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Section 3 Wood Protecting Chemicals

Protection of southern pine using N,N-Naphthaloylhydroxylamine: Field tests, soft-rot cellars and aquatic bioassay leach testing

by

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Protection of Southern Pine using N'N-Napthaloylhydroxylamine: Field tests, soft-rot cellars and aquatic bioassay leach testing

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Recent environmental restrictions are limiting the use of broad-spectrum biocides for wood preservation. There is an urgent need for new, sharply targeted, environmentally benign wood preservatives. N'N-Napthaloylhydroxylamine (NHA), a water-soluble calcium-precipitating agent, has been shown to inhibit decay by brown-rot and white-rot fungi in soil-block tests and prevent damage by Eastern subterranean termites under lab conditions. In order to further evaluate the capacity of NHA to prevent decay and termite damage, SYP stakes were pressure treated with three concentrations of NHA (0.1, 0.5 and 1.00%) and CCA (1.0%) and placed in-ground at the Harrison Experimental Forest, Gulfport, Mississippi, in June, 1997. Similarly, treated sticks were placed in soft-rot fungal cellars at FPL. One percent NHA-treated stakes were also leached for 72 hours in water and the leachates tested by an acute, five dilution bioassay using *Ceriodaphnia dubia* in EPA Protocol 600/4-90/27F. Results to date: 1) NHA stakes (0.5 and 1.0%) are as durable as CCA; 2) NHA does not inhibit soft-rot fungi and; 3) NHA is a relatively benign molecule with LD_{50} 130-fold less than copper for *C. dubia.*

southern pine, field testing, targeted wood preservation, termite damage, environmental testing, soft-rot fungi, aquatic bioassay, stake test

3. Wood Protecting Chemicals

Introduction

Many wood preservatives are being restricted from use because of the long term health hazards of broad-spectrum biocides and their toxic components. Acute and chronic aquatic toxicity testing of new preservatives will help preserve the environment (Van Eetvelde et al., 1997, 1998).

In order to avoid the long term environmental impacts of these preservatives the scientific and industrial communities must develop new models for non-toxic, yet efficacious alternatives. Preferably, these new preservatives would incorporate a simple, water soluble chemical moiety which binds to or precipitates into the wood and protects the wood against a variety of wood decay fungi and termite damage at low concentrations (Highley, 1989).

One such model system is under study in our laboratory: N'N-napthaloylhydroxylamine (NHA) and has previously been shown to protect southern pine from white-rot decay and brown-rot decay in soil block tests and also protect against termite damage under laboratory conditions (Green et al., 1997). Further testing is required to determine the following:

1.) environmental hazards through aquatic toxicity testing
2.) susceptibility to soft rot fungi in cellars
3.) field tests in high decay areas like Gulfport, MS

The results of this paper indicate that NHA is a relatively benign compound with lower toxicity in aquatic environments than copper, has no ability to inhibit soft rot fungi and associated bacteria in fungal cellars, yet is able to protect southern pine from decay fungi and termite damage in a high decay area after one-year in the field.

Materials and Methods

NHA treated southern yellow pine stakes. Six southern yellow pine stakes were sent to the Aquatic Environmental Sciences (Port Townsend, WA) following treatment with NHA. Three of those stakes (numbers 34, 36 and 37) and an untreated control southern yellow pine stake were chosen for this leaching experiment and the subsequent bioassays. Each of the 18" stakes was cut in half to fit into the leaching apparatus. Both halves were leached in the same container. Recorded dimensions were 3.7cm square and 16.1-16.4cm in length and NHA retentions were as follows: 0.416-0.431 pounds per cubic foot (lbs/ft^3).

Leaching conditions. These stakes were leached in a static renewal system consisting of five liter High Density Polyethylene (HDPE) tubs with tightly fitting lids. The NHA treated wood was secured to the lid with stainless steel screws and immersed in distilled water amended with UV treated, five micro filtered well water to give a nominal hardness of 50 mg/L (as $CaCO_3$). The water was changed daily, except on day five. Actual water volume in each system was measured with a graduated cylinder. Volumes varied between 4,230 and 4,310 ml.

Physiochemical measurements. Temperature (ºC) and the initial and final pH of conductivity (µmhos) and dissolved oxygen (mg/L) were measured at the beginning and end of each static renewal.

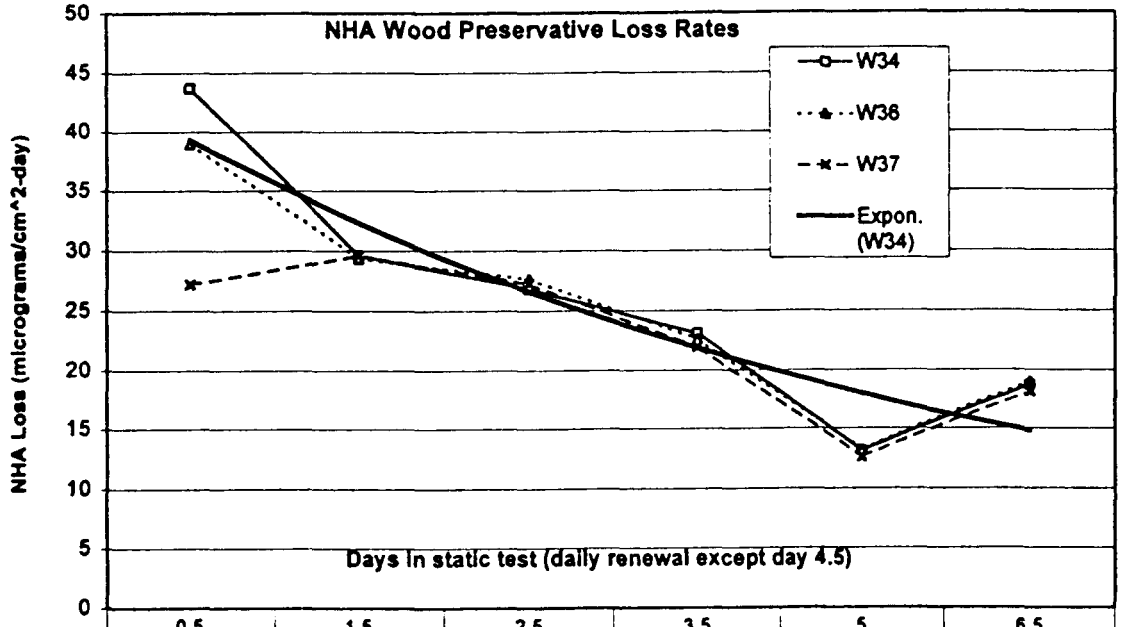
Analysis of N,N-naphthaloylhydroxylamine (NHA). Qualification of the amount of NHA in the leachate was accomplished by the Forest Products Laboratory using the linear part of the response curve and known standards on a Spec 20. The Forest Products Laboratory was provided with 20 ml samples in new borosilicate glass test tubes with screw top caps. Single samples were provided from each leaching container on each test day and for each of the five dilutions tested in the definitive bioassay.

Bioassays. An acute, five dilution, definitive bioassay, using *Ceriodaphnia dubia* in EPA Protocol 600/4-90/027F, was completed on five serial dilutions of the primary leachate (100%, 25%, 6.25%, 1.56% and 0.39%). Sodium chloride was used in a concurrent reference toxicant bioassay. Four of these tests were conducted on Day 0.5 leachate from the three NHA treated stakes and the untreated control.

Soft-rot fungal cellars. The fungal cellar evaluation was conducted at the USDA, Forest Service, Forest Products Laboratory, Madison, Wisconsin. Four bundles of twenty southern yellow pine stakes were treated with three concentrations of NHA and one concentration of CCA. The stakes were 3-mm thick (transverse) by 19-mm (radial direction) by 150 mm (parallel to the grain) (0. 118 by 0.748 by 5.91 in.). Stakes were exposed in soil that is maintained at a moisture

content of 50%-70% of water holding capacity to promote growth of soft-rot fungi (Nicholas et al ., 1991). The soil bed was maintained in a controlled environment at 26°C and a relative humidity of 86% to 90%. At 3-month intervals, for 2 years, wood specimens were removed from the soft-rot fungal cellar (soil bed), cleaned with a brush to remove excess soil, and placed in water-tight plastic bags until evaluation for strength loss using the bending strength apparatus. Strength loss was determined as described by Crawford (1994).

Field stake testing. Field trials (graveyard tests), in which the durability of experimentally treated wood is ascertained by monitoring the duration that those members resist attack of naturally occurring microflora and fauna, is the standard method for evaluating wood preservatives. Field trials were established in the southern USA. Plots were established in a randomized block design consistent with AWPA Standard E7 (AWPA, 1995).



	0.5	1.5	2.5	3.5	5	6.5
W34	43.7	29.63	26.82	23.1	13.1	18.66
W36	38.96	29.35	27.63	22.61	13.19	18.97
W37	27.2	29.58	27.24	21.92	12.57	18.089

Figure 1. Loss rates in micrograms NHA/cm^2-day observed from southern yellow pine stakes immersed in fresh water with a hardness of 50 mg/L ($CaCO_3$). This was a static renewal test with water renewals at the end of 1.0, 2.0, 3.0, 4.0, 6.0 and 7.0 days. The solid-bold line is an exponential fit to the data.

Results

Leaching results. The results of the leaching experiment are summarized in Figure (1). Initial NHA loss was variable depending on the sample. There is a strong correlation between the Day 0.5 NHA loss rates and retention. However, the differences in retention were small (0.431 pcf for sample 34 and 0.416 pcf for sample 37). There mayor may not be a cause and effect relationship between these small differences in initial retention and the significant differences in loss rates observed on the first day (43.7 µg/cm^2-day for stake 34 and 27.2 µg/cm^2-day for stake 37).

High variability in NHA loss was also observed druing the first leaching experiment (119.6 to 162.6 µg/cm^2-day) on the first day. In both leaching experiments, the variation in loss rates between samples decreased significantly by the second day. It seems reasonable to suggest that surface residues may vary between stakes and that those surface residues of NHA are responsible for the initially high variability.

Bioassay results. The daphnia population at Aquatic Environmental Sciences was stressed just prior to the study. Rather than delay the study, it was decided to have the bioassays conducted at Parametrix, Inc. Parametric is also a Department of Ecology accredited laboratory and the bioassays were conducted using an identical protocol. The use of *Ceriodaphnia dubia* instead of *Daphnia magna* is acceptable and provides comparable results. No abnormalities were observed in the results of these bioassays.

Mortality was observed only in the 100% (full strength) leachinate. The concentration of NHA responsible for 50% mortality in 48 hours (48 hr - LC_{50}) is provided in Table (1).

Table 1. No observed effect concentration (NOEC), lowest observed effect concentration (LOEC) and the concentration killing 50% of the test organisms in 48 hours (LC_{50}) associated with NHA treated southern yellow pine immersed in freshwater at 50 ppm hardness for 24 hours.

Treatment	Control	Stake 34	Stake 36	Stake 37
NOEC	100%	100%	25%	25%
LOEC	>100%	>100%	100%	100%
LC_{50}	>100%	>100%	59.5%	56.5%
NHA (µg/L)	ND	5,500 µg/L	5,375 µg/L	3,425 µg/L
LC_{50}(for NHA)	ND	>5,500 µg/L	3,198 µg/L	1,935 µg/L

Geometric mean 48 hr. NHA LC_{50} = 3,240 µg/L

Modulus of elasticity (MOE) results taken at 3-6 month intervals on stakes in the soft-rot fungal cellar test showed i) NHA treated stakes performed no better than controls and ii) CCA treated stakes performed better than either NHA or controls (Fig. 1). After one year exposure of 3/4 X 3/4 field stakes pressure-treated with CCA and NHA showed that the two higher retentions in NHA (0.5 and 1.0%) performed as well as CCA treated stakes (Fig. 3).

DISCUSSION

Comparison of NHA toxicity with copper toxicity. Copper toxicity varies with water hardness. Reported copper LC_{50} values range from 18 µg/L at an alkalinity of 12.5 mg/L and hardness of 110 mg/L (Meador, 1991) to 36 to 44 µg/L at a hardness of 374 mg/L (Buchanan and Solomon (1990)). These NHA bioassays were conducted at 50 mg/L hardness and the copper LC_{50} would be in the range of 25 µg/L. This suggests that NHA was 3,250/25 = 130 times less toxic than copper.

Chronic water quality criteria are developed following extensive gioassay testing on a suite of animals. As a rule of thumb, the chronic criteria will be about 10% of the acute value. In this case, the chronic criterion for NHA would be ca. 325 µg/L. The EPA chronic copper criterion for fresh water is given in Equation (1).

Equation 1. EPA chronic copper criterion $\leq 0.960 \times \exp^{0.854[\ln(\text{hardness})]-1.465}$

The chronic copper criterion at 50 mg/L hardness is 6.3 µg Cu/L. This copper criterion is 52 times less than the rough estimate given for NHA. The point is that NHA is likely two orders of magnitude less toxic than copper (at least for this daphnid).

Comparison of NHA loss rates with those of CCA, ACQ-B and ACZA. Initial copper losses from CCA-C, ACQ-B and ACZA are compared with the loss of NHA in Table (2). The data for other preservatives was generated at neutral pH (7.0) in freshwater.

ACQ-B	25 µg Cu/cm^2-day
ACZA	105 µg Cu/cm^2-day
CCA-C	1 µg Cu/cm^2-day
NHA	36 µg NHA/cm^2-day

The NHA copper loss rate is 36 times higher than the copper loss rate from CCA-C on the first day of immersion. Coupled with the estimate that a reasonable NHA chronic criterion is 52 times less than that for copper, this suggests (as a roough first cut) that fewer restrictions would be placed on the use of NHA in aquatic environments than are imposed on CCA-C. I should add that there are very few aquatic environments where CCA-C poses significant environmental risk.

NHA has previously been shown to protect southern yellow pine (syp) from white-rot and brown-rot decay in soil block tests and termite damage by Eastern subterranean termites (Green et al., 1997). NHA does not inhibit sapstain fungi, molds, Ascomycetes fungi or formosan termites (unpublished results) which supports the hypothesis that NHA is not a broad spectrum biotide. The failure of NHA to effect any inhibition of soil-rot decay could be due to i) leachability of NHA or ii) not inhibitory to soft-rot fungi and bacterial metabolism. In light of the one year field test data from Gulport, MS (Fig. 2) as well as leach test data from soil block tests it is more likely that NHA is not inhibtory to the ascomycete and deuteromycete fungi causing soil-rot (Daniel and Wielsson, 1998). NHA has recently been shown to inhibit weight loss and depolymerization of cotton cellulose by brown-rot and white-rot fungi which further supports a direct effect on Basidiomycete metabolism, possibly calcium metabolism (Green and Kuster, 1999).

Opportunities for targeted control of wood decay and the development of designer preservatives will avail themselves as a more precise understanding of the biochemistry of decay mechanisms is elucidated.

Summary

The following conclusions are based on the results of the leaching study and the *Ceriodaphnia dubia* bioassays.

- NHA is toxic to *Ceriodaphnia dubia* with a 48 hr.-LC_{50} of 1,935 to > 5,500 µg NHA/L. The geometric mean LC_{50} was 3,240 µg/L. This is 130 times less toxic than copper.

- Minor decreases in dissolved oxygen and pH were observed in untreated control and NHA treated leachate. These consistent decreases appear to have been associated with the wood and were not increased in the NHA treated samples when compared with the untreated controls. Similar decreases were not observed in the water only controls.

- NHA loss rates (µg NHA/cm^2-day) are about equal to copper losses from ACQ-B and are lower than initial copper losses from ACZA in fresh water. Initial (Day 0.5) losses of NHA were significanlty higher than copper losses from CCA-C treated wood in fresh water.

- The overall toxicity to *Ceriodaphnia dubia* associated with NHA preserved wood immersed in fresh water is therefore judged to be similar or slightly lower than predicted for CCA-C. It should be noted that environmental restrictions are seldom imposed on CCA-C use in freshwater systems. It should also be noted that background levels of copper, chrome and arsenic must be added to the losses from CCA-C, ACZA or ACQ-B treated wood.

Acknowledgements

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Fig 1. Results from soft-rot test using 3x19x150mm fungal stakes.

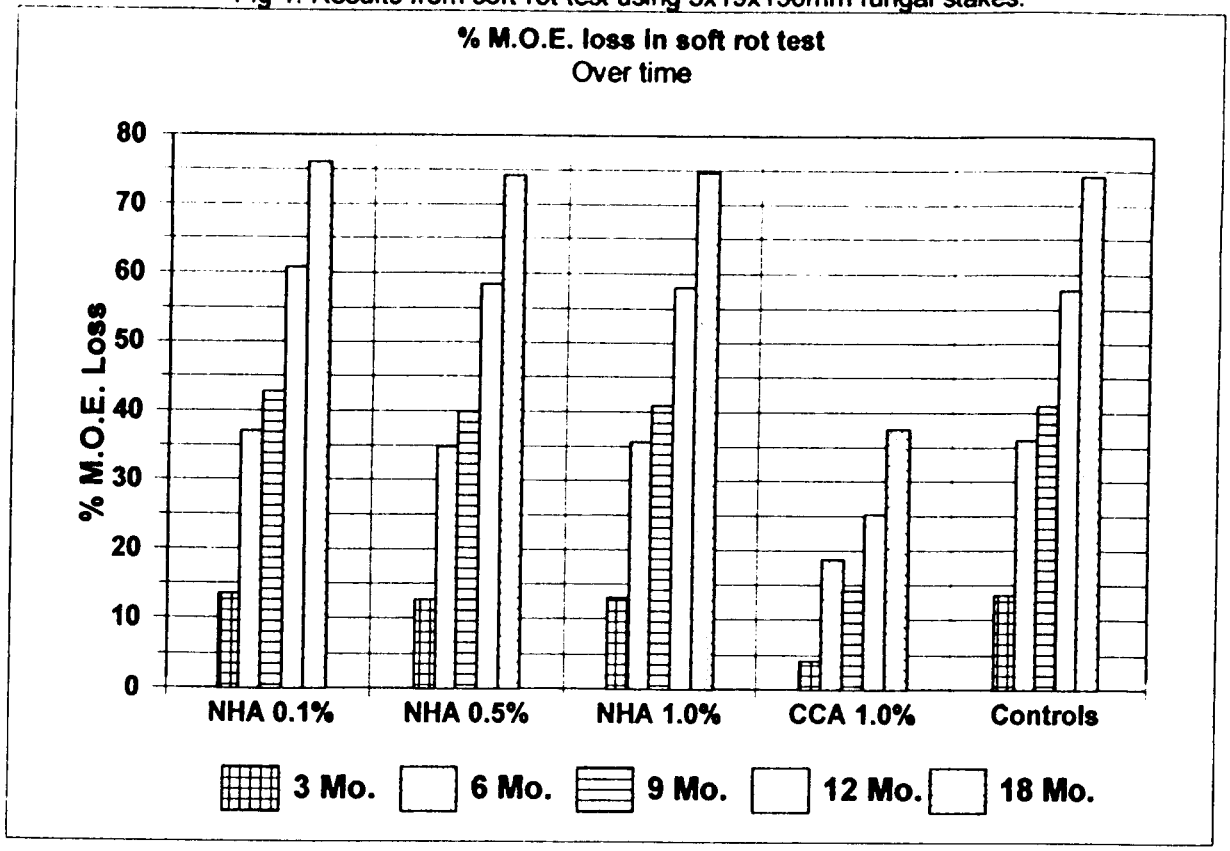

Fig 2. Index of condition of 3/4 in stakes after one-year of exposure in ground contact at HEF.