WONDER MAKERS
ENVIRONMENTAL

Benefect Decon 30 Study

Conducted by
Wonder Makers Environmental

Project Number GC13-11834

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August, 2013
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1.0 Abstract

Since many water intrusion events in buildings involve water contaminated with pathogens, a strictly controlled test was conducted to determine the effectiveness of a new botanical cleaner disinfectant when used as part of the standard cleaning process. Botanical Decon 30 by Benefect was tested using methods designed to replicate real-world conditions.

Wonder Makers Environmental developed the test design and conducted experiments on a contract basis for the manufacturer. Testing involved saturation with raw sewage of simulated wall cavities constructed of standard lumber and orient strand board (OSB). Two methods of chemical application were utilized—spray and foam. No scrubbing, wiping, rinsing, or other physical cleaning was conducted. Two types of tests were utilized to determine the effectiveness of the product—swabs with analysis by a direct read adenosine triphosphate (ATP) instrument and sterile sponges, which were sent to a lab for culturing of the collected bacteria. The standard sewage screen of *Enterococcus*, total coliform, and *E. coli* bacteria was used for each of the sponge samples.

Testing indicated that, when used according to the manufacturer's directions, the product Decon 30 was effective in addressing high levels of bacterial contamination in porous materials. (In the restoration industry wooden framing members are often referred to as "semi-porous"; however, for the purpose of this study the more traditional definition of a porous material is used, which includes lumber and OSB.) The application of Decon 30 on unfinished wood lumber reduced bacterial contamination by 73.8% to 100% without agitation, rinsing, re-application, or other commonly employed cleaning procedures. The test results showed little effectual difference between the two forms of application, with similar pathogen destruction when applied to the surface as a liquid spray or longer-lasting foam. Finally, varied test results for the intermediate sampling serve to emphasize that the process of surface decontamination when using Decon 30 continues to reduce pathogens until it has dried.

2.0 Introduction

Having recently introduced Benefect Botanical Decon 30 Disinfectant to the remediation industry with encouraging results, a study was undertaken to evaluate the performance of Decon

30 with residential remediation of unfinished wood framing after being contaminated by "black water" (sewage water).

Several questions were to be considered:

- How does the chemical respond to cleaning sewage from unfinished wood-framed assemblies?
- What is the most effective way to apply the chemical (sprayed-on liquid or foam spray)?
- How does the performance of Decon 30 vary with the time that the chemical is left on the contaminated surface?
- How does the performance of the liquid or foam application vary with the orientation of the surface? (vertical or horizontal)

Wonder Makers Environmental, Inc. was contracted by Benefect to provide an independent study, incorporating the questions listed above. A protocol was designed and implemented by Wonder Makers Environmental in May 2013 to provide objective data and answers to these questions.

3.0 Test Design

Although extensive testing is conducted on all products that are registered by the U.S. Environmental Protection Agency (EPA) or Health Canada's Pest Management Regulatory Agency (PMRA) as antimicrobial or disinfectants, those tests are closely controlled laboratory experiments. As such, different results may occur when the products are used in "real-world" settings.

For example, classification as a disinfectant is based on specific tests of the chemical on hard, non-porous surfaces. While this standardizes the comparison of the test data between chemicals, it does not necessarily represent the full range of surfaces on which chemicals such as Decon 30 are utilized during the restoration process.

In an effort to provide a test process that more closely represents typical use of antimicrobial products in a water loss restoration scenario, a test design was proposed that included application of the Benefect product on classic framing members (porous wooden studs) in a wall cavity configuration that is typically present after flood cuts (horizontal and vertical framing members). The inclusion of tested surfaces in a vertical orientation is especially important since liquid products that are sprayed or misted on upright studs have a tendency to run down the framing—a natural process that can significantly reduce the contact time between the chemical and the surface being treated. The inclusion of vertical surfaces to be tested also prompted the test design to include two different application methods for Decon 30: spray and foam.

Another important difference between testing protocols for American and Canadian registration and the proposed test design was the use of actual sewage as the contaminant for the test. Registration testing of disinfectants utilizes "simulated sewage", a standardized mix of known bacterial types. In contrast, the type and level of contaminants in sewage from a wastewater treatment plant are constantly changing. In order to assure a high level of contamination in the liquid saturated on the test surfaces prior to application of Decon 30, two buckets of untreated sewage were collected from the local wastewater treatment plant on two different days. A sample of the actual wastewater from the second application on the test wall cavities was submitted for analysis so that a baseline of bacterial contamination could be determined.

All told, a considerable number of steps were incorporated into the test design in an effort to more accurately evaluate how Decon 30 would perform in real life restoration scenarios. The test parameters were incorporated to determine how the product would perform under a "worst case" scenario (*i.e.*, raw, undiluted sewage as the test agent; no physical cleaning along with the chemical application; etc.). The following table summarizes the distinctions between the standard test parameters for chemical registration purposes in the United States and Canada and those utilized for this evaluation of the new Benefect product. Additional details regarding the testing process are included in sections 4.0 and 5.0 of this report.

Summary of EPA Test Parameters for Disinfectants	**Summary of Industry Representative Test Parameters for Benefect Decon 30**
• Standardized, hard, non-porous, inanimate surfaces (smooth)	• Typical restoration project test surface of porous, untreated, unsealed wood (uneven)
• Horizontal surfaces only	• Horizontal & vertical surfaces (significantly reduced contact time)
• Pre-cleaning required (~95% soil removal)	• No spray washing, scrubbing, wiping, rinsing or other physical cleaning of visible soil that was deposited with the application of the sewage
• Spray application	• Spray & foam application (clings for longer contact time but contains air bubbles)
• 10 minutes contact time prior to air dry	• Variable contact time from depending on surface orientation and application method before being allowed to air dry
• Standardized contaminant (simulated sewage)	• Real-world raw sewage from wastewater plant, no standardization (includes particulate)
• Standardized, consistent application of contaminant	• Multiple saturations of real sewage on real wood, no standardization

4.0 Materials and Equipment

An extensive array of equipment, materials, and supplies were marshaled to support the testing process. The primary items utilized for this project are briefly described here. Additional details regarding the equipment and setup can be gleaned from the photo log that is included as appendix 10.1.

4.1 Wood frame assembly. A wood frame assembly was constructed from 2x4 unfinished lumber and ½" unfinished oriented strand board (OSB) sheathing. The assembly was approximately 27" wide by 48" tall. The OSB was secured to one side of the panel, and an OSB "foot" was constructed at the base to provide additional stability to the upright panel. Prior to contaminating the panel it was HEPA-vacuumed to ensure that no residual construction dust was present that could skew the sampling and laboratory analysis.

4.2 Sewage (black water), bucket, brush. The local wastewater treatment plant provided raw sewage that was post-screening but pre-treatment, so that biological pathogens would still be present in the liquid. Sewage was acquired on two separate occasions so that the wood frame assemblies could be saturated multiple times as a simulation of a flooded building. Approximately three gallons of sewage was obtained in a 5-gallon bucket each time and then brought to the study site where it was used within a day. Using a soft-bristled brush with a long handle, the sewage was mopped onto the assembly and left to dry. A liquid sample of the sewage was obtained for lab analysis.

4.3 Garden sprayer. A standard airless pressurized garden sprayer was used to apply Decon 30 in liquid form. The one-gallon sprayer was manufactured by GardenPlus, model #27151.

4.4 Foam sprayer. The foam application of Decon 30 was performed using the ten-gallon battery-operated Foam-It unit manufactured by Innovative Cleaning Equipment, item #FI-10N-13.

4.5 ATP luminometer, swabs. A portable meter was used to assess the effectiveness of surface decontamination immediately after cleaning. The field portable direct read device uses special swabs to collect and measure adenosine triphosphate (ATP), the universal energy molecule found in all animal, plant, bacteria, yeast, and mold cells. When ATP is brought into contact with the chemical reagent in the sample collection swab, light is emitted in direct proportion to the amount of ATP present. The system measures the amount of light generated and provides

information on the level of contamination after 15 seconds. The higher the reading, the more contamination present.

The ATP luminometer used was manufactured by Hygiena, model "SystemSURE Plus", a portable palm-sized unit. Test swabs used in the study were "UltraSnap", also manufactured by Hygiena. They had an expiration date of June 3, 2014, marked on each unit. Use of the ATP luminometer and swabs followed manufacturer's guidelines.

The size of the area sampled was 1"x1" as marked on the wood frame assembly with a black permanent marker prior to the contamination of the panel. Samples were collected both prior to and after cleaning of the sewage-contaminated panels. The sample collected after cleaning was taken from an area similar in material and orientation as was sampled prior to cleaning. Until they were used the swabs were kept refrigerated.

4.6 Bacterial sponge samples. Bacterial samples were collected using SR18-10LET-G, SampleRight Sponge Samplers (with attached glove packet), manufactured by World Bioproducts. The product number on each bag was SR18-10LET-G18 oz., 100/ca. Lot No. SR18-10184-00546 Exp. Date 03/18/2014. The biocide-free cellulose sponge used for sampling was pre-moistened by the manufacturer with 10 ml of Letheen broth to maintain the viability of the bacteria.

Using sterile gloves, the sponge was removed from the sterile plastic sample bag. A sample was collected by gently rubbing the sponge over the surface of a 100 cm^2 area of the selected location as marked on the wood frame assembly with a black marker prior to contamination of the panel. Each sponge was then re-inserted into its plastic bag and the bag was sealed. Samples were placed in a cooler containing ice packs for overnight shipment to Summit Laboratory for analysis.

Samples were collected both prior to and after cleaning of the sewage-contaminated panels. The sample collected after cleaning was taken from an area similar in material and orientation as was sampled prior to cleaning.

For analysis, the levels of total viable coliform, *Escherichia coli*, and *Enterococcus* were determined and reported as colony forming units per 100 milliliters of swab solution (cfu/100 mL). Because of the specifics of the analytical process used for *Enterococcus* bacteria the results from those tests are reported as "most probable number" per 100 milliliters of swab solution (MPN/100 mL). This result has a close correlation to colony forming units but is

stated differently because the analytical technique requires multiple repetitions of the diagnostic process and the selection sample result that is most representative of the entire batch. The selection of these analytical parameters to properly represent sewage/black water contamination was based on research done for previous studies related to sewage contamination of contents. These particular bacterial contaminants are used by multiple laboratories, and even government agencies, as surrogates for identifying sewage contamination in water and porous materials.

5.0 Procedures

A specific series of steps were followed to implement the sampling plan developed as part of the investigative process. The primary stages of the testing process included:

5.1 Following the assembly of the wood panels, they were HEPA-vacuumed and then marked to indicate a consistent area for analysis. Four areas were marked and numbered for each sampling method, each orientation (horizontal or vertical), and each form of cleaning solution (liquid or foam). The areas for ATP analysis were 1"x1" and marked using a permanent marker. The areas for bacterial sponge analysis were 16 square inches (100 square centimeters) and marked using a permanent marker. This made a total of 16 ATP sample locations and 16 bacterial sponge sample locations.

5.2 Initial contamination of the panels occurred out-of-doors in a parking lot and involved setting the panels on their backs and mopping all exposed surfaces of the wood assemblies with sewage, using a soft-bristled brush and long handle. Both panels were mopped with sewage four times over the course of the 24 hours prior to testing. Shortly after mopping the panels were set upright to dry.

5.3 The contaminated panels were moved to an indoor polyethylene-lined work area that was under negative pressure using a HEPA-filtered negative air machine. One additional rag-mopping with sewage (fifth application) was applied within 15 minutes before the application of Decon 30. Each of the panels was sampled prior to cleaning to provide a comparative reading for post-cleaning samples. Sampling methods included both ATP and bacterial sponge.

5.4 Decon 30 was sprayed on one half of one of the contaminated panels using the Foam-It sprayer to test how foamed Decon 30 performed on vertically-oriented wood surfaces. Within 30 seconds the Decon 30 foam was wiped away from two of the pre-marked sample locations (a larger square for a sponge sample and a smaller square for an ATP swab sample) with a gloved hand. Both ATP and

bacterial sponge samples were collected for the first round of analysis. The foam was then allowed to "dwell" on the wood framing (12 minutes 15 seconds) until the foam adequately dissipated for additional sampling to take place by both methods. The panel was then allowed to dry before a fourth set of samples was collected, approximately 1½ hours later.

5.5 Decon 30 was sprayed on the second half of the first contaminated panel using the Foam-It sprayer in order to test how foamed Decon 30 performed on horizontally-oriented wood surfaces. Within 30 seconds the Decon 30 foam was wiped away from two test locations with a gloved hand and both ATP and bacterial sponge samples were collected for the first round of analysis. The foam was then allowed to "dwell" on the wood framing (25 minutes 30 seconds) until the foam adequately dissipated for additional sampling to take place by both methods. The panel was then allowed to dry before a fourth set of samples was collected, approximately 1½ hours later.

5.6 Decon 30 was sprayed on one half of the second contaminated panel using the garden sprayer in order to test how liquid Decon 30 performed on vertically-oriented wood surfaces. Within 30 seconds both ATP and bacterial sponge samples were collected for the first round of analysis. After approximately four minutes the second set of samples was collected. The panel was then allowed to dry before a fourth set of samples was collected, approximately 1½ hours later.

5.7 Decon 30 was sprayed on the second half of the second contaminated panel using the garden sprayer to test how liquid Decon 30 performed on horizontally-oriented wood surfaces. Within 30 seconds both ATP and bacterial sponge samples were collected for the first round of analysis. After approximately four minutes the second set of samples was collected. The panel was then allowed to dry before a fourth set of samples was collected, approximately 1½ hours later.

5.8 The bacterial sponge samples were placed into a cooler with a chain of custody form and an ice pack, and then were sent to Summit Laboratory via overnight UPS.

5.9 The collected ATP sampling data was inserted into the sample collection form.

5.10 Photos taken during the process were downloaded to the project file.

5.11 All surfaces of the work enclosure and the two contaminated panels were sprayed with Decon 30 using the Foam-It sprayer. The following day residual liquid was wiped from the enclosure and dried.

5.12 On June 4 and June 6, 2013, data from the analysis of the bacteria sponge samples was received from Summit Laboratory. The information was recorded on the sample collection form.

6.0 Data Presentation Format

The detailed results for all the tests are presented in the appendices, along with a simplified summary of the data obtained from this study. The summary includes ATP readings and lab results from samples collected using bacterial sponges prior to cleaning and after the surfaces were dry. In the summary table the intermediate sample results were excluded as the label directions for Decon 30 indicate that the product is to be applied and allowed to air dry. Therefore, the summary table compares the pre- and post-cleaning results that could be expected if the user follows the manufacturer's instructions.

7.0 Results

A number of clear trends were revealed by a simple review of the test results. Overall, a substantial reduction in biological contamination was observed after the application of Decon 30. Some specific notable outcomes include:

7.1 Significant reductions in contamination levels as measured with the ATP meter and swabs. ATP levels were reduced by greater than 97% for all tests of porous wood whether the application method was foam or liquid Decon 30. These reductions were observed regardless of whether the surface was vertical or horizontal.

7.1.1 The greatest reduction recorded by ATP sampling was on a horizontal surface where Benefect Decon 30 was sprayed as a liquid, and the readings went from 859 RLU on the contaminated surface to zero on the cleaned wood—a 100% decrease.

7.1.2 The smallest reduction recorded by ATP for the complete process (including drying) was when the chemical was foamed onto a vertical surface and the readings went from 859 RLU on the contaminated surface to 21 RLU on the cleaned wood—a 97.5% decrease.

7.2 *Enterococcus* levels were reduced by greater than 99% for all tests.

7.2.1 The greatest reduction recorded for *Enterococcus* bacteria was achieved on three tests (spray application on a vertical surface, spray application on a horizontal surface, and foam application on a vertical surface) where the comparison readings went from above the upper limit of detection (24,196,000 of bacteria per hundred milliliters of solution) on the contaminated surface to an end result of less than 1,000 bacteria in the solution on the cleaned wood—essentially a 100% decrease since the final number was less than the laboratory's lower limit of detection for that contaminant.

7.2.2 The least reduction recorded for *Enterococcus* bacteria after the cleaning process was complete was foam application on a horizontal surface, where the readings went from greater than 24,196,000 on the contaminated surface to 8,600 on the cleaned wood—a 99.97% decrease.

7.3 *Escherichia coli* and other coliform levels were reduced by greater than 97% in all but two tests.

7.3.1 The greatest reduction recorded by sponge sampling for total coliforms was on the horizontal surface with a foam application where the readings went from 66,000,000 colony forming units per milliliter (cfu/100 mL) on the contaminated surface to 100,000 cfu/100 mL on the cleaned wood—a 99.83% decrease.

7.3.2 The smallest reduction recorded by sponge sampling for total coliforms was on a horizontal surface that was sprayed with the Benefect product and allowed to dry. In that situation the recorded levels went from 84,000,000 cfu/100 mL on the contaminated surface to 22,000,000 cfu/100 mL on the cleaned wood—a 73.81% decrease.

7.3.2.1 It is interesting to note that this is the same set of samples where reduction in total coliforms (which includes *E. coli*) was also the lowest, yet the reduction of *Enterococcus* bacteria was the highest at essentially 100%. In addition, they also correspond with the lowest recorded ATP levels of all the samples with a 100% reduction. This lack of correlation can be the result of several factors such as the lack of pre-cleaning any visible soil (*i.e.*, spray washing).

7.3.3 The greatest reduction recorded by sponge sampling for *E. coli* was on the horizontal surface where Decon 30 was applied as foam. In that case the readings went from 8,000,000 cfu/100 mL on the contaminated surface to 13,000 cfu/100 mL on the cleaned wood after it was dry—a 99.84% decrease.

7.3.4 The smallest reduction recorded by sponge sampling for *E. coli* was on a horizontal wood stud with spray application, where the readings went from 8,000,000 cfu/100 mL on the contaminated surface to 2,200,000 cfu/100 mL on the cleaned wood—a 72.50% reduction.

7.3.4.1 It is interesting to note that this is the same set of samples where reduction in total coliforms (which includes *E. coli)* was also the lowest, yet the reduction of *Enterococcus* bacteria was the highest at essentially 100%. In addition, they also correspond with the lowest recorded ATP levels of all the samples with a 100% reduction. This lack of correlation can be the result of several factors such as the lack of pre-cleaning any visible soil (*i.e.*, spray washing).

7.4 Laboratory tests confirmed the elimination of specific marker organisms contaminating raw sewage: coliforms, *Escherichia coli*, and *Enterococcus*. Although the specific marker bacteria were cultured to gauge the cleaning efficiency of Decon 30, it is critical to keep in mind that raw sewage contains a plethora of microorganisms, including bacterial, viral, and even fungal materials. The ATP test, which is not bacteria specific, verified extensive removal of all microorganisms.

7.5 For specific sample information refer to the appendices Summary of Lab Results and Summit Laboratory Data.

8.0 Conclusions

8.1 The use of Decon 30 on unfinished wood lumber is effective in substantially reducing bacterial contamination as demonstrated by a 73.8% to 100% reduction of sewage indicator microbes on surfaces that represent typical wood stud framing.

8.1.1 These results were achieved with a straightforward application of the product as a spray or foam, without any additional cleaning steps.

8.1.2 Standard cleaning procedures that are often used when dealing with areas of known bacterial contamination (such as vacuuming, drying, agitation with brushes, wiping, etc.) may affect final results or intermediate readings.

8.1.2.1 Generally, it would be expected that any activities that supplement the chemical action of Decon 30 with physical cleaning could improve the already positive results.

8.2 The varied test results for the intermediate sampling serves to emphasize that the application of Decon 30 continues to reduce pathogens until it has dried.

8.2.1 These results are consistent with the manufacturer's label directions for proper use of the product except pre-cleaning directions in case of potential visible soil or debris.

8.2.2 In several cases the sample results from intermediate tests (after application of Decon 30 but prior to air drying) actually showed an increase in bacterial contamination.

8.2.2.1 It is speculated that the increase in bacterial counts on intermediate samples is a result of the product pulling saturated contamination to the surface.

8.2.3 Using cleaned surfaces or closing off exposed wall cavities before the Decon 30 has fully dried may not result in as high a level of bacteria reduction as would occur if application was in conformance with the label directions.

8.3 Decon 30 was applied as both liquid and foam. Test results show little effectual difference between the two forms of application.

8.3.1 It was clear from the testing that the foam application increased the visibility of Decon 30 on the surfaces. Nevertheless, it took approximately the same amount of time for both treated test models to air dry to the touch. Therefore, the application on the product as foam did not significantly increase the dwell time of the decontaminating chemical on the wood surfaces.

8.3.2 The investigators observed no differences when using Decon 30 liquid and foam.

8.3.2.1 None of the individuals involved in the testing noted any adverse reaction to the chemical throughout its use and handling.

8.3.2.2 VOCs seemed negligible, skin reaction minimal, and leave-behind ghosting or film on treated surfaces were not significant.

8.4 There is a strong correlation between the laboratory results and the ATP sample numbers when the pre-clean and post-clean/dry numbers are compared.

8.4.1 The correlation between the laboratory and the ATP numbers is not as strong for one set of the intermediate samples (the samples that showed 100% reduction with the ATP meter, and a 100% reduction of *Enterococcus*, but 72.5% *E. coli* reduction and 73.8% total coliform

reduction), suggesting that additional study be undertaken to further verify that ATP testing is an appropriate field testing methodology for evaluating the effectiveness of black water restoration projects.

9.0 Recommendations

Although the design of the test of the effectiveness of Decon 30 on porous materials that are typically impacted as part of black water losses was detailed and comprehensive, no single testing process can answer all the questions related to a product or process. Indeed, such testing often leads to additional questions regarding the efficacy of the product.

9.1 The fact that Decon 30, manufactured by Benefect, was shown to be effective in reducing black-water-style contamination on porous building materials (oriented both horizontally and vertically) should be shared with the restoration industry.

9.1.1 Even though EPA registration of disinfectants is for hard, non-porous surfaces, many antimicrobial products are applied to porous building materials by restoration professionals as part of the remediation process.

9.1.1.1 Industry guidance documents, such as the Standard and Reference Guide for Professional Water restoration (IICRC S500), recommend the application of antimicrobials as part of the restoration process for category 2 or category 3 water losses.

9.1.2 This study offers evidence that Decon 30 can be effective when used as part of a water loss response, even if the material being treated is porous.

9.2 Utilization of field test devices such as ATP meters can be recommended with some reliance for evaluating the effectiveness of Decon 30 on water-damaged dimensional lumber as long as proper precautions are provided to users.

9.2.1 Individuals choosing to use an ATP meter to help determine the effectiveness of decontamination after water loss should be warned that the results may show an increase in relative light units as the cleaning process is underway due to the release of contaminants from inside the porous structural materials.

9.2.2 Instruction should be provided that pre-cleaning sample results should be compared to post-cleaning and post-dry conditions for the most accurate understanding of the decontamination process.

9.3 In this particular case the increase in the number of bacteria cultured from the intermediate samples collected while the surfaces were still wet seems to indicate

that the surfactants in Benefect Decon 30 are deep cleaning the wood materials by bringing contaminants to the surface.

9.3.1 An additional test protocol, perhaps involving the collection of core samples from the wood stud and the analysis of specific layers of the cores, would have to be undertaken to verify this hypothesis.

9.4 As noted previously, both dimensional lumber and OSB absorb water when wetted. As such, by definition they are both considered to be porous materials. Nevertheless, in the restoration industry dimensional lumber is often characterized as semi-porous, while OSB is typically treated as porous material. While it is expected that Decon 30 would perform similarly on OSB as compared to dimensional lumber, testing comparable to what was conducted in this investigation could confirm that hypothesis.

10.0 Appendices

As discussed in several sections above, a number of appendices are included with this report that supply significantly more detail. Of particular interest is the photo log (appendix 10.1), as it provides more comprehensive visual documentation of the project.

The two summarized tables of results and the actual analytical data from the laboratory that conducted analysis of the bacterial sponges are attached as appendices 10.2 through 10.4.

11.0 Certifications

Michael Pinto provided oversight and generated the report for this study. Mr. Pinto's post-graduate training is in Public Administration and Environmental Engineering, and, in addition to his scholastic achievements, he holds the titles of Certified Safety Professional and Certified Mold Professional. He is a member of the American Society of Safety Engineers, Restoration Industry Association, American Industrial Hygiene Association, Indoor Air Quality Association, and the Cleaning Industry Research Institute. Mr. Pinto is the author of over 150 published technical articles and has successfully conducted industrial hygiene/indoor air quality investigations since 1988.

Michael A. Pinto, CSP, CMP
CEO

Appendix 10.1

Photograph Log

Wonder Makers Environmental

PHOTOGRAPH LOG

PROJECT:	GC13-11834	**DATE:**	May 31, 2013
PROJECT NAME:	Benefect Decon 30 Study	**SPECIALIST:**	T. Kloosterman

1. Wood stud panels were constructed of standard lumber 2x4's and orient strand board (OSB) to simulate a standard wall cavity of a stick-built house.

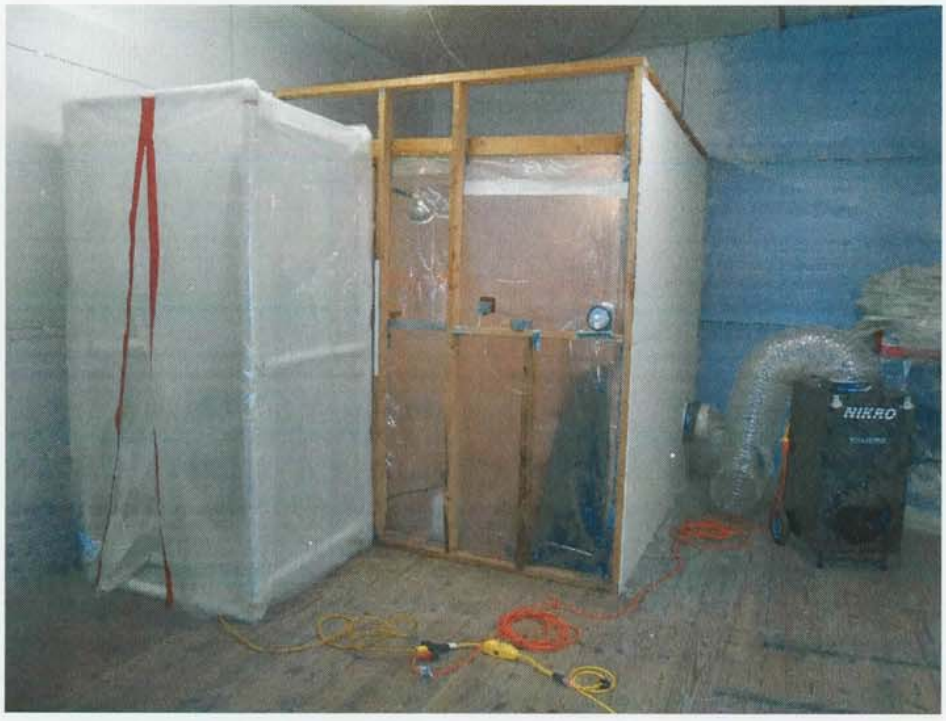
2. The two wall cavity panels were cleaned to ensure that no visible fungal growth was present prior to the test procedure. The panels were then marked to indicate a consistent area for sample collection. Areas were marked and numbered for each sampling method (ATP and bacterial sponge), each orientation (horizontal and vertical), and each form of cleaning product (liquid and foam). The areas for ATP sample collection were 1"x1", and the areas for bacterial sponge samples were 16 square inches (100 square centimeters).

3. In order to simulate wall areas that have been impacted by a black water loss, the wood panels were repeatedly soaked with raw sewage. The primary contamination of the panels occurred out-of-doors, where excess runoff could be more easily cleaned. Wonder Makers personnel that had the potential for direct contact with raw sewage utilized appropriate personal protective equipment including protective body covering, respiratory protection, boots, and gloves.

4. Sewage was obtained from the Kalamazoo County wastewater treatment facility. A five-gallon bucket of sewage that had been screened for large debris but had not been mechanically or chemically treated was provided.

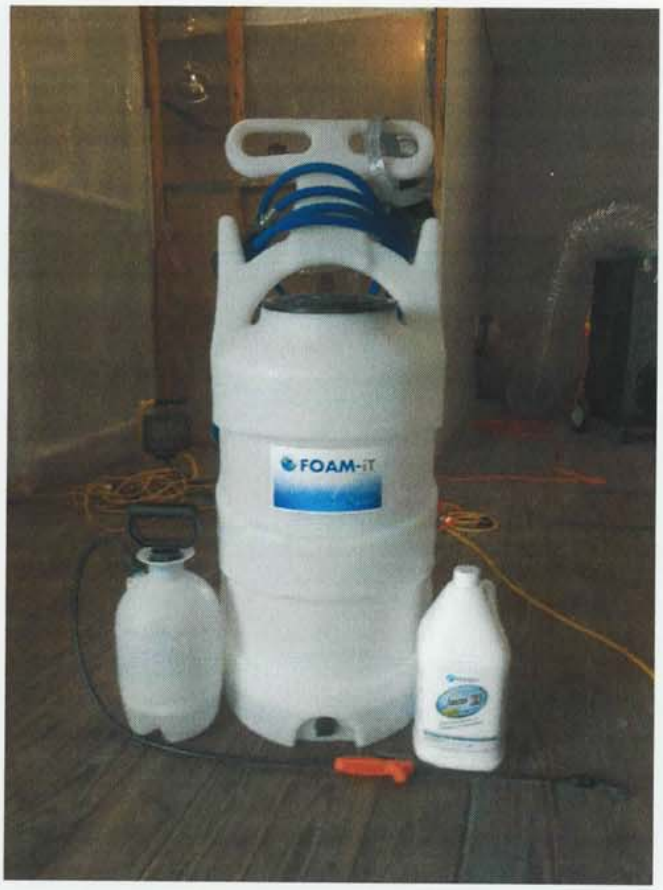
5. A sample of the sewage used for contaminating the wood panels was collected for laboratory analysis. The laboratory results verified that the sewage was contaminated with bacteria levels over 1 million colony forming units per 100 mL of solution.

6. Contamination of the panels involved mopping all exposed surfaces of the wood assemblies with the sewage using a soft-bristled brush and long handle. Both panels were mopped with sewage four times over the course of the 24 hours before testing.



7. One additional rag mopping with sewage (fifth application) was applied within 15 minutes before the application of Decon 30. For this last application of contaminants a second "fresh" container of post-screening pre-treatment sewage was obtained from the local water reclamation plant.

8. The contaminated panels were moved to an indoor polyethylene-lined work area that was under negative pressure using a HEPA-filtered negative air machine.



9. The primary materials and equipment utilized for the testing were a new pump-up garden sprayer, a battery-operated foam generator manufactured by Innovative Cleaning Equipment, and Decon 30 disinfectant cleaner manufactured by Benefect.

10. Each of the panels was sampled prior to cleaning to provide a comparison for post-cleaning samples. After the initial sampling, Benefect Decon 30 was applied to some of the vertical members of the wood panels, using the foam generating machine in accordance with the manufacturer's directions.

11. In order to determine if there is a difference in how Decon 30 performs, both horizontal and vertical studs were treated with foam.

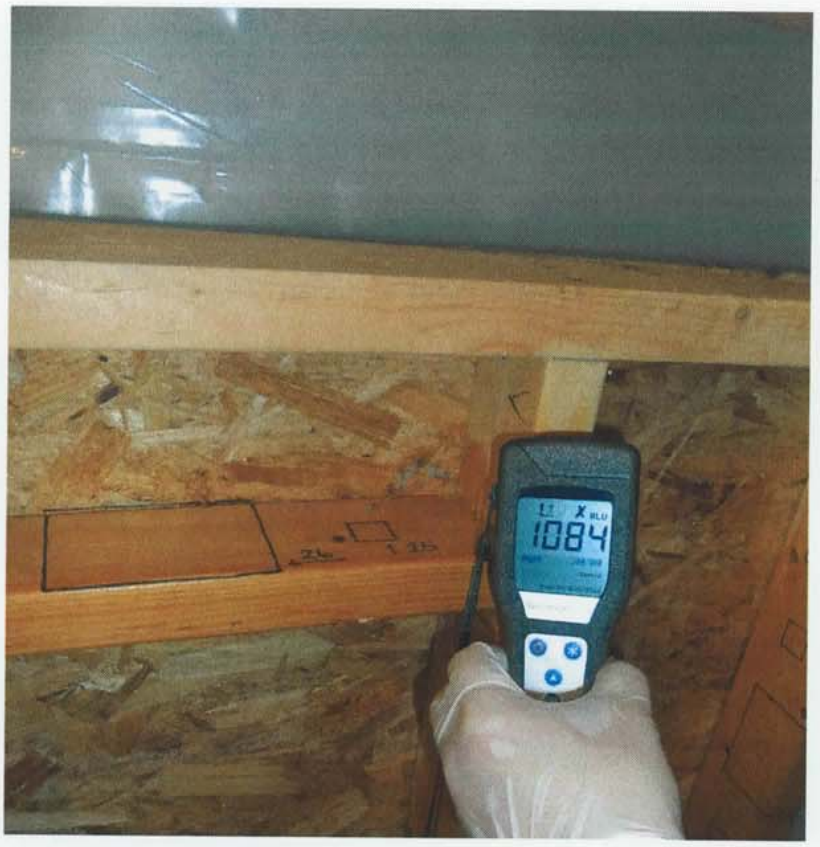
12. In a similar fashion, Decon 30 was applied to horizontal studs using the more traditional application method of the garden sprayer.

13. Vertical studs were also sprayed with liquid Decon 30.

14. Sampling methods included both ATP and bacterial sponge. The ATP meter and sample collection swabs allow for feedback on site with a 15 second analysis time after the sample has been collected on the swab and prepared for insertion into the meter.

15. Having sample locations pre-marked and pre-numbered allowed for quick and efficient collection of samples. Here, an ATP swab sample was collected from a vertical member after it had been sprayed with Decon 30 and allowed to dry.

16. Decon 30 was sprayed on one of the contaminated panels using the Foam-It sprayer to test how the foamed product performed on horizontally- and vertically-oriented wood surfaces. Although the manufacturer's directions call for the material to air dry as part of the decontamination process, interim samples were collected 30 seconds after application and after the foam/spray had dissipated in order to determine how the product was reacting with the contaminated surface. This ATP sample of the horizontal surface where the chemical had been applied as foam was collected after 25 minutes 30 seconds (the time it took for the foam to dissipate).



17. ATP sample results were documented with a photo as well as written records. In this case the pre-cleaning sample of the horizontal surface that was to be tested with the foam application had a sample result of 1,084 relative light units.

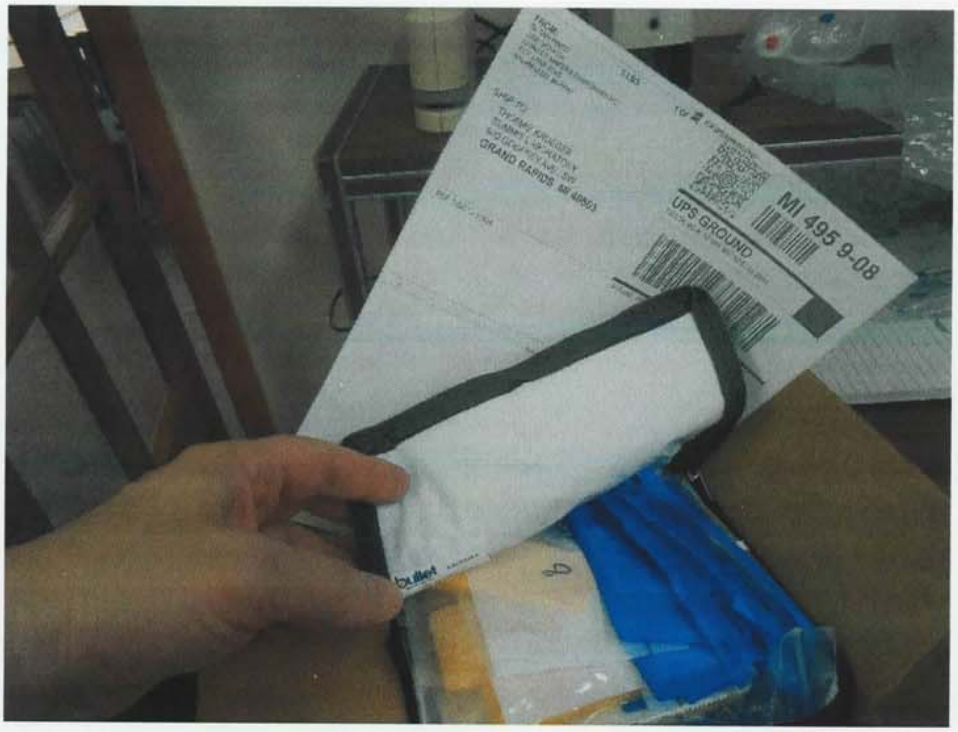
18. The ATP sample result for the horizontal surface where Decon 30 had been sprayed and then allowed to dwell for four minutes showed a substantial decrease, down to 13 relative light units.

19. On the horizontal wood surface where the chemical had been sprayed as a liquid the ATP result after application and drying was zero.

20. In order to generate more precise data, sterile sponges pre-saturated with Letheen broth were used to sample horizontal and vertical surfaces. The sponges came with their own gloves and were kept cool after sample collection.

21. As with the ATP samples, sponge samples were collected from both horizontal and vertical sections of the wood studs. Samples were also collected from surfaces where Decon 30 was applied as a foam and then then allowed to "dwell" on the wood until it had dissipated to the point where sampling of the substrate was possible without picking up visible foam. For this sample, the dwell time was approximately four minutes.

22. Because of the number of samples that had to be taken on a precise schedule, multiple individuals were involved in the testing process. From this photo it is clear that multiple samples were being collected at the same time.



23. The bacterial sponge samples were placed into a cooler with a chain of custody form and an ice pack, and then were sent to Summit Laboratory via overnight UPS.

24. Following the collection of all samples, all surfaces of the work enclosure and the wood panels were sprayed with Decon 30 foam and left to dry overnight. ATP sampling was conducted to confirm that no residual bacteria was present in the negative pressure enclosure used for testing.

Appendix 10.2

Summary of Laboratory Results – Pre- and Post-Cleaning

Sprayed Decon30 (liquid) - Vertical wood surfaces						
ATP		Bacterial sponge samples				
#	Reading	#	Total Coliform (cfu/mL of swab solution)	E.coli (cfu/mL of swab solution)	Enterococcus (MPN/100mL of swab solution)	Conditions
1	260	2	88,000,000	14,000,000	> 24,196,000	Soiled with sewage (pre-cleaning)
7	5	8	500,000	5,000	< 1,000	Dried after D30 (liq) application
	98.08%		**99.43%**	**99.96%**	**> 99.99%**	Percentage Reduction

Sprayed Decon30 (liquid) - Horizontal wood surfaces						
ATP		Bacterial sponge samples				
#	Reading	#	Total Coliform (cfu/mL of swab solution)	E.coli (cfu/mL of swab solution)	Enterococcus (MPN/100mL of swab solution)	Conditions
9	859	10	84,000,000	8,000,000	> 24,196,000	Soiled with sewage (pre-cleaning)
15	0	16	22,000,000	2,200,000	< 1,000	Dried after D30 (liq) application
	100.00%		**73.81%**	**72.50%**	**> 99.99%**	Percentage Reduction

Foamed Decon30 - Vertical wood surfaces						
ATP		Bacterial sponge samples				
#	Reading	#	Total Coliform (cfu/mL of swab solution)	E.coli (cfu/mL of swab solution)	Enterococcus (MPN/100mL of swab solution)	Conditions
17	838	18	20,000,000	700,000	> 24,196,000	Soiled with sewage (pre-cleaning)
23	21	24	80,000	19,000	< 1,000	Dried after D30 (foam) application
	97.49%		**99.60%**	**97.29%**	**> 99.99%**	Percentage Reduction

Foamed Decon30 - Horizontal wood surfaces						
ATP		Bacterial sponge samples				
#	Reading	#	Total Coliform (cfu/mL of swab solution)	E.coli (cfu/mL of swab solution)	Enterococcus (MPN/100mL of swab solution)	Conditions
25	1084	26	66,000,000	8,000,000	> 24,196,000	Soiled with sewage (pre-cleaning)
31	1	32	110,000	13,000	8,600	Dried after D30 (foam) application
	99.91%		**99.83%**	**99.84%**	**99.96%**	Percentage Reduction

Lab analysis of sewage sample						
ATP		Bacterial sponge samples				
#	Reading	#	Total Coliform (cfu/mL of swab solution)	E.coli (cfu/mL of swab solution)	Enterococcus (MPN/100mL of swab solution)	Conditions
33	2228	34	1,100,000	30,000	103,900	n/a

Field Blank						
ATP		Bacterial sponge samples				
#	Reading	#	Total Coliform (cfu/mL of swab solution)	E.coli (cfu/mL of swab solution)	Enterococcus (MPN/100mL of swab solution)	Conditions
35	n/a	36	< 10	< 10	< 1,000	n/a

Comments:

1 Application of Decon30 included only the application using the method indicated. No scrubbing, rinsing, or re-application was used.

2 Intermediate samples were also collected during the drying of the panels to monitor efficacy over time.

3 Samples were collected of the dried panels approximately 2.5 - 3 hours after application of Decon 30.

Appendix 10.3

Summary of Laboratory Results – With Intermediate Samples

Sprayed Decon30 (liquid) - Vertical wood surfaces

ATP		Bacterial sponge samples				
#	Reading	#	Total Coliform (cfu/mL of swab solution)	E.coli (cfu/mL of swab solution)	Enterococcus (MPN/100mL of swab solution)	Sampling Period
1	260	2	88,000,000	14,000,000	> 24,196,000	Soiled with sewage (pre-cleaning)
3	9	4	86,000,000	10,000,000	> 24,196,000	30 seconds after D30 (liq) application
5	57	6	90,000,000	14,000,000	> 24,196,000	4 minutes after D30 (liq) application
7	5	8	500,000	5,000	< 1,000	Dried after D30 (liq) application

Sprayed Decon30 (liquid) - Horizontal wood surfaces

ATP		Bacterial sponge samples				
#	Reading	#	Total Coliform (cfu/mL of swab solution)	E.coli (cfu/mL of swab solution)	Enterococcus (MPN/100mL of swab solution)	Sampling Period
9	859	10	84,000,000	8,000,000	> 24,196,000	Soiled with sewage (pre-cleaning)
11	0	12	92,000,000	12,000,000	> 24,196,000	30 seconds after D30 (liq) application
13	13	14	84,000,000	12,000,000	> 24,196,000	4 minutes after D30 (liq) application
15	0	16	22,000,000	2,200,000	< 1,000	Dried after D30 (liq) application

Foamed Decon30 - Vertical wood surfaces

ATP		Bacterial sponge samples				
#	Reading	#	Total Coliform (cfu/mL of swab solution)	E.coli (cfu/mL of swab solution)	Enterococcus (MPN/100mL of swab solution)	Sampling Period
17	838	18	20,000,000	700,000	> 24,196,000	Soiled with sewage (pre-cleaning)
19	162	20	70,000,000	14,000,000	1,986,300	30 seconds after D30 (foam) application
21	158	22	24,000,000	3,100,000	49,500	12:15 minutes after D30 (foam) application
23	21	24	80,000	19,000	< 1,000	Dried after D30 (foam) application

Foamed Decon30 - Horizontal wood surfaces

ATP		Bacterial sponge samples				
#	Reading	#	Total Coliform (cfu/mL of swab solution)	E.coli (cfu/mL of swab solution)	Enterococcus (MPN/100mL of swab solution)	Sampling Period
25	1084	26	66,000,000	8,000,000	> 24,196,000	Soiled with sewage (pre-cleaning)
27	285	28	26,000,000	1,700,000	> 24,196,000	30 seconds after D30 (foam) application
29	37	30	48,000,000	4,700,000	1,119,900	25:30 minutes after D30 (foam) application
31	1	32	110,000	13,000	8,600	Dried after D30 (foam) application

Lab analysis of sewage sample

ATP		Bacterial sponge samples				
#	Reading	#	Total Coliform (cfu/mL of swab solution)	E.coli (cfu/mL of swab solution)	Enterococcus (MPN/100mL of swab solution)	Sampling Period
33	2228	34	1,100,000	30,000	103,900	n/a

Field Blank

ATP		Bacterial sponge samples				
#	Reading	#	Total Coliform (cfu/mL of swab solution)	E.coli (cfu/mL of swab solution)	Enterococcus (MPN/100mL of swab solution)	Sampling Period
35	n/a	36	< 10	< 10	< 1,000	n/a

Appendix 10.4

Summit Laboratories Data

SUMMIT LABORATORY, LLC

900 Godfrey Avenue SW
Grand Rapids, MI 49503

Ph 616-245-3818
1-800-213-9589
Fax 616-245-3884

Client: Wonder Makers Environmental, Inc
PO Box 50209
Kalamazoo, MI 49005-0209

Report Date: June 6, 2013

Report prepared by: Michael Snarski

Summit Laboratory job #: 1305309

Contact: Mr. Tom Kloosterman

Samples Collected: 5/29 and 30/13 btw 2:00PM and 4:45PM by client
Samples Received: 5/31/13 @ 1:45PM
Analyses Started: 5/31/13 @ 4:30PM and 6/4/13

Analysis Requested: Quantification of *Enterococcus* (US EPA Method 1600)
Quantification of *Escherichia coli* and Total Coliform Bacteria (AOAC Official Method 991.14)

Following are the analytical results for the "**Environmental**" samples submitted:

Sample No:	Sample ID #	Sample Description		Total coliform results: (cfu/mL of swab solution)	*Escherichia coli* results: (cfu/mL of swab solution)	*Enterococcus* results: (MPN/100mL of swab solution)
1	11834-02	Decon 30 Liquid, vert wood, before	=	880×10^5	140×10^5	>24,196,000
2	11834-04	Decon 30 Liquid, vert wood, 30 sec	=	860×10^5	100×10^5	>24,196,000
3	11834-06	Decon 30 Liquid, vert wood, 4 min	=	900×10^5	140×10^5	>24,196,000
4	11834-08	Decon 30 Liquid, vert wood, dried	=	5.0×10^5	5.0×10^3	<1,000
5	11834-10	Decon 30 Liquid, horiz. wood, before	=	840×10^5	80×10^5	>24,196,000
6	11834-12	Decon 30 Liquid, horiz. wood, 30 sec	=	920×10^5	120×10^5	>24,196,000
7	11834-14	Decon 30 Liquid, horiz. wood, 4 min	=	840×10^5	120×10^5	>24,196,000
8	11834-16	Decon 30 Liquid, horiz. wood, dried	=	220×10^5	22×10^5	<1,000
9	11834-18	Decon 30 Foam, vert. wood, before	=	200×10^5	7.0×10^5	>24,196,000
10	11834-20	Decon 30 Foam, vert. wood, 30 sec	=	700×10^5	140×10^5	1,986,300
11	11834-22	Decon 30 Foam, vert. wood, 12m 15s	=	240×10^5	31×10^5	49,500
12	11834-24	Decon 30 Foam, vert. wood, dried	=	8.0×10^4	19×10^3	<1,000
13	11834-26	Decon 30 Foam, horiz. wood, before	=	660×10^5	80×10^5	>24,196,000
14	11834-28	Decon 30 Foam, horiz. wood, 30 sec	=	260×10^5	17×10^5	>24,196,000
15	11834-30	Decon 30 Foam, horiz. wood, 25m 30s	=	480×10^5	47×10^5	1,119,900
16	11834-32	Decon 30 Foam, horiz. wood, dried	=	1.1×10^5	13×10^3	8,600
17	11834-34	Sewage water sample, Kalamazoo	=	11×10^5	3.0×10^4	103,900
18	11834-36	Field Blank	=	< 10	< 10	<1,000
Summit Laboratory QC Dilution Blank			=	< 1	< 1	< 1

Analyses are in accordance with the Manual of Environmental Microbiology, 2nd Edition, 2002 and/or current AOAC methodologies. Results reported are provided "as is" and relate only to samples tested.

Report approved by:

Joel Steenstra
Laboratory Analyst

"The fusion of science and service"