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FINAL PROJECT REPORT

March 21, 2011

Michael Reed
Rhino Hide, LLC
3135 Reynolds Rd.
Lakeland, Florida 33803

Re: Project UltraBan Studies

Dear Mr. Reed:

You have asked that RealTime Laboratories, LLC, Carrollton, Texas, conduct studies on your product, UltraBan, for purposes of determining if fungal growth and mycotoxins are present after treatment of wood products with your product.

The entire study was 28 days in length.

Objective:
To determine if UltraBan treated lumber can inhibit fungal growth and production of mycotoxins (aflatoxins, ochratoxins, and tricothecenes) when comparing to lumber that is not treated.

Hypothesis: UltraBan inhibits the growth of fungal elements and the production of mycotoxins.

Background:

Growth of mold on lumber and other materials used in construction as well as growth of the same in water damaged homes is a health concern and hazard for individuals who live in homes or buildings with contaminated material.

Materials:

A. Samples were supplied by Rhino Hide, Florida, were submitted by chain of custody and were labeled as:

1. 3" X 3" X 5/8" piece of wood labeled "untreated".

2. 3" X 3" X 5/8" piece of wood labeled "treated".

3. Solution labeled "UltraBan, Liquid Coating for Test, Rhino Hide".

B. The following organisms were used to inoculate all portions of samples:

1. *Fusarium solani*
2. *Chaetomium sp.*
3. *Penicillium chrysogenum*
4. *Stachybotrys echinata*
5. *Aspergillus niger*
6. *Penicillium verrucosum*
7. *Aspergillus fumigatus*
8. *Curvularia sp.*
9. *Penicillium corylophilicum*
10. *Penicillium crustosum*

All organisms were obtained from American Type Culture Collection (ATCC) or College of American Pathology (CAP) or from environmental samples which previously had the fungal organisms present. All samples are maintained in pure culture at RealTime Laboratories, LLC.

C. Samples were incubated at 28 degrees Centigrade for 2 weeks after initial testing and inoculation. Humidity was maintained at 40-50% in the enclosed incubator.

Procedure:

A. Initial testing: The submitted samples were swabbed with sterile saline moistened swabs and plated on Potato Dextrose Media (PDM). Plates were examined at 7 and 12 days for fungal and/or bacterial growth. Final readings were conducted at 28 days. Also, samples were swabbed with sterile saline moistened swabs and the swabs were placed in 1.0 ml of Phosphate Buffered Saline (PBS) with 10% Methanol. These samples were used to conduct Enzyme Linked ImmunoSorbant Assays (ELISA) testing for mycotoxins. The mycotoxins tested were: Aflatoxin B1, B2, G1, G2; Ochratoxin A, and macrocyclic trichothecenes. Samples were read on an ELISA reader at 450 A and 650 A. Tests were conducted using RTL proprietary procedures and reported in parts per billion (ppb) or ng/ml.

Minimal Levels of Detection (LOD) for each mycotoxin is as follows:

Aflatoxins - 1.0 ppb (ng/ml)
Ochratoxins- 2.0 ppb (ng/ml)
Tricothecenes - 0.2 ppb (ng/ml)

B. Inoculation of samples: Samples were then inoculated with known control organisms (listed above).All fungal organisms were initially cultured on PDM for 14 days prior to diluting in sterile distilled water. An inocula from the PDM was taken and placed in 3 cc. of sterile distilled water. Each inoculum was then counted using a hemocytometer and inoculums were adjusted to approximately 450 spores/ml. The final dilution (450 spores/ml) was labeled at "neat" or undiluted. The organism concentration was determined to be a final concentration of 450 spores/ml in the mixture used to inoculate each board. 100 µl (0.1 cc) of the final concentration was placed on each piece of sample. For example: 0.1 cc of the neat solution was placed on the upper left corner for a final volume of 450 spores. The assumption of the final concentration would be if ANY spore is present in the solution, it should either grow or be inhibited.

Samples were placed in the incubator and evaluated weekly for a total of 28 days Samples were evaluated for visible mold growth and reported as positive or negative growth for purposes of this interim report. Actual numbers of colonies were not counted on the material because of the confluent growth on the wood. ELISA tests for mycotoxin presence were conducted at the end of the study (Day 28).

C. UltraBan Studies: A solution of Liquid coating UltraBan was also provided. The solution was diluted in the following manner:

1. 1:10 in sterile phosphate buffered saline (PBS, pH 7.0-7.2);
2. 1:100 in sterile PBS;
3. 1:1000 in sterile PBS.

One ml of the spores prepared in paragraph B, was placed in each dilution of UltraBan. Spores were held in the solution for 1 hr, 4, hrs, 8 hrs, 12, hrs, and 24 hrs. At the end of that holding time, 0.1 ml of the solution was plated on PDM media and incubated for 28 days. Results of cultures after 28 days are shown in Results section.

Results:

A. <u>Initial Culture</u>: Results of Cultures conducted upon receipt at RTL show no fungal elements are noted on the UltraBan treated wood sample as well as the raw wood.

B. <u>Initial mycotoxin ELISA testing</u>: Results of ELISA testing for the total mycotoxin panel (Aflatoxin, Ochratoxin, and Tricothecenes) showed no mycotoxins present on any of the samples submitted.

C. Final Culture Results: Results of the cultures conducted show no fungal growth on the UltraBan treated wood. Control wood (non-treated) shows fungal growth at all concentrations of fungi. No bacteria were noted on either sample.

D. Final ELISA tests for mycotoxins: Conducted at the end of the project (Day 28). No Mycotoxins (Aflatoxins, Ochratoxins, and Tricothecenes) detected.

E. Dilutions of UltraBan: Dilutions of UltraBan solution were made at 1:10; 1:100; and 1:1000 and then mixed with fungal elements. All solutions were then plated on fungal media. There was no growth of fungal elements after 28 days incubation. Control cultures without UltraBan were positive for fungal elements.

CONCLUSIONS: Final Report (Day 28)

1. UltraBan inhibits the growth of fungal elements on treated wood products when compared to non treated wood. Specifically, those toxin producing fungal elements inhibited are:

Fusarium solani
Chaetomium sp.
Penicillium chrysogenum
Stachybotrys echinata
Aspergillus niger
Penicillium verrucosum
Aspergillus fumigatus
Curvularia sp.
Penicillium corylophilicum
Penicillium crustosum

2. Because UltraBan inhibited the growth of toxin producing fungal elements in 28 days, it was assumed that no mycotoxins were produced. Testing using ELISA tests on swabs from the treated wood showed no mycotoxins present. ELISA tests on the untreated wood showed the presence of Tricothecenes, Aflatoxins, and Ochratoxins.

COMMENTS:

This final report on a study of UltraBan is significant in that it demonstrates that fungal elements do not grow on treated wood samples when compared to non-treated wood samples. The final results after 28 days show no fungal growth and no presence of mycotoxins on the treated wood with growth and mycotoxins present on the untreated sample of wood.

Respectfully submitted,

Dennis G. Hooper

Dennis G. Hooper, M.D., Ph.D.