 stakes were placed in the 20 to 25-litre sample volumes (seawater), let stand for 24 hours, prior to addition of the Stickleback to initiate the test period. Untreated Hemlock, Lifetime Treated Wood, Plastic Lumber and Bois Ecotherm and CCA were not toxic to Stickleback. Based on the test results, Creosote would be marginally less toxic than ACQ which is the most toxic with an LC50 for the wood treated at 6.4 Kg/m^3 of 12.4 cm^2/L.

Table 4
Threespine Stickleback in Salt Water

Material Tested	SA/vol (cm^2/L)	% Mortality	LC50 (cm^2/L)
ACQ 4 kg/m^3	47.3 28.4 9.47 0	100 100 0 0	16.4 (9.47 – 28.4)
ACQ 6.4 kg/m^3	47.3 28.4 9.47 0	100 100 10 0	12.4 (10.9 – 14.1)
Bois Ecotherm Wood	59.2 35.5 11.8 0	10 0 0 0	> 59.2
Untreated Hemlock	28.4	0	>28.4
Lifetime® Treated Wood	28.4	0	>28.4
Plastic Wood	28.4	0	>28.4
CCA 6.4 kg/m^3	28.4	0	>28.4
CCA 22.4 kg/ m^3	47.3 28.4 9.47	0 0 0	> 47.3
Creosote 40.04 kg/ m^3	47.3 28.4	100 40	30.9 (24.8 – 28.5)

3.5 Interpretation of the Significance of the Test Results

The cause of the observed toxicity in the test solutions has not been determined in this preliminary study because of the lack of a budget for chemical analysis. The results do show that in both freshwater and marine conditions, some of the treatments (Lifetime Wood Treatment and the untreated wood and Plastic Lumberare not acutely toxic under the conditions of this study. However, for other treatments(CCA, ACQ and Creosote) toxic chemicals in toxic concentrations can leach out of the treated wood under the conditions of this study. Only acute effects were measured in this study, and it would be reasonable to expect sublethal toxic effects at lower dosage rates under longer term exposure regimes. The potential for toxic effects in the marine and freshwater environment has thus been established for the construction materials tested.

The main value of this study is that it has demonstrated the comparative toxic potential of different materials used in aquatic construction, and allows us to rank their potential effects when alternative materials are available for use. To our knowledge, this type of study has not been conducted previously, and we plan to continue the study with a variety of other materials used in aquatic construction. The report will be updated as new data becomes available.

4.0 CONCLUSIONS

As a brief summary, this toxicity data indicates that Plastic Wood, Untreated Hemlock, Ecotherm Wood and wood treated with Lifetime Wood Treatment are non acutely toxic to the test species in fresh water or the marine environment. In all of the tests conducted for this toxicity assessment of the various construction materials that may be used in aquatic construction, the data indicates that creosote, is similar in toxic potential to CCA and less toxic that ACQ.

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