UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
CHEMICAL SAFETY
AND POLLUTION
PREVENTION

April 7, 2015

MEMORANDUM

SUBJECT: Efficacy Review for Sanitizer #1;
EPA File Symbol 67603-RE;
DP Barcode: D424257

FROM: Marcus Rindal, Microbiologist
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)

THRU: Mark Perry, Team Leader
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)

TO: John C. Cowden, PM31/Velma Noble
Regulatory Management Branch II
Antimicrobials Division (7510P)

APPLICANT: Sherwin-Williams Diversified Brands
101 West Prospect Ave
Cleveland, Ohio 44115-1075

FORMULATION FROM LABEL:

Active Ingredient(s)	% by wt.
n-Alkyl (C_{14} 50%, C_{12} 40%, C_{16} 10%) Dimethyl benzyl ammonium chloride	0.52%
Other Ingredients:	99.48%
Total	100.00%

I BACKGROUND

The product, Sanitizer #1 (EPA File Symbol 67603-RE), is a new end-use product intended for use as an antimicrobial paint. The product is an interior architectural paint which functions as a dry film sanitizer. With this submission, the registrant seeks to make efficacy claims against *Staphylococcus aureus*, *Enterobacter aerogenes*, Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Enterococcus faecalis* (VRE), and *Escherichia coli* O157:H7 after 2 hours. This product may only be used on indoor hard non-porous surfaces where cleaning practices are consistent in non-critical areas of hospitals and other commercial, institutional, and residential environments. Studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Eagan, MN 55121.

This data package contained a letter from the applicant's representative to EPA (dated October 30, 2014), 24 study reports (19 efficacy studies and 5 summaries), Statements of No Data Confidentiality Claims for the studies, and the proposed label.

II USE DIRECTIONS

Directions on the proposed label provided the use of the architectural paint/coating on interior walls, doors, and trim of nursing homes, assisted living facilities or other group homes, day care centers, doctor and dentist offices, residences, and non-critical areas of hospitals such as waiting rooms, examination rooms, hallways, walkways, emergency rooms, offices, etc. with the exclusion of floors and exterior surfaces.

The proposed label provides guidance for surface care and repaint schedule and claims the surface (applied film) may be cleaned up to once a month without impacting bacterial reduction performance. Users are directed to repaint any surface if film becomes damaged or within 4 years.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

Test Requirements for the Sanitization Efficacy of Product as an Antimicrobial Coated Surface

Registrant Designated Test Method #1: Test Method for Determining the Efficacy of Antimicrobial Coated Surfaces as Sanitizers (EPA Approved Protocol 577PA1, DP Barcode 402958, Final Memo dated August 20, 2012).

The Agency approved protocol is based on the EPA Test Method for Efficacy of Copper Alloy Surfaces as a Sanitizer which includes the following requirements. Sanitizer efficacy testing must be conducted against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048), before additional organisms or claims (residual self-sanitizing activity and continuous reduction) are considered. Claims are limited to indoor, hard, non-porous surfaces where cleaning practices are consistent.

To support a supplemental sanitization claim on an antimicrobial surface, a 99.9% reduction in numbers of the test organism(s) must be obtained as compared to the carrier quantitation control. Efficacy data can support the claim "kills greater than 99.9% of bacteria* within two hours (* Includes list of tested organisms). Claims are limited to indoor, hard, non-porous surfaces where cleaning practices are consistent. The following language is required on

the registered products, the use of an antimicrobials surface coating is a supplement to and not a substitute for standard infection control practices; user must continue to follow all current infection control practices, including those practices related to cleaning and disinfection of environmental surfaces. The study controls must perform according to the criteria detailed in the study controls description section for purity controls, organic soil sterility control, carrier sterility control, neutralizing subculture medium sterility control, viability control, neutralization confirmation control, inoculum count, and carrier quantitation control.

Registrant Designated Test Method #2: Test Method for Continuous Reduction of Organisms on Antimicrobial Coated Surfaces (EPA Approved Protocol 577PA3, DP Barcode 402956, Final Memo dated August 20, 2012).

The Agency approved protocol is based on the EPA Test Method for the Continuous Reduction of Bacterial Contamination on Copper Alloy Surfaces which includes the following requirements. Acceptable efficacy testing is required against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048) as a non-food contact sanitizer before additional microorganisms or claims can be granted, or for claims of Continuous Reduction and/or Residual Self-Sanitizing Activity. Therefore, a 99.9% initial reduction within 60 minutes of exposure (supplemental sanitization claim) followed by continuous reduction of 90% thereafter is required. Claims are limited to indoor, hard, non-porous surfaces where cleaning practices are consistent.

To support a claim for continuously reducing bacteria on an antimicrobial surface, a minimum of 90% reduction in numbers of the test organism(s) on the test surface compared to the number of test organism(s) on the control surface must be achieved at all recovery times over the 24 hour inoculation and exposure period.

Efficacy data can support the claim that the surface continuously reduces bacterial (includes list of tested organisms) contamination: surface provides continuous/ongoing/persistent antimicrobial action even with repeated exposures, surface continuously kills over 90% of bacteria (includes list of tested organisms) after repeated exposures during a day, surface prevents the buildup of disease-causing bacteria (includes list of tested organisms), and surface delivers continuous, long-lasting antibacterial (includes list of tested organisms) activity. Claims are limited to indoor, hard, non-porous surfaces where cleaning practices are consistent.

The study controls must perform according to the criteria detailed in the study controls description section for purity controls, organic soil sterility control, carrier sterility control, neutralizing subculture medium sterility control, viability control, neutralization confirmation control, inoculum count, and carrier quantitation control.

Registrant Designated Test Method #3: Test Method for Determining the Efficacy of Antimicrobial Coated Surfaces as Residual Self Sanitizers (EPA Approved Protocol 577PA2, DP Barcode 402957, Final Memo dated August 20, 2012).

The proposed method is based on the EPA Test Method for Efficacy of Copper Alloy Surfaces as a Sanitizer and Test Method for Residual Self-Sanitizing Activity of Copper Alloy Surfaces which includes the following requirements. Sanitizer efficacy testing must be conducted against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048), before additional organisms or claims (residual self-sanitizing activity and continuous reduction) are considered. Claims are limited to indoor, hard, non-porous surfaces where cleaning practices are consistent. The study controls must perform according to the criteria detailed in the study controls description section for purity controls, organic soil sterility control,

carrier sterility control, neutralizing subculture medium sterility control, viability control, neutralization confirmation control, inoculum count, and carrier quantitation control.

To be defined as a residual self-sanitizer, the test material must reduce the total number of organisms by at least 99.9% on the surface within the prescribed exposure time. In order to demonstrate effective sanitization on a worn surface representative of end of use life, one set of material per lot will be subjected to wear procedure designed to simulate at least four years of quarterly washing of the painted wall surface.

IV COMMENTS ON THE SUBMITTED EFFICACY STUDIES

Test Method #1: Test Method for Determining the Efficacy of Antimicrobial Coated Surfaces as Sanitizers (EPA Approved Protocol 577PA1, DP Barcode 402958, Final Memo dated August 20, 2012). The objective of this study was to evaluate the antimicrobial efficacy of coated surfaces as a sanitizer against specific test organisms.
Performance Criteria: To support a claim for surface as a sanitizer, a 99.9% reduction in numbers of the test organism(s) must be obtained as compared to the carrier quantitation control for the product to be effective.

Test Method #2: Test Method for Continuous Reduction of Organisms on Antimicrobial Coated Surfaces (EPA Approved Protocol 577PA3, DP Barcode 402956, Final Memo dated August 20, 2012). The objective of this study was to determine the effectiveness of antimicrobial coated surfaces to continuously reduce test organism contamination after multiple re-inoculations over extended time periods.
Performance Criteria: To support a claim for continuously reducing test organisms on a treated surface, a minimum of a 90% reduction in numbers of the test organism on the test surface compared to the number of test organism on the control surface must be achieved at all recovery times over the 24 hour inoculation and exposure period.

Test Method #3: Test Method for Determining the Efficacy of Antimicrobial Coated Surfaces as Residual Self Sanitizers (EPA Approved Protocol 577PA2, DP Barcode 402957, Final Memo dated August 20, 2012). The objective of this study was to evaluate the antimicrobial efficacy of coated surfaces as a residual self sanitizer against specific test organisms.
Performance Criteria: To support a claim for a residual self sanitizer surface, a 99.9% reduction I numbers of the test organism(s) must be obtained as compared to the carrier quantitation control for both the unworn carriers (initial sanitizer) and the worn carriers (final sanitizer) for the product to be an effective residual self sanitizer.

Preparation of Painted Carriers

The Test Material Paint(s) as well as the Quantitation Control paint, were applied to a plastic support base in order to generate hard surface carriers for efficacy testing. Paint was applied to scrub chart panels in accordance with the Direction for Use on the draft product label for Sanitizer #1. After curing, painted scrub chart panels were cut into 1 inch squares to produce carriers for efficacy testing. In advance of testing, these carriers were sterilized by exposure to UV light for 15±2 minutes. Sterile painted carriers were used to evaluate the antimicrobial efficacy of Sanitizer #1 in Test Method #1, Test Method #2, and Test Method #3 as described.

1. **MRID 494203-14 "Test Method for Determining Efficacy," against *Staphylococcus aureus* (ATCC 6538) of Sanitizer #1 (EPA File Symbol 67603-RE) by Matthew Sathe. Study conducted at ATS Labs. Study completion date – May 31, 2013. Project Number A14718.**

This study was conducted against *Staphylococcus aureus* (ATCC 6538). This study was conducted using Test Method #1 against the base paint tinted with 10 different colorants (1 untinted + 10 different tinted versions for a total of 11 treatments) each prepared at the rate of 3 fl. oz. tint per gallon of white base. The purpose of testing the tinted formulations was to assess the potential for colorants to alter antimicrobial efficacy through dilution effects and/or chemical interactions with the active ingredient. A single lot of white base paint (Batch #2011-130:110) prepared at the nominal concentration for the active ingredient was used to prepare the 10 different tint preparations used in this study. Testing followed ATS Protocol Number SHE09021113.CNFS.7 (copy provided). A 48±4 hour culture of *S. aureus* was supplemented with fetal bovine serum organic load (5% final concentration) and Triton X-100 (0.01% final concentration). Sterilized carriers coated with test material and Quantitation Control paint formulations were inoculated with 40 µL of final test culture. Each of the 10 tint versions and controls were evaluated in sets of five carriers each. After 120 minutes of exposure at 25±2°C and 60±2% relative humidity, carriers were transferred to 20 mL of Letheen broth neutralizer medium and sonicated for 5 minutes. Serial dilutions of the sonicated neutralizer/carrier suspensions were plated in duplicate on TSA/5% Blood agar plates and incubated for 48±4 hours at 35-37°C. Resulting colonies were enumerated and used for calculations. Controls included neutralizer confirmation, carrier quantitation, carrier sterility, and initial inoculum count.

2. **MRID 494203-19 "Test Method for Determining Efficacy," against *Staphylococcus aureus* (ATCC 6538) of Sanitizer #1 (EPA File Symbol 67603-RE) by Matthew Sathe. Study conducted at ATS Labs. Study completion date – June 17, 2013. Project Number A14731.**

This study was conducted against *Staphylococcus aureus* (ATCC 6538). This study was conducted using Test Method #1 against three lots of base white paint each prepared at or below the Lower Certified Limit for active ingredient concentration (Batch #2011-130:124, #2011-130:125, and #2011-130:126). Testing followed ATS Protocol Number SHE09021113.CNFS.1 (copy provided). A 48±4 hour culture of *S. aureus* was supplemented with fetal bovine serum organic load (5% final concentration) and Triton X-100 (0.01% final concentration). Sterilized carriers coated with test material and Quantitation Control paint formulations were inoculated with 40 µL of final test culture. Each of the three test lots and controls were evaluated in sets of five carriers each. After 120 minutes of exposure at 25±2°C and 60±2% relative humidity, carriers were transferred to 20 mL of Letheen broth neutralizer medium and sonicated for 5 minutes. Serial dilutions of the sonicated neutralizer/carrier suspensions were plated in duplicate on TSA/5% Blood agar plates and incubated for 48±4 hours at 35-37°C. Resulting colonies were enumerated and used for calculations. Controls included neutralizer confirmation, carrier quantitation, carrier sterility, and initial inoculum count.

3. **MRID 494203-24 "Test Method Two for Determining Efficacy," against *Staphylococcus aureus* (ATCC 6538) of Sanitizer #1 (EPA File Symbol 67603-RE) by Matthew Sathe. Study conducted at ATS Labs. Study completion date – June 21, 2013. Project Number A14894.**

This study was conducted against *Staphylococcus aureus* (ATCC 6538). This study was conducted using Test Method #2 against three lots of base white paint each prepared at or below the Lower Certified Limit for active ingredient concentration (Batch #2011-130:124, #2011-130:125, and #2011-130:126). The untreated batch was #201-130:109. Testing followed ATS Protocol Number SHE09041613.CCR.1 (copy provided). The objective of this study was to determine the effectiveness of antimicrobial coated surfaces to continuously reduce test organism contamination after multiple re-inoculations over extended time periods. The exposure times were 2 hours following inoculation with recovery at 2, 5, 11, 17 and 24 hours. A 48±4 hour culture of *S. aureus* was supplemented with fetal bovine serum organic load (5% final concentration) and Triton X-100 (0.01% final concentration) to facilitate organism spreading. For the inoculation of treated test and untreated control carriers, five sets of Sponsor supplied treated test and untreated control carriers were used in the study. At time zero, all carrier sets were inoculated. At 3, 6, 9, 12, 15, 18, and 22 hours, carrier sets not removed for quantitative recovery were reinoculated. Each carrier was inoculated at staggered intervals with 40 µL of culture using a calibrated pipettor. Exposure began when the inoculum was first applied to the carrier. The carriers were held covered at 25±2°C and 60±2% relative humidity for the duration of the exposure. Sets of carriers were removed for quantitative recovery two hours after the corresponding inoculation point outlined in the chart below.

Test System Inoculation & Recovery Chart			
Carrier Set	Inoculation Time(s) in hours	Recovery Time in hours	Total # of Inoculations
1	0	2	1
2	0, 3	5	2
3	0, 3, 6, 9	11	4
4	0, 3, 6, 9, 12, 15	17	6
5	0, 3, 6, 9, 12, 15, 18, 22	24	8

At each recovery time, the carriers were transferred to 20 mL of neutralizer (10^0) following the same staggered intervals used during inoculation. Each neutralized vessel was sonicated for 5 minutes to suspend and survivors from the carriers and was rotated to mix. Serial dilutions of the sonicated neutralizer/carrier suspensions were prepared and duplicate 1.00 mL aliquots of 10^0 through 10^{-4} were plated onto agar. The plates and incubated for 48±4 hours at 35-37°C. Resulting colonies were enumerated and used for calculations. Controls included neutralizer confirmation, carrier quantitation, carrier sterility, and initial inoculum count.

4. **MRID 494203-28 "Test Method Three for Determining Efficacy," against *Staphylococcus aureus* (ATCC 6538) of Sanitizer #1 (EPA File Symbol 67603-RE) by Matthew Sathe. Study conducted at ATS Labs. Study completion date – June 20, 2013. Project Number A14702.**

This study was conducted against *Staphylococcus aureus* (ATCC 6538). This study was conducted using Test Method #3 against three lots of base white paint each prepared at or below the Lower Certified Limit for active ingredient concentration (Batch #2011-130:124, #2011-130:125, and #2011-130:126). The untreated batch was #201-130:109. Testing followed ATS Protocol Number SHE09021813.CRS.1 (copy provided). The objective of this study was to evaluate the antimicrobial efficacy of coated surfaces as a residual self sanitizer against *Staphylococcus aureus*. The exposure time tested was 2 hours. For the preparation of carriers, simulated wear cycling was initiated after the final coat of paint on each test panel had been allowed to cure for at least 24 hours. The wear procedure was intended to simulate cleaning of a vertical surface; therefore, relatively low volumes of cleaner were applied to the test panels at a rate where the cleaner would not drip down to the floor if applied to a vertical

surface. The cleaning solution, Best Yet Citrus Cleaner, was prepared at the manufacturer's recommended dilution ration. A common sponge was soaked in the prepared cleaner solution prior to the initial simulated wear cycle. The appropriate test or control panel was placed on the Gardco Washability Tester tray. The sponge was removed from the solution, wrung of excess solution and positioned under the 1000±10 gram weigh boat. The cycle was started and two cycles were performed, representing one complete wear cycle (equivalent to 4 washes or one month of cleaning). The panel was removed and placed on a horizontal surface to dry at room temperature. This procedure was repeated until a total of 50 wear cycles (total of 200 washes) had been performed on each panel. The washed carriers were prepared as previously described. A 48±4 hour culture of *S. aureus* was supplemented with fetal bovine serum organic load (5% final concentration) and Triton X-100 (0.01% final concentration) to facilitate organism spreading. Two sets of treated carriers per lot, representing both worn and unworn surfaces were evaluated on the day of the test. The unworn surfaces were used to assess the initial sanitizing action of the surface. The worn surfaces were used to assess the sanitizing action of the surface at the end of its usable life. Each test and control carrier was inoculated at staggered intervals with 40 µL of culture using a calibrated pipettor. Exposure began when the inoculum was first applied to the carrier. The carriers were allowed to expose at 25±2°C and 60±2% relative humidity for 2 hours. Following exposure, the carriers were transferred to 20 mL of neutralizer (10^0) following the same staggered intervals used during inoculation. Each neutralized vessel was sonicated for 5 minutes to suspend and survivors from the carriers and was rotated to mix. Serial dilutions of the sonicated neutralizer/carrier suspensions were prepared and duplicate 0.100 mL aliquots of 10^0 through 10^{-3} were plated onto agar. The plates and incubated for 48±4 hours at 35-37°C. Resulting colonies were enumerated and used for calculations. Controls included neutralizer confirmation, carrier quantitation, carrier sterility, and initial inoculum count.

5. **MRID 494203-15 "Test Method for Determining Efficacy," against *Enterobacter aerogenes* (ATCC 13048) of Sanitizer #1 (EPA File Symbol 67603-RE) by Matthew Sathe. Study conducted at ATS Labs. Study completion date – June 5, 2013. Project Number A14719.**

This study was conducted against *Enterobacter aerogenes* (ATCC 13048). This study was conducted using Test Method #1 against the base paint tinted with 10 different colorants (1 untinted + 10 different tinted versions for a total of 11 treatments) each prepared at the rate of 3 fl. oz. tint per gallon of white base. The purpose of testing the tinted formulations was to assess the potential for colorants to alter antimicrobial efficacy through dilution effects and/or chemical interactions with the active ingredient. A single lot of white base paint (Batch #2011-130:110) prepared at the nominal concentration for the active ingredient was used to prepare the 10 different tint preparations used in this study. Testing followed ATS Protocol Number SHE09021113.CNFS.6 (copy provided). A 48±4 hour culture of *E. aerogenes* was supplemented with fetal bovine serum organic load (5% final concentration) and Triton X-100 (0.01% final concentration). Sterilized carriers coated with test material and Quantitation Control paint formulations were inoculated with 40 µL of final test culture. Each of the 10 tint versions and controls were evaluated in sets of five carriers each. After 120 minutes of exposure at 25±2°C and 60±2% relative humidity, carriers were transferred to 20 mL of Letheen broth neutralizer medium and sonicated for 5 minutes. Serial dilutions of the sonicated neutralizer/carrier suspensions were plated in duplicate on agar plates and incubated for 48±4 hours at 25-30°C. Resulting colonies were enumerated and used for calculations. Controls included neutralizer confirmation, carrier quantitation, carrier sterility, and initial inoculum count.

6. **MRID 494203-20 "Test Method for Determining Efficacy," against *Enterobacter aerogenes* (ATCC 13048) of Sanitizer #1 (EPA File Symbol 67603-RE) by Matthew Sathe. Study conducted at ATS Labs. Study completion date – June 17, 2013. Project Number A14732.**

This study was conducted against *Enterobacter aerogenes* (ATCC 13048). This study was conducted using Test Method #1 against three lots of base white paint each prepared at or below the Lower Certified Limit for active ingredient concentration (Batch #2011-130:124, #2011-130:125, and #2011-130:126). Testing followed ATS Protocol Number SHE09021113.CNFS.2 (copy provided). A 48±4 hour culture of *E. aerogenes* was supplemented with fetal bovine serum organic load (5% final concentration) and Triton X-100 (0.01% final concentration). Sterilized carriers coated with test material and Quantitation Control paint formulations were inoculated with 40 µL of final test culture. Each of the three test lots and controls were evaluated in sets of five carriers each. After 120 minutes of exposure at 25±2°C and 60±2% relative humidity, carriers were transferred to 20 mL of Letheen broth neutralizer medium and sonicated for 5 minutes. Serial dilutions of the sonicated neutralizer/carrier suspensions were plated in duplicate on agar plates and incubated for 48±4 hours at 25-30°C. Resulting colonies were enumerated and used for calculations. Controls included neutralizer confirmation, carrier quantitation, carrier sterility, and initial inoculum count.

7. **MRID 494203-25 "Test Method Two for Determining Efficacy," against *Enterobacter aerogenes* (ATCC 13048) of Sanitizer #1 (EPA File Symbol 67603-RE) by Matthew Sathe. Study conducted at ATS Labs. Study completion date – June 24, 2013. Project Number A14895.**

This study was conducted against *Enterobacter aerogenes* (ATCC 13048). This study was conducted using Test Method #2 against three lots of base white paint each prepared at or below the Lower Certified Limit for active ingredient concentration (Batch #2011-130:124, #2011-130:125, and #2011-130:126). The untreated batch was #201-130:109. Testing followed ATS Protocol Number SHE09041613.CCR.2 (copy provided). The objective of this study was to determine the effectiveness of antimicrobial coated surfaces to continuously reduce test organism contamination after multiple re-inoculations over extended time periods. The exposure times were 2 hours following inoculation with recovery at 2, 5, 11, 17 and 24 hours. A 48±4 hour culture of *E. aerogenes* was supplemented with fetal bovine serum organic load (5% final concentration) and Triton X-100 (0.01% final concentration) to facilitate organism spreading. For the inoculation of treated test and untreated control carriers, five sets of Sponsor supplied treated test and untreated control carriers were used in the study. At time zero, all carrier sets were inoculated. At 3, 6, 9, 12, 15, 18, and 22 hours, carrier sets not removed for quantitative recovery were reinoculated. Each carrier was inoculated at staggered intervals with 40 µL of culture using a calibrated pipettor. Exposure began when the inoculum was first applied to the carrier. The carriers were held covered at 25±2°C and 60±2% relative humidity for the duration of the exposure. Sets of carriers were removed for quantitative recovery two hours after the corresponding inoculation point outlined in the chart below.

Test System Inoculation & Recovery Chart			
Carrier Set	Inoculation Time(s) in hours	Recovery Time in hours	Total # of Inoculations
1	0	2	1
2	0, 3	5	2
3	0, 3, 6, 9	11	4
4	0, 3, 6, 9, 12, 15	17	6

5	0, 3, 6, 9, 12, 15, 18, 22	24	8

At each recovery time, the carriers were transferred to 20 mL of neutralizer (10^0) following the same staggered intervals used during inoculation. Each neutralized vessel was sonicated for 5 minutes to suspend and survivors from the carriers and was rotated to mix. Serial dilutions of the sonicated neutralizer/carrier suspensions were prepared and duplicate 1.00 mL aliquots of 10^0 through 10^{-4} were plated onto agar. The plates and incubated for 48±4 hours at 25-30°C. Resulting colonies were enumerated and used for calculations. Controls included neutralizer confirmation, carrier quantitation, carrier sterility, and initial inoculum count.

8. **MRID 494203-29 "Test Method Three for Determining Efficacy," against *Enterobacter aerogenes* (ATCC 13048) of Sanitizer #1 (EPA File Symbol 67603-RE) by Matthew Sathe. Study conducted at ATS Labs. Study completion date – June 18, 2013. Project Number A14703.**

This study was conducted against *Enterobacter aerogenes* (ATCC 13048). This study was conducted using Test Method #3 against three lots of base white paint each prepared at or below the Lower Certified Limit for active ingredient concentration (Batch #2011-130:124, #2011-130:125, and #2011-130:126). The untreated batch was #201-130:109. Testing followed ATS Protocol Number SHE09021813.CRS.2 (copy provided). The objective of this study was to evaluate the antimicrobial efficacy of coated surfaces as a residual self sanitizer against *Enterobacter aerogenes*. The exposure time tested was 2 hours. For the preparation of carriers, simulated wear cycling was initiated after the final coat of paint on each test panel had been allowed to cure for at least 24 hours. The wear procedure was intended to simulate cleaning of a vertical surface; therefore, relatively low volumes of cleaner were applied to the test panels at a rate where the cleaner would not drip down to the floor if applied to a vertical surface. The cleaning solution, Best Yet Citrus Cleaner, was prepared at the manufacturer's recommended dilution ration. A common sponge was soaked in the prepared cleaner solution prior to the initial simulated wear cycle. The appropriate test or control panel was placed on the Gardco Washability Tester tray. The sponge was removed from the solution, wrung of excess solution and positioned under the 1000±10 gram weigh boat. The cycle was started and two cycles were performed, representing one complete wear cycle (equivalent to 4 washes or one month of cleaning). The panel was removed and placed on a horizontal surface to dry at room temperature. This procedure was repeated until a total of 50 wear cycles (total of 200 washes) had been performed on each panel. The washed carriers were prepared as previously described. A 48±4 hour culture of *E. aerogenes* was supplemented with fetal bovine serum organic load (5% final concentration) and Triton X-100 (0.01% final concentration) to facilitate organism spreading. Two sets of treated carriers per lot, representing both worn and unworn surfaces were evaluated on the day of the test. The unworn surfaces were used to assess the initial sanitizing action of the surface. The worn surfaces were used to assess the sanitizing action of the surface at the end of its usable life. Each test and control carrier was inoculated at staggered intervals with 40 µL of culture using a calibrated pipettor. Exposure began when the inoculum was first applied to the carrier. The carriers were allowed to expose at 25±2°C and 60±2% relative humidity for 2 hours. Following exposure, the carriers were transferred to 20 mL of neutralizer (10^0) following the same staggered intervals used during inoculation. Each neutralized vessel was sonicated for 5 minutes to suspend and survivors from the carriers and was rotated to mix. Serial dilutions of the sonicated neutralizer/carrier suspensions were prepared and duplicate 0.100 mL aliquots of 10^0 through 10^{-3} were plated onto agar. The plates and incubated for 48±4 hours at 25-30°C. Resulting colonies were enumerated and used for calculations. Controls included neutralizer confirmation, carrier quantitation, carrier sterility, and

initial inoculum count.

9. **MRID 494203-17 "Test Method for Determining Efficacy," against *Staphylococcus aureus*-MRSA (ATCC 33592) of Sanitizer #1 (EPA File Symbol 67603-RE) by Matthew Sathe. Study conducted at ATS Labs. Study completion date – June 5, 2013. Project Number A14721.**

This study was conducted against *Staphylococcus aureus*-MRSA (ATCC 33592). This study was conducted using Test Method #1 against the base paint tinted with 10 different colorants (1 untinted + 10 different tinted versions for a total of 11 treatments) each prepared at the rate of 3 fl. oz. tint per gallon of white base. The purpose of testing the tinted formulations was to assess the potential for colorants to alter antimicrobial efficacy through dilution effects and/or chemical interactions with the active ingredient. A single lot of white base paint (Batch #2011-130:110) prepared at the nominal concentration for the active ingredient was used to prepare the 10 different tint preparations used in this study. Testing followed ATS Protocol Number SHE09021113.CNFS.10 (copy provided). A 48±4 hour culture of *S. aureus*-MRSA was supplemented with fetal bovine serum organic load (5% final concentration) and Triton X-100 (0.01% final concentration). Sterilized carriers coated with test material and Quantitation Control paint formulations were inoculated with 40 µL of final test culture. Each of the 10 tint versions and controls were evaluated in sets of five carriers each. After 120 minutes of exposure at 25±2°C and 60±2% relative humidity, carriers were transferred to 20 mL of Letheen broth neutralizer medium and sonicated for 5 minutes. Serial dilutions of the sonicated neutralizer/carrier suspensions were plated in duplicate on TSA/5% Blood agar plates and incubated for 48±4 hours at 35-37°C. Resulting colonies were enumerated and used for calculations. Controls included neutralizer confirmation, carrier quantitation, carrier sterility, and initial inoculum count.

10. **MRID 494203-22 "Test Method for Determining Efficacy," against *Staphylococcus aureus*-MRSA (ATCC 33592) of Sanitizer #1 (EPA File Symbol 67603-RE) by Matthew Sathe. Study conducted at ATS Labs. Study completion date – June 19, 2013. Project Number A14736.**

This study was conducted against *Staphylococcus aureus*-MRSA (ATCC 33592). This study was conducted using Test Method #1 against two lots of base white paint each prepared at or below the Lower Certified Limit for active ingredient concentration (Batch #2011-130:124 and #2011-130:125). Testing followed ATS Protocol Number SHE09021113.CNFS.1 (copy provided). A 48±4 hour culture of *S. aureus*-MRSA was supplemented with fetal bovine serum organic load (5% final concentration) and Triton X-100 (0.01% final concentration). Sterilized carriers coated with test material and Quantitation Control paint formulations were inoculated with 40 µL of final test culture. Each of the three test lots and controls were evaluated in sets of five carriers each. After 120 minutes of exposure at 25±2°C and 60±2% relative humidity, carriers were transferred to 20 mL of Letheen broth neutralizer medium and sonicated for 5 minutes. Serial dilutions of the sonicated neutralizer/carrier suspensions were plated in duplicate on TSA/5% Blood agar plates and incubated for 48±4 hours at 35-37°C. Resulting colonies were enumerated and used for calculations. Controls included neutralizer confirmation, carrier quantitation, carrier sterility, and initial inoculum count.

11. **MRID 494203-26 "Test Method Two for Determining Efficacy," against *Staphylococcus aureus*-MRSA (ATCC 33592) of Sanitizer #1 (EPA File Symbol 67603-RE) by Matthew Sathe. Study conducted at ATS Labs. Study completion**

date – June 21, 2013. Project Number A14925.

This study was conducted against *Staphylococcus aureus*-MRSA (ATCC 33592). This study was conducted using Test Method #2 against two lots of base white paint each prepared at or below the Lower Certified Limit for active ingredient concentration (Batch #2011-130:124 and #2011-130:125). The untreated batch was #201-130:109. Testing followed ATS Protocol Number SHE09041613.CCR.4 (copy provided). The objective of this study was to determine the effectiveness of antimicrobial coated surfaces to continuously reduce test organism contamination after multiple re-inoculations over extended time periods. The exposure times were 2 hours following inoculation with recovery at 2, 5, 11, 17 and 24 hours. A 48±4 hour culture of *S. aureus*-MRSA was supplemented with fetal bovine serum organic load (5% final concentration) and Triton X-100 (0.01% final concentration) to facilitate organism spreading. For the inoculation of treated test and untreated control carriers, five sets of Sponsor supplied treated test and untreated control carriers were used in the study. At time zero, all carrier sets were inoculated. At 3, 6, 9, 12, 15, 18, and 22 hours, carrier sets not removed for quantitative recovery were reinoculated. Each carrier was inoculated at staggered intervals with 40 µL of culture using a calibrated pipettor. Exposure began when the inoculum was first applied to the carrier. The carriers were held covered at 25±2°C and 60±2% relative humidity for the duration of the exposure. Sets of carriers were removed for quantitative recovery two hours after the corresponding inoculation point outlined in the chart below.

Test System Inoculation & Recovery Chart			
Carrier Set	Inoculation Time(s) in hours	Recovery Time in hours	Total # of Inoculations
1	0	2	1
2	0, 3	5	2
3	0, 3, 6, 9	11	4
4	0, 3, 6, 9, 12, 15	17	6
5	0, 3, 6, 9, 12, 15, 18, 22	24	8

At each recovery time, the carriers were transferred to 20 mL of neutralizer (10^0) following the same staggered intervals used during inoculation. Each neutralized vessel was sonicated for 5 minutes to suspend and survivors from the carriers and was rotated to mix. Serial dilutions of the sonicated neutralizer/carrier suspensions were prepared and duplicate 1.00 mL aliquots of 10^0 through 10^{-4} were plated onto agar. The plates and incubated for 48±4 hours at 35-37°C. Resulting colonies were enumerated and used for calculations. Controls included neutralizer confirmation, carrier quantitation, carrier sterility, and initial inoculum count.

12. MRID 494203-30 "Test Method Three for Determining Efficacy," against *Staphylococcus aureus*-MRSA (ATCC 33592) of Sanitizer #1 (EPA File Symbol 67603-RE) by Matthew Sathe. Study conducted at ATS Labs. Study completion date – June 18, 2013. Project Number A14705.

This study was conducted against *Staphylococcus aureus*-MRSA (ATCC 33592). This study was conducted using Test Method #3 against two lots of base white paint each prepared at or below the Lower Certified Limit for active ingredient concentration (Batch #2011-130:124 and #2011-130:125). The untreated batch was #201-130:109. Testing followed ATS Protocol Number SHE09021813.CRS.4 (copy provided). The objective of this study was to evaluate the antimicrobial efficacy of coated surfaces as a residual self sanitizer against *S. aureus*-MRSA. The exposure time tested was 2 hours. For the preparation of carriers, simulated wear cycling was initiated after the final coat of paint on each test panel had been allowed to cure for at least 24 hours. The wear procedure was intended to simulate cleaning of a vertical surface;

therefore, relatively low volumes of cleaner were applied to the test panels at a rate where the cleaner would not drip down to the floor if applied to a vertical surface. The cleaning solution, Best Yet Citrus Cleaner, was prepared at the manufacturer's recommended dilution ration. A common sponge was soaked in the prepared cleaner solution prior to the initial simulated wear cycle. The appropriate test or control panel was placed on the Gardco Washability Tester tray. The sponge was removed from the solution, wrung of excess solution and positioned under the 1000±10 gram weigh boat. The cycle was started and two cycles were performed, representing one complete wear cycle (equivalent to 4 washes or one month of cleaning). The panel was removed and placed on a horizontal surface to dry at room temperature. This procedure was repeated until a total of 50 wear cycles (total of 200 washes) had been performed on each panel. The washed carriers were prepared as previously described. A 48±4 hour culture of *S. aureus*-MRSA was supplemented with fetal bovine serum organic load (5% final concentration) and Triton X-100 (0.01% final concentration) to facilitate organism spreading. Two sets of treated carriers per lot, representing both worn and unworn surfaces were evaluated on the day of the test. The unworn surfaces were used to assess the initial sanitizing action of the surface. The worn surfaces were used to assess the sanitizing action of the surface at the end of its usable life. Each test and control carrier was inoculated at staggered intervals with 40 µL of culture using a calibrated pipettor. Exposure began when the inoculum was first applied to the carrier. The carriers were allowed to expose at 25±2°C and 60±2% relative humidity for 2 hours. Following exposure, the carriers were transferred to 20 mL of neutralizer (10^0) following the same staggered intervals used during inoculation. Each neutralized vessel was sonicated for 5 minutes to suspend and survivors from the carriers and was rotated to mix. Serial dilutions of the sonicated neutralizer/carrier suspensions were prepared and duplicate 0.100 mL aliquots of 10^0 through 10^{-3} were plated onto agar. The plates and incubated for 48±4 hours at 35-37°C. Resulting colonies were enumerated and used for calculations. Controls included neutralizer confirmation, carrier quantitation, carrier sterility, and initial inoculum count.

13. MRID 494203-18 "Test Method for Determining Efficacy," against Vancomycin Resistant *Enterococcus faecalis*-VRE (ATCC 51575) of Sanitizer #1 (EPA File Symbol 67603-RE) by Matthew Sathe. Study conducted at ATS Labs. Study completion date – June 5, 2013. Project Number A14722.

This study was conducted against Vancomycin Resistant *Enterococcus faecalis*-VRE (ATCC 51575). This study was conducted using Test Method #1 against the base paint tinted with 10 different colorants (1 untinted + 10 different tinted versions for a total of 11 treatments) each prepared at the rate of 3 fl. oz. tint per gallon of white base. The purpose of testing the tinted formulations was to assess the potential for colorants to alter antimicrobial efficacy through dilution effects and/or chemical interactions with the active ingredient. A single lot of white base paint (Batch #2011-130:110) prepared at the nominal concentration for the active ingredient was used to prepare the 10 different tint preparations used in this study. Testing followed ATS Protocol Number SHE09021113.CNFS.8 (copy provided). A 48±4 hour culture of *Enterococcus faecalis*-VRE was supplemented with fetal bovine serum organic load (5% final concentration) and Triton X-100 (0.01% final concentration). Sterilized carriers coated with test material and Quantitation Control paint formulations were inoculated with 40 µL of final test culture. Each of the 10 tint versions and controls were evaluated in sets of five carriers each. After 120 minutes of exposure at 25±2°C and 60±2% relative humidity, carriers were transferred to 20 mL of Letheen broth neutralizer medium and sonicated for 5 minutes. Serial dilutions of the sonicated neutralizer/carrier suspensions were plated in duplicate on agar plates and incubated for 48±4 hours at 35-37°C. Resulting colonies were enumerated and used for calculations. Controls included neutralizer confirmation, carrier quantitation, carrier sterility, and initial inoculum count.

14. MRID 494203-23 "Test Method for Determining Efficacy," against Vancomycin Resistant *Enterococcus faecalis*-VRE (ATCC 51575) of Sanitizer #1 (EPA File Symbol 67603-RE) by Matthew Sathe. Study conducted at ATS Labs. Study completion date – June 20, 2013. Project Number A14737.

This study was conducted against Vancomycin Resistant *Enterococcus faecalis*-VRE (ATCC 51575). This study was conducted using Test Method #1 against two lots of base white paint each prepared at or below the Lower Certified Limit for active ingredient concentration (Batch #2011-130:124 and #2011-130:125). Testing followed ATS Protocol Number SHE09021113.CNFS.5 (copy provided). A 48±4 hour culture of *Enterococcus faecalis*-VRE was supplemented with fetal bovine serum organic load (5% final concentration) and Triton X-100 (0.01% final concentration). Sterilized carriers coated with test material and Quantitation Control paint formulations were inoculated with 40 µL of final test culture. Each of the three test lots and controls were evaluated in sets of five carriers each. After 120 minutes of exposure at 25±2°C and 60±2% relative humidity, carriers were transferred to 20 mL of Letheen broth neutralizer medium and sonicated for 5 minutes. Serial dilutions of the sonicated neutralizer/carrier suspensions were plated in duplicate on agar plates and incubated for 48±4 hours at 35-37°C. Resulting colonies were enumerated and used for calculations. Controls included neutralizer confirmation, carrier quantitation, carrier sterility, and initial inoculum count.

15. MRID 494203-27 "Test Method Two for Determining Efficacy," against Vancomycin Resistant *Enterococcus faecalis*-VRE (ATCC 51575) of Sanitizer #1 (EPA File Symbol 67603-RE) by Matthew Sathe. Study conducted at ATS Labs. Study completion date – June 21, 2013. Project Number A14926.

This study was conducted against Vancomycin Resistant *Enterococcus faecalis*-VRE (ATCC 51575). This study was conducted using Test Method #2 against two lots of base white paint each prepared at or below the Lower Certified Limit for active ingredient concentration (Batch #2011-130:124 and #2011-130:125). The untreated batch was #201-130:109. Testing followed ATS Protocol Number SHE09041613.CCR.5 (copy provided). The objective of this study was to determine the effectiveness of antimicrobial coated surfaces to continuously reduce test organism contamination after multiple re-inoculations over extended time periods. The exposure times were 2 hours following inoculation with recovery at 2, 5, 11, 17 and 24 hours. A 48±4 hour culture of *Enterococcus faecalis*-VRE was supplemented with fetal bovine serum organic load (5% final concentration) and Triton X-100 (0.01% final concentration) to facilitate organism spreading. For the inoculation of treated test and untreated control carriers, five sets of Sponsor supplied treated test and untreated control carriers were used in the study. At time zero, all carrier sets were inoculated. At 3, 6, 9, 12, 15, 18, and 22 hours, carrier sets not removed for quantitative recovery were reinoculated. Each carrier was inoculated at staggered intervals with 40 µL of culture using a calibrated pipettor. Exposure began when the inoculum was first applied to the carrier. The carriers were held covered at 25±2°C and 60±2% relative humidity for the duration of the exposure. Sets of carriers were removed for quantitative recovery two hours after the corresponding inoculation point outlined in the chart below.

Test System Inoculation & Recovery Chart			
Carrier Set	Inoculation Time(s) in hours	Recovery Time in hours	Total # of Inoculations
1	0	2	1
2	0, 3	5	2
3	0, 3, 6, 9	11	4

4	0, 3, 6, 9, 12, 15	17	6
5	0, 3, 6, 9, 12, 15, 18, 22	24	8

At each recovery time, the carriers were transferred to 20 mL of neutralizer (10^0) following the same staggered intervals used during inoculation. Each neutralized vessel was sonicated for 5 minutes to suspend and survivors from the carriers and was rotated to mix. Serial dilutions of the sonicated neutralizer/carrier suspensions were prepared and duplicate 1.00 mL aliquots of 10^0 through 10^{-4} were plated onto agar. The plates and incubated for 48±4 hours at 35-37°C. Resulting colonies were enumerated and used for calculations. Controls included neutralizer confirmation, carrier quantitation, carrier sterility, and initial inoculum count.

16. MRID 494203-31 "Test Method Three for Determining Efficacy," against Vancomycin Resistant *Enterococcus faecalis*-VRE (ATCC 51575) of Sanitizer #1 (EPA File Symbol 67603-RE) by Matthew Sathe. Study conducted at ATS Labs. Study completion date – June 19, 2013. Project Number A14706.

This study was conducted against Vancomycin Resistant *Enterococcus faecalis*-VRE (ATCC 51575). This study was conducted using Test Method #3 against two lots of base white paint each prepared at or below the Lower Certified Limit for active ingredient concentration (Batch #2011-130:124 and #2011-130:125). The untreated batch was #201-130:109. Testing followed ATS Protocol Number SHE09021813.CRS.5 (copy provided). The objective of this study was to evaluate the antimicrobial efficacy of coated surfaces as a residual self sanitizer against *Enterococcus faecalis*-VRE. The exposure time tested was 2 hours. For the preparation of carriers, simulated wear cycling was initiated after the final coat of paint on each test panel had been allowed to cure for at least 24 hours. The wear procedure was intended to simulate cleaning of a vertical surface; therefore, relatively low volumes of cleaner were applied to the test panels at a rate where the cleaner would not drip down to the floor if applied to a vertical surface. The cleaning solution, Best Yet Citrus Cleaner, was prepared at the manufacturer's recommended dilution ration. A common sponge was soaked in the prepared cleaner solution prior to the initial simulated wear cycle. The appropriate test or control panel was placed on the Gardco Washability Tester tray. The sponge was removed from the solution, wrung of excess solution and positioned under the 1000±10 gram weigh boat. The cycle was started and two cycles were performed, representing one complete wear cycle (equivalent to 4 washes or one month of cleaning). The panel was removed and placed on a horizontal surface to dry at room temperature. This procedure was repeated until a total of 50 wear cycles (total of 200 washes) had been performed on each panel. The washed carriers were prepared as previously described. A 48±4 hour culture of *Enterococcus faecalis*-VRE was supplemented with fetal bovine serum organic load (5% final concentration) and Triton X-100 (0.01% final concentration) to facilitate organism spreading. Two sets of treated carriers per lot, representing both worn and unworn surfaces were evaluated on the day of the test. The unworn surfaces were used to assess the initial sanitizing action of the surface. The worn surfaces were used to assess the sanitizing action of the surface at the end of its usable life. Each test and control carrier was inoculated at staggered intervals with 40 µL of culture using a calibrated pipettor. Exposure began when the inoculum was first applied to the carrier. The carriers were allowed to expose at 25±2°C and 60±2% relative humidity for 2 hours. Following exposure, the carriers were transferred to 20 mL of neutralizer (10^0) following the same staggered intervals used during inoculation. Each neutralized vessel was sonicated for 5 minutes to suspend and survivors from the carriers and was rotated to mix. Serial dilutions of the sonicated neutralizer/carrier suspensions were prepared and duplicate 0.100 mL aliquots of 10^0 through 10^{-3} were plated onto agar. The plates and incubated for 48±4 hours at 35-37°C. Resulting colonies were

enumerated and used for calculations. Controls included neutralizer confirmation, carrier quantitation, carrier sterility, and initial inoculum count.

17. MRID 494203-16 "Test Method for Determining Efficacy," against *Escherichia coli* O157:H7 (ATCC 35150) of Sanitizer #1 (EPA File Symbol 67603-RE) by Matthew Sathe. Study conducted at ATS Labs. Study completion date – June 10, 2013. Project Number A14720.

This study was conducted against *Escherichia coli* O157:H7 (ATCC 35150). This study was conducted using Test Method #1 against the base paint tinted with 10 different colorants (1 untinted + 10 different tinted versions for a total of 11 treatments) each prepared at the rate of 3 fl. oz. tint per gallon of white base. The purpose of testing the tinted formulations was to assess the potential for colorants to alter antimicrobial efficacy through dilution effects and/or chemical interactions with the active ingredient. A single lot of white base paint (Batch #2011-130:110) prepared at the nominal concentration for the active ingredient was used to prepare the 10 different tint preparations used in this study. Testing followed ATS Protocol Number SHE09021113.CNFS.9 (copy provided). A 48±4 hour culture of *Escherichia coli* O157:H7 was supplemented with fetal bovine serum organic load (5% final concentration) and Triton X-100 (0.01% final concentration). Sterilized carriers coated with test material and Quantitation Control paint formulations were inoculated with 40 µL of final test culture. Each of the 10 tint versions and controls were evaluated in sets of five carriers each. After 120 minutes of exposure at 25±2°C and 60±2% relative humidity, carriers were transferred to 20 mL of Letheen broth neutralizer medium and sonicated for 5 minutes. Serial dilutions of the sonicated neutralizer/carrier suspensions were plated in duplicate on agar plates and incubated for 48±4 hours at 35-37°C. Resulting colonies were enumerated and used for calculations. Controls included neutralizer confirmation, carrier quantitation, carrier sterility, and initial inoculum count.

18. MRID 494203-21 "Test Method for Determining Efficacy," against *Escherichia coli* O157:H7 (ATCC 35150) of Sanitizer #1 (EPA File Symbol 67603-RE) by Matthew Sathe. Study conducted at ATS Labs. Study completion date – June 19, 2013. Project Number A14733.

This study was conducted against *Escherichia coli* O157:H7 (ATCC 35150). This study was conducted using Test Method #1 against two lots of base white paint each prepared at or below the Lower Certified Limit for active ingredient concentration (Batch #2011-130:124 and #2011-130:125). Testing followed ATS Protocol Number SHE09021113.CNFS.3 (copy provided). A 48±4 hour culture of *Escherichia coli* O157:H7 was supplemented with fetal bovine serum organic load (5% final concentration) and Triton X-100 (0.01% final concentration). Sterilized carriers coated with test material and Quantitation Control paint formulations were inoculated with 40 µL of final test culture. Each of the three test lots and controls were evaluated in sets of five carriers each. After 120 minutes of exposure at 25±2°C and 60±2% relative humidity, carriers were transferred to 20 mL of Letheen broth neutralizer medium and sonicated for 5 minutes. Serial dilutions of the sonicated neutralizer/carrier suspensions were plated in duplicate on agar plates and incubated for 48±4 hours at 35-37°C. Resulting colonies were enumerated and used for calculations. Controls included neutralizer confirmation, carrier quantitation, carrier sterility, and initial inoculum count.

19. MRID 494203-32 "Test Method Three for Determining Efficacy," against *Escherichia coli* O157:H7 (ATCC 35150) of Sanitizer #1 (EPA File Symbol 67603-RE) by Matthew Sathe. Study conducted at ATS Labs. Study completion date –

June 20, 2013. Project Number A14914.

This study was conducted against *Escherichia coli* O157:H7 (ATCC 35150). This study was conducted using Test Method #3 against two lots of base white paint each prepared at or below the Lower Certified Limit for active ingredient concentration (Batch #2011-130:124 and #2011-130:125). The untreated batch was #201-130:109. Testing followed ATS Protocol Number SHE09041513.CRS (copy provided). The objective of this study was to evaluate the antimicrobial efficacy of coated surfaces as a residual self sanitizer against *Escherichia coli* O157:H7. The exposure time tested was 2 hours. For the preparation of carriers, simulated wear cycling was initiated after the final coat of paint on each test panel had been allowed to cure for at least 24 hours. The wear procedure was intended to simulate cleaning of a vertical surface; therefore, relatively low volumes of cleaner were applied to the test panels at a rate where the cleaner would not drip down to the floor if applied to a vertical surface. The cleaning solution, Best Yet Citrus Cleaner, was prepared at the manufacturer's recommended dilution ration. A common sponge was soaked in the prepared cleaner solution prior to the initial simulated wear cycle. The appropriate test or control panel was placed on the Gardco Washability Tester tray. The sponge was removed from the solution, wrung of excess solution and positioned under the 1000±10 gram weigh boat. The cycle was started and two cycles were performed, representing one complete wear cycle (equivalent to 4 washes or one month of cleaning). The panel was removed and placed on a horizontal surface to dry at room temperature. This procedure was repeated until a total of 50 wear cycles (total of 200 washes) had been performed on each panel. The washed carriers were prepared as previously described. A 48±4 hour culture of *Escherichia coli* O157:H7 was supplemented with fetal bovine serum organic load (5% final concentration) and Triton X-100 (0.01% final concentration) to facilitate organism spreading. Two sets of treated carriers per lot, representing both worn and unworn surfaces were evaluated on the day of the test. The unworn surfaces were used to assess the initial sanitizing action of the surface. The worn surfaces were used to assess the sanitizing action of the surface at the end of its usable life. Each test and control carrier was inoculated at staggered intervals with 40 µL of culture using a calibrated pipettor. Exposure began when the inoculum was first applied to the carrier. The carriers were allowed to expose at 25±2°C and 60±2% relative humidity for 2 hours. Following exposure, the carriers were transferred to 20 mL of neutralizer (10^0) following the same staggered intervals used during inoculation. Each neutralized vessel was sonicated for 5 minutes to suspend and survivors from the carriers and was rotated to mix. Serial dilutions of the sonicated neutralizer/carrier suspensions were prepared and duplicate 0.100 mL aliquots of 10^0 through 10^{-3} were plated onto agar. The plates and incubated for 48±4 hours at 35-37°C. Resulting colonies were enumerated and used for calculations. Controls included neutralizer confirmation, carrier quantitation, carrier sterility, and initial inoculum count.

V RESULTS

Staphylococcus aureus (ATCC 6538)		
MRID 494203-14		
2 Hour Contact Time		
Batch #	Survivors Log_{10} (survivors/carrier)	Percent Reduction
2011-130:110 version 12	<1.46	>99.9
2011-130:110 version 13	<1.50	>99.9
2011-130:110 version 14	<1.58	>99.9
2011-130:110 version 15	<1.74	>99.9
2011-130:110 version 16	<1.40	>99.9
2011-130:110 version 17	<1.46	>99.9
2011-130:110 version 18	<1.94	>99.9
2011-130:110 version 19	<1.36	>99.9
2011-130:110 version 20	<1.30	>99.9
2011-130:110 version 21	<1.30	>99.9
2011-130:110 version 22	<1.42	>99.9
Carrier Quantitation Control Results		
2011-130:109*	6.59	--
* Base white paint formulation with no active ingredient		

Staphylococcus aureus (ATCC 6538)		
MRID 494203-19		
2 Hour Contact Time		
Batch #	Log_{10} Reduction	Percent Reduction
2011-130:124	>4.20	>99.9
2011-130:125	>4.20	>99.9
2011-130:126	>4.20	>99.9
Carrier Quantitation Control Results		
2011-130:109*	6.59	--
* Base white paint formulation with no active ingredient		

MRID 494203-24 Log Reduction Over 24 Hours for 3 Test Lots			
Time Point In hours	Test Lots		
	2011-130:124	2011-130:125	2011-130:126
2	4.80	4.95	4.85
5	4.76	4.78	4.80
11	4.57	4.53	4.49
17	4.28	4.21	4.18
24	3.58	3.24	3.27

MRID 494203-28 Log Reduction after 2 Hour Exposure		
Test Substance	Log Reduction	
	Worn Carriers	Unworn Carriers
2011-130:124	4.55	4.54
2011-130:125	3.74	4.22
2011-130:126	3.79	4.42

Enterobacter aerogenes (ATCC 13048)		
MRID 494203-15		
2 Hour Contact Time		
Batch #	Survivors Log_{10} (survivors/carrier)	Percent Reduction
2011-130:110 version 12	<1.30	>99.9
2011-130:110 version 13	<1.30	>99.9
2011-130:110 version 14	<1.30	>99.9
2011-130:110 version 15	<1.30	>99.9
2011-130:110 version 16	<1.30	>99.9
2011-130:110 version 17	<1.30	>99.9
2011-130:110 version 18	<1.30	>99.9
2011-130:110 version 19	<1.30	>99.9
2011-130:110 version 20	<1.30	>99.9
2011-130:110 version 21	<1.30	>99.9
2011-130:110 version 22	<1.30	>99.9
Carrier Quantitation Control Results		
2011-130:109*	6.48	--
* Base white paint formulation with no active ingredient		

Enterobacter aerogenes (ATCC 13048)		
MRID 494203-20		
2 Hour Contact Time		
Batch #	Log_{10} Reduction	Percent Reduction
2011-130:124	>4.85	>99.9
2011-130:125	>4.85	>99.9
2011-130:126	>4.85	>99.9
Carrier Quantitation Control Results		
2011-130:109*	6.15	--
* Base white paint formulation with no active ingredient		

MRID 494203-25 Log Reduction Over 24 Hours for 3 Test Lots			
Time Point In hours	Test Lots		
	2011-130:124	2011-130:125	2011-130:126
2	4.91	4.91	4.91
5	3.92	2.99	3.39
11	1.93	1.74	1.83
17	2.54	1.71	2.04
24	1.78	1.56	1.49

MRID 494203- 29 Log Reduction after 2 Hour Exposure		
Test Substance	Log Reduction	
	Worn Carriers	Unworn Carriers
2011-130:124	>3.62	>4.09
2011-130:125	>3.58	>4.09
2011-130:126	>3.22	>4.09

Staphylococcus aureus-MRSA (ATCC 33592)		
MRID 494203-17		
2 Hour Contact Time		
Batch #	Survivors Log_{10} (survivors/carrier)	Percent Reduction
2011-130:110 version 12	2.20	>99.9
2011-130:110 version 13	2.26	>99.9
2011-130:110 version 14	2.24	>99.9
2011-130:110 version 15	2.64	>99.9
2011-130:110 version 16	<1.78	>99.9
2011-130:110 version 17	1.80	>99.9
2011-130:110 version 18	<1.72	>99.9
2011-130:110 version 19	<1.84	>99.9
2011-130:110 version 20	<1.30	>99.9
2011-130:110 version 21	<1.75	>99.9
2011-130:110 version 22	<1.40	>99.9
Carrier Quantitation Control Results		
2011-130:109*	6.59	--
* Base white paint formulation with no active ingredient		

Staphylococcus aureus-MRSA (ATCC 33592)		
MRID 494203-22		
2 Hour Contact Time		
Batch #	Log_{10} Reduction	Percent Reduction
2011-130:124	>4.61	>99.9
2011-130:125	>4.43	>99.9
Carrier Quantitation Control Results		
2011-130:109*	6.33	--
* Base white paint formulation with no active ingredient		

MRID 494203-26 Log Reduction Over 24 Hours for 2 Test Lots			
Time Point In hours	Test Lots		
	2011-130:124	2011-130:125	2011-130:126
2	3.34	4.03	--
5	5.06	5.14	--
11	3.11	3.11	--
17	3.39	3.49	--
24	3.09	3.11	--

MRID 494203-30 Log Reduction after 2 Hour Exposure		
Test Substance	Log Reduction	
	Worn Carriers	Unworn Carriers
2011-130:124	3.32	4.44
2011-130:125	3.25	4.30
2011-130:126	--	--

Vancomycin Resistant *Enterococcus faecalis*-VRE (ATCC 51575)		
MRID 494203-18		
2 Hour Contact Time		
Batch #	Survivors Log_{10} (survivors/carrier)	Percent Reduction
2011-130:110 version 12	<1.50	>99.9
2011-130:110 version 13	<1.30	>99.9
2011-130:110 version 14	<1.52	>99.9
2011-130:110 version 15	<1.83	>99.9
2011-130:110 version 16	<1.30	>99.9
2011-130:110 version 17	<1.58	>99.9
2011-130:110 version 18	<1.64	>99.9
2011-130:110 version 19	<1.42	>99.9
2011-130:110 version 20	<1.67	>99.9
2011-130:110 version 21	<1.30	>99.9
2011-130:110 version 22	<1.40	>99.9
Carrier Quantitation Control Results		
2011-130:109*	6.89	--
* Base white paint formulation with no active ingredient		

Vancomycin Resistant *Enterococcus faecalis*-VRE (ATCC 51575)		
MRID 494203-23		
2 Hour Contact Time		
Batch #	Log_{10} Reduction	Percent Reduction
2011-130:124	>4.64	>99.9
2011-130:125	>4.74	>99.9
2011-130:126	--	--
Carrier Quantitation Control Results		
2011-130:109*	6.04	--
* Base white paint formulation with no active ingredient		

MRID 494203-27 Log Reduction Over 24 Hours for 3 Test Lots			
Time Point In hours	Test Lots		
	2011-130:124	2011-130:125	2011-130:126
2	>3.47	3.02	--
5	5.24	4.97	--
11	3.70	3.64	--
17	3.43	3.16	--
24	3.21	2.81	--

MRID 494203-31 Log Reduction after 2 Hour Exposure		
Test Substance	Log Reduction	
	Worn Carriers	Unworn Carriers
2011-130:124	>4.42	>4.14
2011-130:125	>4.36	>4.46
2011-130:126	--	--

Escherichia coli O157:H7 (ATCC 35150)		
MRID 494203-16		
2 Hour Contact Time		
Batch #	Survivors Log_{10} (survivors/carrier)	Percent Reduction
2011-130:110 version 12	<1.30	>99.9
2011-130:110 version 13	<1.30	>99.9
2011-130:110 version 14	<1.30	>99.9
2011-130:110 version 15	<1.30	>99.9
2011-130:110 version 16	<1.30	>99.9
2011-130:110 version 17	<1.30	>99.9
2011-130:110 version 18	<1.30	>99.9
2011-130:110 version 19	<1.30	>99.9
2011-130:110 version 20	<1.30	>99.9
2011-130:110 version 21	<1.30	>99.9
2011-130:110 version 22	<1.30	>99.9
Carrier Quantitation Control Results		
2011-130:109*	6.53	--
* Base white paint formulation with no active ingredient		

Escherichia coli O157:H7 (ATCC 35150)		
MRID 494203-21		
2 Hour Contact Time		
Batch #	Log_{10} Reduction	Percent Reduction
2011-130:124	>5.24	>99.9
2011-130:125	>5.24	>99.9
2011-130:126	--	--
Carrier Quantitation Control Results		
2011-130:109*	6.54	--
* Base white paint formulation with no active ingredient		

MRID 494203-32 Log Reduction after 2 Hour Exposure		
Test Substance	Log Reduction	
	Worn Carriers	Unworn Carriers
2011-130:124	>4.61	>5.36
2011-130:125	>4.61	>5.46
2011-130:126	--	--

VI CONCLUSIONS

1. The following submitted efficacy data support the use of the product, Sanitizer #1, to yield a coated (painted) surface with efficacy as a sanitizer with bactericidal activity against *Staphylococcus aureus* and *Enterobacter aerogenes* after a 2 hour exposure period at room temperature. Testing was conducted with inoculum containing 5% organic soil.

Staphylococcus aureus	ATCC 6538	MRID 494203-19
Enterobacter aerogenes	ATCC 13048	MRID 494203-20

The necessary 99.9% reduction in numbers of the test organism(s) were obtained as compared to the carrier quantitation control. The necessary three lots of product (certified to be at the LCL) were evaluated. The study controls performed according to the criteria detailed in the study controls description section for purity controls, organic soil sterility control, carrier sterility control, neutralizing subculture medium sterility control, viability control, neutralization confirmation control, inoculum count, and carrier quantitation control.

2. The following submitted efficacy data support the use of the product, Sanitizer #1, to yield a coated (painted) surface with efficacy as a sanitizer with bactericidal activity against the listed organisms after a 2 hour exposure period at room temperature. Testing was conducted with inoculum containing 5% organic soil.

Staphylococcus aureus-MRSA	ATCC 33592	MRID 494203-22
Vancomycin Resistant *Enterococcus faecalis*-VRE	ATCC 51575	MRID 494203-23
Escherichia coli O157:H7	ATCC 35150	MRID 494203-21

The necessary 99.9% reduction in numbers of the test organism(s) were obtained as compared to the carrier quantitation control. The necessary two lots of product (certified to be at the LCL) were evaluated. The study controls performed according to the criteria detailed in the study controls description section for purity controls, organic soil sterility control, carrier sterility control, neutralizing subculture medium sterility control, viability control, neutralization confirmation control, inoculum count, and carrier quantitation control.

3. The following submitted efficacy data support the use of the product, Sanitizer #1, to yield a coated (painted) surface with efficacy for the continuous reduction of microorganisms against the listed bacteria after a 2 hour exposure period at room temperature. Testing was conducted with inoculum containing 5% organic soil.

Staphylococcus aureus	ATCC 6538	MRID 494203-24
Enterobacter aerogenes	ATCC 13048	MRID 494203-25

The necessary minimum of 90% reduction in numbers of the test organism(s) on the test surface compared to the number of test organism(s) on the control surface was achieved at all recovery times over the 24 hour inoculation and exposure period. The necessary three lots of product (certified to be at the LCL) were evaluated. The study controls performed according to the criteria detailed in the study controls description section for

purity controls, organic soil sterility control, carrier sterility control, neutralizing subculture medium sterility control, viability control, neutralization confirmation control, inoculum count, and carrier quantitation control.

4. The following submitted efficacy data support the use of the product, Sanitizer #1, to yield a coated (painted) surface with efficacy for the continuous reduction of microorganisms against the listed bacteria after a 2 hour exposure period at room temperature. Testing was conducted with inoculum containing 5% organic soil.

Staphylococcus aureus-MRSA	ATCC 33592	MRID 494203-26
Vancomycin Resistant *Enterococcus faecalis*-VRE	ATCC 51575	MRID 494203-27

The necessary minimum of 90% reduction in numbers of the test organism(s) on the test surface compared to the number of test organism(s) on the control surface was achieved at all recovery times over the 24 hour inoculation and exposure period. The necessary two lots of product (certified to be at the LCL) were evaluated. The study controls performed according to the criteria detailed in the study controls description section for purity controls, organic soil sterility control, carrier sterility control, neutralizing subculture medium sterility control, viability control, neutralization confirmation control, inoculum count, and carrier quantitation control.

5. The following submitted efficacy data support the use of the product, Sanitizer #1, to yield a coated (painted) surface with efficacy as a residual self sanitizer with bactericidal activity against *Staphylococcus aureus* and *Enterobacter aerogenes* after a 2 hour exposure period at room temperature. Testing was conducted with inoculum containing 5% organic soil.

Staphylococcus aureus	ATCC 6538	MRID 494203-28
Enterobacter aerogenes	ATCC 13048	MRID 494203-29

The test material reduced the total number of organisms by at least 99.9% on the surface within the prescribed exposure time. In order to demonstrate effective sanitization on a worn surface representative of end of use life, one set of material per lot was subjected to wear procedure designed to simulate at least four years of quarterly washing of the painted wall surface. The necessary three lots of product (certified to be at the LCL) were evaluated. The study controls performed according to the criteria detailed in the study controls description section for purity controls, organic soil sterility control, carrier sterility control, neutralizing subculture medium sterility control, viability control, neutralization confirmation control, inoculum count, and carrier quantitation control.

6. The following submitted efficacy data support the use of the product, Sanitizer #1, to yield a coated (painted) surface with efficacy as a residual self sanitizer with bactericidal activity against *Staphylococcus aureus* and *Enterobacter aerogenes* after a 2 hour exposure period at room temperature. Testing was conducted with inoculum containing 5% organic soil.

Staphylococcus aureus-MRSA	ATCC 33592	MRID 494203-30
Vancomycin Resistant *Enterococcus faecalis*-VRE		

	ATCC 51575	MRID 494203-31
Escherichia coli O157:H7	ATCC 35150	MRID 494203-32

The test material reduced the total number of organisms by at least 99.9% on the surface within the prescribed exposure time. In order to demonstrate effective sanitization on a worn surface representative of end of use life, one set of material per lot was subjected to wear procedure designed to simulate at least four years of quarterly washing of the painted wall surface. The necessary two lots of product (certified to be at the LCL) were evaluated. The study controls performed according to the criteria detailed in the study controls description section for purity controls, organic soil sterility control, carrier sterility control, neutralizing subculture medium sterility control, viability control, neutralization confirmation control, inoculum count, and carrier quantitation control.

VII RECOMMENDATION

1. The label claims are acceptable regarding the use the product, Sanitizer #1, to yield a coated (painted) surface with efficacy as a sanitizer with bactericidal activity against *Staphylococcus aureus*, *Enterobacter aerogenes*, *Staphylococcus aureus*-MRSA, Vancomycin Resistant *Enterococcus faecalis*, and *Escherichia coli* O157:H7 are acceptable. These claims are supported by the submitted data.
   EXAMPLE: Kills 99.9% of *Staphylococcus aureus*, *Enterobacter aerogenes*, *Staphylococcus aureus*-MRSA, Vancomycin Resistant *Enterococcus faecalis*, and *Escherichia coli* O157:H7 after 2 hours on a painted surface.

2. The label claims are acceptable regarding the use the product, Sanitizer #1, to yield a coated (painted) surface with efficacy for the continuous reduction of microorganisms against the listed bacteria after a 2 hour exposure period and continue to kill 90% of bacteria even after repeated contamination for the following:
   Staphylococcus aureus
   Enterobacter aerogenes
   Staphylococcus aureus-MRSA
   Vancomycin Resistant *Enterococcus faecalis*
   Data to support the continuous reduction (Test Method #2) claims against *Escherichia coli* O157:H7 were not submitted.

Label Comments:

- Page 3, OPTIONAL LABEL CLAIMS:
  Second claim, "Painted surfaces continuously reduce bacteria...," statement must be qualified by listing the 4 organisms in 2 above. *Escherichia coli* O157:H7 should not be included in the list as not data were submitted.
- Page 4, the first three bulleted claims at the top of the page must be qualified. The second and third claims are supported by Test Method #2 and should list the 4 organisms tested. *Escherichia coli* O157:H7 should not be included in the list as not data were submitted.
- Indicate under DIRECTIONS FOR USE or WHERE TO USE that claims are limited to indoor hard non-porous surfaces where cleaning practices are consistent.
- Page 3, Under repaint if film becomes damaged – indicate damaged (cracked, chipped, etc.) or if paint becomes covered (i.e., film, wax, oils, other paints, crayons, etc.).