UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION,
PESTICIDES
AND TOXIC
SUBSTANCES

September 26, 2011

MEMORANDUM

Subject: Response to Protocol Review for 84542-PA12, Cupron Enhanced Hard Surfaces as a Residual Self-Sanitizer; No DP Barcode Assigned

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Formulation from the Label: revised Label does not include formulation statement

I BACKGROUND

Through the current submission, Cupron is addressing questions from the Agency's protocol review (dated July 1, 2011). The current submission also includes a revised protocol for Agency review. The proposed protocol is based on an accepted Agency protocol, and this review is for surface equivalency only.

II USE DIRECTIONS

Directions on the proposed label provided the following instructions for the preparation and use of the product:

The use of Antimicrobial Cupron Enhanced Hard Surface does not replace standard infection control procedures and good hygienic practice. Antimicrobial Cupron Enhanced Hard Surface must be cleaned and sanitized according to standard practice. Health care facilities must maintain the product in accordance with infection control guidelines; users must continue to follow all current infection control practices, including those practices related to disinfection of environmental surfaces. In order for the Antimicrobial Cupron Enhanced Hard Surface have proper antimicrobial effect, the product must be cleaned and maintained according to the directions included on this label.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

Test Requirements for Residual Self-Sanitizing Activity of Copper Surfaces

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. 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. For claims of Continuous Reduction and/or Residual Self-Sanitizing Activity, initial efficacy testing against *Staphylococcus aureus* and *Enterobacter aerogenes* is required before additional microorganisms are granted. 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. Claims are limited to indoor, hard, non-porous surfaces where cleaning practices are consistent. The acceptable claim is this surface kills greater than 99.9% of bacteria* for 24 hours.

*Includes list of tested organisms.

The following language is required on the registered products, the use of a copper surface 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 copper surface material has been shown to reduce microbial contamination, but does not necessarily prevent cross contamination.

Proper Care and Use of Antimicrobial Copper Surfaces: The use of copper surfaces does not replace standard infection control procedures and good hygienic practices. Antimicrobial copper surfaces must be cleaned and sanitized according to standard practice. Heath care facilities must maintain the product in accordance with infection control guidelines; users must continue to follow all current infection control practices, including those practices related to disinfection of environmental surfaces.

Cleaning Directions: Routine cleaning to remove dirt and filth is necessary for good sanitization and to assure the effective antibacterial performance of the copper surfaces. Cleaning agents typically used for traditional touching surfaces are permissible; the appropriate cleaning agent depends on the type of soiling and the measure of sanitization required.

This product must not be waxed, painted, lacquered, varnished, or otherwise coated.

IV REGISTRANT'S RESPONSES TO AGENY'S INITIAL PROTOCOL REVIEW

Agency's Initial Response 1. The registrant must explain the distribution of Cupron when impregnated with Corian or other polymers. The registrant must include detailed information regarding additional polymers for which Cupron will be impregnated. Additionally, test carriers must represent all proposed polymer blends. Furthermore, efficacy data must be generated using both non-pigmented and pigmented carrier types.
Registrant's Response: The distribution of Cupron within the hard surface polymer is homogenously distributed throughout the active area as shown in the figures provided. The Figure 1 corresponds to an area on the hard surface and the Figure 2 is the EDS mapping of the same area. The purple dots in the Figure 2 represent copper which is uniformly distributed in the polymer. The Cupron impregnated surfaces will be constructed of only one polymer blend. The single polymer Cupron will be incorporated into will be a polyester isophthalic and acrylic ester blended resin. That exact blend will then be tested. Cupron understands that any other polymer blend must be specifically tested. Cupron assumes that any pigment will be acceptable for nontested pigments (in other words a test on a blue colored polymer slab will constitute efficacy for a red colored slab of the same material). Please note Corian is a registered trademark and Cupron is not authorized to use this mark.
Agency's Follow-up Response: No additional information is required.

Agency's Initial Response 2. The registrant stated in the submitted letter (dated February 21, 2010), the use sites are "similar to Corian". Corian is limited to kitchen countertops, bathroom vanity tops, and wall cladding in showers. Several of the proposed use sites are not typically composed of Corian or Corian-liked polymers. The registrant must review the label, and remove or provide a rationale for those sites that are not composed of Corian or Corian-liked polymers.
Registrant's Response: As noted in response 1, the items claimed on the label are all composed of only one polymer blend. That blend will be tested with this protocol. Please note Corian is a registered trademark and Cupron is not authorized to use this mark. The manufacturing process (cast or injection molding) used to produce solid products from polyester isophthalic and acrylic ester blended resins are capable of forming flat surface products such as counter tops and table tops and more complex shaped objects such as sinks, tubs and rails amongst others.

Agency's Follow-up Response: No additional information is required

Agency's Initial Response 3. Food contact surfaces are not acceptable use sites for this protocol.
Registrant's Response: All surfaces on the label that potentially could be food contact surfaces have been specified to be non-food contact surfaces. The abstract of the protocol specifically states nonfood contact surfaces.
Agency's Follow-up Response: No additional information is required.

Agency's Initial Response 4. 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) can be considered. For claims of Continuous Reduction and/or Residual Self-Sanitizing Activity, initial efficacy testing against *Staphylococcus aureus* and *Enterobacter aerogenes* is required before additional microorganisms are granted.
Registrant's Response: Sanitizer efficacy data will be demonstrated against *Staphylococcus aureus* (ATCC# 6538) and *Enterobacter aerogenes* (ATCC# 13048), before additional organisms or claims will be pursued.
Agency's Follow-up Response: No additional information is required.

Agency's Initial Response 5. Contact time should not exceed 24 hours.
Registrant's Response: The efficacy contact time is proposed to be 2 hours with observation periods of 24 to 48 hours for confirmation.
Agency's Follow-up Response: The Agency remains firm in its position that the contact time should not exceed 24 hours.

V SYNOPSIS OF SUBMITTED PROTOCOL

Protocol Title: Test Method for Residual Self-Sanitizing Activity of Cupron Enhanced Hard Surfaces, (MRID No. 484337-01); MRID Number was not assigned

Test Organisms: *Staphylococcus aureus* (ATCC 6538)
Enterobacter aerogenes (ATCC 13048)

Additional Test Organisms: *Pseudomonas aeruginosa* (ATCC 15442)
Methicillin Resistant *Staphylococcus aureus* (ATCC 33592)
Escherichia coli O157:H7 (ATCC 35150)

Test System

Preparation of Test Surfaces: Cut each Cupron Enhanced Hard Surfaces into individual 1" x 1" square carriers. Identical hard surfaces without Cupron (1" x 1") must be incorporated into the test system. Cupron Enhanced Hard Surfaces will be utilized as the test carriers and identical hard surfaces without Cupron as control carriers for this assay. Clean all metal surfaces with alcohol and rinse thoroughly in deionized water and allow to air dry. Sterilize carriers prior to use in test. After sterilization, place each carrier into a plastic Petri dish matted with two pieces of filter paper using sterile forceps.

Preparation of Test Organisms

Staphylococcus aureus, Pseudomonas aeruginosa, Methicillin Resistant *Staphylococcus aureus*: From stock cultures, inoculate tubes of the appropriate broth with organism, and incubate for 24±2 hours at 35-37°C. Using a 4-mm inside diameter disposable sterile plastic transfer loop, transfer at least three consecutive daily cultures in appropriate broth prior to use as inoculum. Transfer two (2) loopfuls of culture into 10 ml broth medium. Transfers more than 15 days away from stock culture should not be used for the inocula for this test. Use 48±4 hour cultures of the inocula on the day of testing. On the day of use, aspirate pellicle from the *Pseudomonas aeruginosa* culture.

Enterobacter aerogenes: From stock cultures, inoculate tubes of Tryptic Soy Broth and incubate for 24±2 hours of 25-30°C. Using a 4-mm inside diameter disposable sterile plastic transfer loop, perform at least three consecutive daily transfers of cultures in Tryptic Soy Broth prior to use as inoculum. Transfer two (2) loopfuls of culture into 10 ml broth medium. Transfers more than 15 days away from stock culture should not be used for the inocula for this test.

a) For the initial sanitizer and final sanitizer inoculum, vortex mix a 48-54 hour culture and allow to stand for 15±1 minutes. Add a volume of serum to equal 5% organic soil load containing Triton X-100 (to aid in spreading of the inoculum), (0.25 ml serum + 0.05 ml Triton X-100 + 4.70 ml bacteria suspension). Decant the upper two thirds of this suspension, and transfer to a sterile tube for use in testing.
b) For the inoculation and reinoculations of the carriers used in the simulated wear tests, use an 18-24 hour culture to ensure that no culture is allowed to stand with organic soil for longer than eight hours. Vortex mix an 18-24 hour culture, and allow to stand for 15±1 minutes. Perform two (2) 0.1 ml to 9.9 ml serial dilutions and one final dilution of 5.0 ml to 5.0 ± 0.2 ml in sterile deionized water. Add a volume of serum to equal 5% organic soil containing Trition X-100 (0.25 ml serum + 0.05 ml Triton X-100 + 4.70 ml bacteria suspension). Vortex mix the suspension, and allow to stand 15 ± 1 minutes before being used to inoculate.

Antimicrobial Susceptibility Testing : Antimicrobial susceptibility testing is required when utilizing a resistant organism. On the day of testing, verify the antimicrobial resistance pattern of Methicillin Resistant *Staphylococcus aureus* (MRSA). Subculture the organism onto a Blood Agar plate (BAP), and incubate for approximately 24 hours at 35-37°C. Following incubation, make a suspension of the test organism equal to 0.5 McFarland Standard in 0.85% sterile saline. Streak the suspension onto Mueller Hinton agar. Place an oxacillin disc in the center of the inoculated Mueller Hinton plate. Invert and incubate for ≥ 24 hours at 35-37°C. Following incubation, measure the zone of inhibition using a calibrated caliper. Concurrently run *Staphylococcus aureus* (ATCC 25923), a control organism, with the test organism to confirm the validity of the assay. The interpretation of the zone of inhibition is based on established National Committee for Clinical Laboratory Standards (NCCLS) performance standards.

Initial Sanitizer Evaluation: Inoculate four (4) sterile carriers of each Cupron Enhanced Hard Surface and hard surfaces without Cupron control carrier with a 10 µl aliquot of the 48-54 hour "sanitizer" organism suspension at staggered intervals. Spread the inoculum to within ⅛ inch of the edge using a bent inoculating needle, and allow to dry for 30-40 minutes at 35-37°C, at a 38-42% relative humidity.

Immediately after drying, the 120 minute exposure period will begin at ambient temperature.

After the 120 minute exposure period, using sterile forceps, transfer the test or control surfaces, at the same staggered intervals used for inoculation, to 30 ml of Letheen broth (or appropriate neutralizer broth) in jars. Repeat this until all the test surfaces and control surfaces have been transferred.

Following the transfer, sonicate the neutralized samples for 20 ± 2 seconds in a sonicating waterbath. Mix the samples on an orbital shaker for 3-4 minutes at 250 rpm.

Serial dilute the test and control samples in 9.0 ± 0.1 ml of sterile deionized water. Serial dilutions (10^{-2} through 10^{-4} dilutions for the control samples and 10^{0} to 10^{-2} dilutions for the test samples) were made in duplicate and plated within approximately one hour of their transfer to the neutralizer broth.

Incubate plates at 35-37°C for *S. aureus* (other test microorganisms) and 25-30°C for *E. aerogenes* for 48±4 hours prior to evaluation. If possible, count and record the plates containing between 30 and 300 CFU. Determine the number of surviving organisms per carrier of each test and control sample by multiplying the number of recovered test organisms by the dilution factor and multiplying by 30 (to account for broth volume) and divide by the volume plated. The control plates must have minimum of 2×10^{4} CFU/carrier for a valid test.

<u>Inoculation, Simulated Wear and Reinoculation of the Test and Control Surfaces:</u>
Prior to carrier inoculation, set the abrasion tester to a speed of 2.25 to 2.5 for a total surface contact time of approximately 4-5 seconds for one complete cycle. Measure the speed with a calibrated stopwatch. Calibrate the machine's cycles by adjusting the number counter to 1, 5, 10, 20 and verifying cycle time. Provide one pass on the abrasion tester with the surfaces for a contact time of approximately 2 seconds. A wear cycle equals one pass to the left and a return pass to the right.

A minimum of fifteen minutes after the wear cycle, reinoculate each carrier as described above and dry at ambient temperature for at least 30 minutes.

Decontaminate the surface holder on the Gardner apparatus with absolute ethanol between each set of surface wears to prevent carryover contamination. Allow the alcohol to completely evaporate before proceeding. Replace the foam liner and the cotton cloth between each set of surface wears.

Alternate the wet-wears with the dry-wears. For the wet wear cycles, the boat assembly should include a new foam liner and dry cotton cloth sprayed with sterile deionized water, using a Preval sprayer, from a distance of 75±1 cm for not more than one second. Following the moistening of the cloth, place the abrasion boat on the scrubber and perform a wear cycle (one pass to the left and a return pass to the right).

The following table provides an overview of an example wear and reinoculation procedure, which includes 12 wear cycles. At least 24 hours pass between the initial inoculation and final sanitize

"Wear" and Re-inoculation Procedure
1. Initial inoculation with test organism
2. Wear cycle** with dry cloth (wear #1)
3. Reinoculation with test organism
4. Wear cycle with moist cloth (wear #2)
5. Reinoculation with test organism
6. Wear cycle with dry cloth (wear #3)
7. Reinoculation with test organism
End of first day
8. Wear cycle with moist cloth (wear #4)
9. Reinoculation with test organism
10. Wear cycle with dry cloth (wear #5)
11. Reinoculation with test organism
12. Wear cycle with moist cloth wear (wear #6)
13. Repeated until 12 wear cycles are completed (Day 2 ended after the 9th reinoculation)
14. Sanitizer test performed after the 12th wear cycle and 2 days after the initial inoculation

Final Sanitizer Test: Following the last wear cycle, inoculate Cupron Enhanced Hard Surface and hard surfaces without Cupron control carrier with a 10 µl aliquot of the 48-54 hour "sanitizer" organism suspension at staggered intervals. Spread the inoculum to within ⅛ inch of the edge using a bent inoculating needle, and allow to dry for 30-40 minutes at 35-37°C and 38-42% relative humidity.

Immediately after drying, the 120 minute exposure period begins at ambient temperature.

After the 120 minute exposure period, use sterile forceps to transfer the test or control surfaces, at the same staggered intervals used for inoculation, to 30 ml of Letheen broth (or appropriate neutralizer broth) in jars. Repeat this until all the test surfaces and control surfaces have been transferred.

Following the transfer, sonicate the neutralizer samples for 20 ± 2 seconds in a sonicating waterbath. Mix the samples on an orbital shaker for 3-4 minutes at 250 rpm.

Serial dilute the test and control samples in 9.0 ± 0.1 ml of sterile deionized water. Perform serial dilutions (10^{-2} through 10^{-4} dilutions for the control samples and 10^{0} to 10^{-2} dilutions for the test samples) in duplicate, and plate within approximately one hour of their transfer to the neutralizer broth.

Incubate plates at 35-37°C for *S. aureus* and 25-30°C for *E. aerogenes* for 48±4 hours prior to evaluation. Following incubation and incubation and storage, visually examine the subculture plates by counting plates containing between 30 and 300 CFU, if possible, and record.

Determine the number of surviving organisms per carrier of each test and control sample by multiplying the number of recovered test organisms by the dilution factor and multiplying by 30 (to account for broth volume) and dividing by the volume plated. The control plates must have minimum of 2×10^{4} CFU/carrier for a valid test.

Study Controls

Inoculum Population Controls: Determine the concentration of the sanitizer test inoculum and each 24-hour re-inoculum by serially diluting in sterile deionized water and plating using standard microbiological techniques in duplicate to agar medium plates. Incubate the plates at 35-37°C for *S. aureus* and 25-30°C for *E. aerogenes* for 48±4 hours.

Purity Controls: Perform a "streak plate for isolation" on each organism culture and following incubation examine in order to confirm the presence of a pure culture. The acceptance criterion for this study control is a pure culture demonstrating colony morphology typical of the test organism.

Organic Soil Sterility Control: Culture, incubate, and visually examine the serum used for soil load. The acceptance criterion for this study control is lack of growth.

Carrier Sterility Control: Add a representative uninoculated test and control carrier to the neutralizing subculture medium. Incubate and examine the subculture medium containing each carrier. The acceptance criterion for this study control is lack of growth.

Neutralizing Subculture Medium Sterility Control: Incubate and visually examine a representative sample of uninoculated neutralizing subculture medium. The acceptance criterion for this study control is lack of growth.

Viability Control: Add a representative inoculated control carrier to the subculture medium. Incubate and visually examine the subculture medium containing the carrier for growth. The acceptance criterion for this study control is growth.

Neutralization Confirmation Control: Conduct neutralization efficacy concurrently with testing. Using sterile forceps, transfer sterile carriers (both control and test) to individual bottles containing 30 ml of the sterile neutralizer broth. At time intervals after each surface addition, add a volume of the bacterial suspension (approximately 1000 organisms) to the bottles and mix. At 5 ± 1 minutes, remove 1.0 ± 0.1 ml from each bottle and plate onto appropriate agar. This control is performed with multiple dilutions of the test organism. Evaluate plates after incubation at 35-37°C for *S. aureus* and 25-30°C for *E. aerogenes* for 48±4 hours. Recovery of colonies on the plate indicates the copper test surface has been adequately neutralized by the neutralizer broth. Recoveries from the test surface suspensions should be similar to the controls recovered from the control surface suspensions. The acceptance criterion for this study control is growth within ±1 $\log_{10}$ for the test and control suspensions.

Study Acceptance Criteria

Test Substance Performance Criteria: 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.

Control Acceptance Criteria: The study controls must perform according to the criteria detailed in the study controls description section.

Data Analysis

Calculations

The geometric mean of the number of organisms surviving on four control surfaces of four test surfaces was determined by the following equation:

Geometric Mean= $\frac{\text{Antilog of } (\text{Log}_{10}X1 + \text{Log}_{10}X2 + \text{Log}_{10}X3 + \text{Log}10X4}{4}$

Where X equals the number of organisms surviving per carrier

The percent reduction of organisms surviving on test surfaces over organisms surviving on parallel control surfaces was determined by the following equation:

%Reduction = $\frac{\text{Geometric mean of control survivors} - \text{geometric mean of test survivors}}{\text{Geometric mean control survivors}} \times 100$

Recovery Log_{10} Difference =

(Log_{10} Neutralization Confirmation Numbers Control) – (Log_{10} Neutralizer Control Growth)

VI CONCLUSION AND LABEL RECOMMENDATION

1. The submitted protocol, Test Method for Residual Self-Sanitizing of Cupron Enhanced Hard Surfaces" is acceptable for contact times ≤ 24 hours. The registrant may initiate testing using the approved protocol. The registrant must include the exact type of control carriers identified as "hard surfaces without Cupron" in the protocol. A stewardship program, as described on the Agency website, must be included when efficacy data is submitted.