UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION
PREVENTION

MEMORANDUM

DATE: June 20, 2012

SUBJECT: Efficacy Review for Hitman;
EPA File Symbol 9402-RU
DP Barcode: D399989

FROM: Lorilyn M. Montford
Efficacy Evaluation Team
Antimicrobials Division (7510P)

THRU: Tajah Blackburn, Ph.D., Team Leader
Product Science Branch
Antimicrobials Division (7510P)

TO: Marshall Swindell, PM33/Demson Fuller
Regulatory Management Branch I
Antimicrobials Division (7510P)

APPLICANT: Kimberly-Clark Global Sales, Inc.
2100 Winchester Road, Neenah, WI 54956

FORMULATION FROM LABEL:

Active Ingredient(s)	% by wt.
Hydrogen Peroxide	3.30%
Didecyldimethylammonium carbonate and didecyldimethylammonium bicarbonate	1.38%
Inert Ingredients	95.32%
Total	**100.00%**

I BACKGROUND

The product, Hitman (EPA File Symbol 9402-RU), is a new product. The applicant requested to register the product for use as a bactericide (disinfectant, virucide, and fungicide), sanitizer (non-food contact sanitizer and non-food contact residual self-sanitizer), mildewstat, and deodorizer for use on hard, non-porous surfaces for residential use only. The product is not intended for use in institutional, industrial or commercial establishments. Label directions indicate that the product is a one-step disinfectant which infers that the product is effective in the presence of a soil load. Studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121 and ATL located at 1304 W. Industrial Blvd, in Round Rock, TX, 78681.

This data package contained a letter from the registrant's representative (dated February 16, 2012), Good Laboratory Practice Statements for all studies, Confidential Statement of Formula (EPA Form 8570-4) for basic and 2 alternate formulations, eighteen efficacy studies (MRID 487484-15 through 487484-32), Statements of No Data Confidentiality Claims for all studies, and the proposed label.

II USE DIRECTIONS

The product is designed for sanitizing (hard, non-porous and soft surfaces) and disinfecting hard, non-porous surfaces including: desktops, doorknobs, faucets, chairs, cell phones, computers, soap dispensers, vanities, telephones, shower walls, kitchens, bathrooms, light switches, refrigerator exteriors, garbage cans, mouse pads, offices, laundry rooms, toilets, range hoods, and stove tops. The product is also for use (as a sanitizer) on washable soft surfaces such as, drapes, gym bags, diaper bags, upholstery, uniforms, shower curtains, pillows, sleeping blankets, sofas, oven mitts and rugs. The proposed label indicates that the product may be used on hard, non-porous surfaces, including: aluminum, brass, ceramic, Corian ®, glass, granite, laminate, stainless steel, vinyl, glazed porcelain, painted surfaces, polycarbonate, polypropylene, polyurethane varnish, and silicone rubber. Directions on the proposed label provide the following information regarding use of the product:

Disinfectant: To disinfect hard, non-porous surfaces, spray 6-8 inches from surface until thoroughly wet. Let stand for 5 minutes. Wipe dry. Remove heavy soil prior to disinfection. To disinfect Norovirus, let stand for 6 minutes.

Non-Food Contact Sanitizer: For hard, non-porous, non-food contact surfaces, spray 6-8 inches from surface until thoroughly wet. Let stand for 15 minutes. Wipe dry. Remove heavy soil prior to sanitization.

Residual Self-Sanitizer: To sanitize for 24 hours against *Staphylococcus aureus*, *Enterobacter aerogenes*, and Community Acquired Methicillin Resistant *Staphylococcus aureus* (CA-MRSA) on hard, non-porous surfaces. Spray 6-8 inches from surfaces until thoroughly wet. Let stand for 5 minutes. Wipe dry. This product can be removed with soap and water. Repeat residual self-sanitizing directions to maintain 24 hours sanitization.

Soft Surface Sanitizer: Test a hidden section of fabric. Spray 6-8 inches from surface until moderately damp. DO NOT SATURATE. Fabric must remain wet for 30 seconds. Let air dry.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments

The effectiveness of disinfectants for use on hard surfaces in hospital or medical environments must be substantiated by data derived using the AOAC Use-Dilution Method (for water soluble powders and liquid products) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray products). Sixty carriers must be tested with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old, against *Salmonella enterica* (ATCC 10708; formerly *Salmonella choleraesuis*), *Staphylococcus aureus* (ATCC 6538), and *Pseudomonas aeruginosa* (ATCC 15442). To support products labeled as "disinfectants," killing on 59 out of 60 carriers is required to provide effectiveness at the 95% confidence level.

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments (Additional Bacteria)

Effectiveness of disinfectants against specific bacteria other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Method, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method, must be determined by either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants Method. Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different product lots. To support products labeled as "disinfectants" for specific bacteria (other than those bacteria named in the above test methods), killing of the specific microorganism on all carriers is required.

Virucides

The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level.

Disinfectants for Use as Fungicides (Against Pathogenic Fungi)

The effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data using an appropriate test. The AOAC Use-Dilution Method may be modified to conform with the appropriate elements in the AOAC Fungicidal Test. If the product is intended to be used as a spray product, the AOAC Germicidal Spray Products as Disinfectants Method must be employed. The inoculum in the test must be modified to provide

a concentration of at least 10^6 conidia per carrier. Ten carriers on each of 2 product samples representing 2 different product lots must be employed in the test. Killing of the specific pathogenic fungi on all carriers is required.

Supplemental Claims

An antimicrobial agent identified as a "one-step" disinfectant or as effective in the presence of organic soil must be tested for efficacy with an appropriate organic soil load, such as 5 percent serum. On a product label, the hard water tolerance level may differ with the level of antimicrobial activity (e.g., sanitizer vs. disinfectant) claimed. To establish efficacy in hard water, all microorganisms (i.e., bacteria, fungi, and viruses) claimed to be controlled must be tested by the appropriate Recommended Method at the same hard water tolerance level.

IV COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. MRID 487484-15, "AOAC Germicidal Spray Method", Test Organisms: *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC15442), *Salmonella enterica* (ATCC 10708) for Hitman, by Joshua Luedtke, M.S. Study conducted at ATS Labs located at 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121. Study completion date – January 12, 2012. Project No. A12164.

This study was conducted against *Staphylococcus aureus* (ATCC 6538), *Salmonella enterica* (ATCC 10708) and *Pseudomonas aeruginosa* (ATCC 15442). Two lots of product (Lot # SA1255BLE and #SA1256BLB), Hitman, were tested using the AOAC Germicidal Spray Method, published by Association of Official Analytical Chemists, Method # 961.02 (2000). **Note: The next study (MRID 487484-16) contains the product that is ≥ 60 days old).** The product was received ready-to-use. Cultures of the challenge microorganisms were prepared in accordance with the published AOAC Methods. From a stock slant, an initial tube (10 mL) of culture broth was inoculated. The culture was termed the "initial broth suspension". From this suspension a minimal of three daily transfers were made using 1 loopful (10µL) of culture into 10 mL of culture media were performed on consecutive days prior to use in testing procedure. The appropriate growth medium was used for each test organism and subcultured using daily transfers. In all, a total of 12 daily transfers of each test organism culture were performed before use in testing. A 48-54 hour broth culture incubated at 35-37°C was prepared. For *Pseudomonas aeruginosa*, the pellicle was carefully aspirated from the culture. Each test culture was vortex mixed for 3 to 4 seconds and allowed to stand for≥10 minutes prior to use. After this time, the upper portion of the culture was removed, leaving behind any clumps or debris. The removed portion was transferred to a sterile vessel and used for testing. However, prior to use the final test culture was thoroughly mixed. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of each broth culture to yield a 5% organic soil load. Individual glass slide carriers were each inoculated with 10.0 µL of culture using a calibrated pipettor. The inoculum was uniformly spread over the entire surface of the slide contained in the Petri dish. The dish was covered immediately. For testing performed on 10/31/11, the slides were allowed to dry for 30 minutes at 35-37°C and 51% relative humidity. For testing performed on 11/8/11, the slides were allowed to dry for 30 minutes at a relative humidity of 40%. For each lot of test substance, test carriers were sprayed, in a horizontal position, at staggered intervals with the test substance at a distance of 6-8 inches from the carrier using 3 sprays. After the exposure period of 3 minutes at room temperature, each carrier was transferred using sterile forceps at staggered intervals to 20mL aliquots of Letheen Broth + 0.14% Lecithin + 1.0% Tween 80 + 0.01% Catalase. Carriers were transferred from primary to secondary subcultures containing

20 mL aliquots of Letheen Broth + 0.14% Lecithin +1.0% Tween 80 for ≥ 30 minutes. All subcultures and controls were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, organic sterility, carrier sterility, neutralization confirmation, viability and carrier population. Efficacy data were generated at the lower certified limits, consistent with the CSF.
Note—The results from the October 31, 2011 test data demonstrated 3/60 for *Staphylococcus aureus*.

2. MRID 487484-16, "AOAC Germicidal Spray Method", Test Organisms: *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC15442), *Salmonella enterica* (ATCC 10708) for Hitman, by Joshua Luedtke, M.S. Study conducted at ATS Labs located at 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121. Study completion date – January 11, 2012. Project No. A12251.

This study was conducted against *Staphylococcus aureus* (ATCC 6538), *Salmonella enterica* (ATCC 10708) and *Pseudomonas aeruginosa* (ATCC 15442). One lot (Lot # SA1256BLD; lot was ≥ 60 days old) of the product, Hitman, was tested using the AOAC Germicidal Spray Method, published by Association of Official Analytical Chemists, Method # 961.02 (2000). The product was received ready-to-use. Cultures of the challenge microorganisms were prepared in accordance with the published AOAC Methods. From a stock slant, an initial tube (10 mL) of culture broth was inoculated. The culture was termed the "initial broth suspension". From this suspension a minimal of three daily transfers were made using 1 loopful (10µL) of culture into 10 mL of culture media were performed on consecutive days prior to use in testing procedure. The appropriate growth medium was used for each test organism and subcultured using daily transfers. In all, a total of 12 daily transfers of each test organism culture were performed before use in testing. A 48-54 hour broth culture incubated at 35-37°C was prepared. For *Pseudomonas aeruginosa*, the pellicle was carefully aspirated from the culture. Each test culture was vortex mixed for 3 to 4 seconds and allowed to stand for ≥ 10 minutes prior to use. After this time, the upper portion of the culture was removed, leaving behind any clumps or debris. The removed portion was transferred to a sterile vessel and used for testing. The final test culture was thoroughly mixed. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of each broth culture to yield a 5% organic soil load. Individual glass slide carriers were each inoculated with 10.0 µL of culture using a calibrated pipettor. The inoculum was uniformly spread over the entire surface of the slide contained in the Petri dish. The dish was covered immediately, and the procedure was repeated until all slides were individually inoculated. Slides were allowed to dry for 30 minutes at 35-37°C and a relative humidity of 50%. For each lot of test substance, test carriers were sprayed, in a horizontal position, at staggered intervals with the test substance at a distance of 6-8 inches from the carrier using 3 sprays. After the exposure period of 3 minutes, each carrier was transferred using sterile forceps at staggered intervals to 20mL aliquots of Letheen Broth + 0.14% Lecithin + 1.0% Tween 80 + 0.01% Catalase. Carriers were then transferred from primary to secondary subcultures containing 20 mL aliquots of Letheen Broth + 0.14% Lecithin +1.0% Tween 80 for ≥ 30 minutes. All subcultures and controls were incubated for 48± 4 hours at 35-37°C. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, organic sterility, carrier sterility, neutralization confirmation, viability and carrier population. Efficacy data were generated at the lower certified limits, consistent with the CSF.

3. MRID 487484-17, "AOAC Germicidal Spray Method", Test Organism: *Escherichia coli* O157:H7(ATCC 35150) for Hitman, by Joshua Luedtke, M.S. Study conducted at ATS Labs located at 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121. Study completion date – January 10, 2012. Project No. A12252.

This study was conducted against *Escherichia coli* O157:H7 (ATCC 35150). The product was received ready-to-use. Two lots (Lot # SA1255BLE and #SA1256BLB) of the product Hitman, were tested . Cultures of the challenge microorganism were prepared in accordance with the published AOAC Methods. From a stock slant, an initial tube (10 mL) of culture broth was inoculated. The culture was termed the "initial broth suspension". From this suspension a minimal of three daily transfers were made using 1 loopful (10µL) of culture into 10 mL of culture media were performed on consecutive days prior to use in testing procedure. The appropriate growth medium was used for each test organism and subcultured using daily transfers. In all, a total of 12 daily transfers of each test organism culture were performed before use in testing. A 48-54 hour broth culture incubated at 35-37°C was prepared. The test culture was vortex mixed for 3 to 4 seconds and allowed to stand for ≥ 10 minutes prior to use. After this time, the upper portion of the culture was removed, leaving behind any clumps or debris. The removed portion was transferred to a sterile vessel and used for testing. The final test culture was thoroughly mixed. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of each broth culture to yield a 5% organic soil load. Individual glass slide carriers were each inoculated with 10.0 µL of culture using a calibrated pipettor. The inoculum was uniformly spread over the entire surface of the slide contained in the Petri dish. The dish was covered immediately, and the procedure was repeated until all slides were individually inoculated. Slides were allowed to dry for 31 minutes at 35-37°C and a relative humidity of 51%. For each lot of test substance, test carriers were sprayed, in a horizontal position, at staggered intervals with the test substance at a distance of 6-8 inches from the carrier using 3 sprays. After the exposure period of 3 minutes, each carrier was transferred using sterile forceps at staggered intervals to 20mL aliquots of Letheen Broth + 0.14% Lecithin + 1.0% Tween 80 + 0.01% Catalase. Carriers were then transferred from primary to secondary subcultures containing 20 mL aliquots of Letheen Broth + 0.14% Lecithin +1.0% Tween 80 for≥30 minutes. All subcultures and controls were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, organic sterility, carrier sterility, neutralization confirmation, viability and carrier population. Efficacy data were generated at the lower certified limits, consistent with the CSF.

4. MRID 487484-18, "AOAC Germicidal Spray Method", Test Organism: *Klebsiella pneumoniae* (ATCC 4352) for Hitman, by Joshua Luedtke, M.S. Study conducted at ATS Labs located at 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121. Study completion date – January 11, 2012. Project No. A12253.

This study was conducted against *Klebsiella pneumoniae* (ATCC 4352). The product was received ready-to-use. Two lots (Lot # SA1255BLE and #SA1256BLB) of the product, Hitman, were tested. Cultures of the challenge microorganism were prepared in accordance with the published AOAC Methods. From a stock slant, an initial tube (10 mL) of culture broth was inoculated. The culture was termed the "initial broth suspension". From this suspension a minimal of three daily transfers were made using 1 loopful (10µL) of culture into 10 mL of culture media were performed on consecutive days prior to use in testing procedure. The appropriate growth medium was used for each test organism and subcultured using daily transfers. In all, a

total of 12 daily transfers of each test organism culture were performed before use in testing. A 48-54 hour broth culture incubated at 35-37°C was prepared. The test culture was vortex mixed for 3 to 4 seconds and allowed to stand for ≥ 10 minutes prior to use. After this time, the upper portion of the culture was removed, leaving behind any clumps or debris. The removed portion was transferred to a sterile vessel and used for testing. However, prior to use the final test culture was thoroughly mixed. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of each broth culture to yield a 5% organic soil load. Individual glass slide carriers were each inoculated with 10.0 µL of culture using a calibrated pipettor. The inoculum was uniformly spread over the entire surface of the slide contained in the Petri dish. The dish was covered immediately, and the procedure was repeated until all slides were individually inoculated. Slides were allowed to dry for 30 minutes at 35-37°C and a relative humidity of 51%. For each lot of test substance, test carriers were sprayed, in a horizontal position, at staggered intervals with the test substance at a distance of 6-8 inches from the carrier using 3 sprays. After the exposure period of 3 minutes, each carrier was transferred using sterile forceps at staggered intervals to 20mL aliquots of Letheen Broth + 0.14% Lecithin + 1.0% Tween 80 + 0.01% Catalase. Carriers were then transferred from primary to secondary subcultures containing 20 mL aliquots of Letheen Broth + 0.14% Lecithin +1.0% Tween 80 for≥ 30 minutes. All subcultures and controls were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, organic sterility, carrier sterility, neutralization confirmation, viability and carrier population. Efficacy data were generated at the lower certified limits, consistent with the CSF.

5. MRID 487484-19, "AOAC Germicidal Spray Method", Test Organism: Community Acquired Methicillin Resistant *Staphylococcus aureus* – CA-MRSA Genotype USA 400 (NARSA NRS 123) for Hitman, by Joshua Luedtke, M.S. Study conducted at ATS Labs located at 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121. Study completion date – January 12, 2012. Project No. A12280.

This study was conducted against Community Acquired Methicillin Resistant *Staphylococcus aureus* – CA MRSA Genotype USA 400 (NARSA NRS 123). The product was received ready-to-use. Two lots (Lot # SA1255BLE and #SA1256BLB – both lots were ≥ 60 days old) of the product Hitman were tested. Cultures of the challenge microorganism were prepared in accordance with the published AOAC Methods. From a stock slant, an initial tube (10 mL) of culture broth was inoculated. The culture was termed the "initial broth suspension". From this suspension a minimal of three daily transfers were made using 1 loopful (10µL) of culture into 10 mL of culture media were performed on consecutive days prior to use in testing procedure. The appropriate growth medium was used for each test organism and subcultured using daily transfers. In all, a total of 12 daily transfers of each test organism culture were performed before use in testing. A 48-54 hour broth culture incubated at 35-37°C was prepared. The test culture was vortex mixed for 3 to 4 seconds and allowed to stand for ≥ 10 minutes prior to use. After this time, the upper portion of the culture was removed, leaving behind any clumps or debris. The removed portion was transferred to a sterile vessel and used for testing. However, prior to use the final test culture was thoroughly mixed. A Kirby Bauer Susceptibility assay was performed to verify resistance. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of each broth culture to yield a 5% organic soil load. Individual glass slide carriers were each inoculated with 10.0 µL of culture using a calibrated pipettor. The inoculum was uniformly spread over the entire surface of the slide contained in the Petri dish. The dish was covered immediately, and the procedure was repeated until all slides were individually inoculated. Slides were allowed to dry for 31 minutes at 35-37°C and a relative humidity of 46%. For each lot of test substance, test carriers were sprayed, in a horizontal

position, at staggered intervals with the test substance at a distance of 6-8 inches from the carrier using 3 sprays. After the exposure period of 3 minutes, each carrier was transferred using sterile forceps at staggered intervals to 20mL aliquots of Letheen Broth + 0.14% Lecithin + 1.0% Tween 80 + 0.01% Catalase. Carriers were then transferred from primary to secondary subcultures containing 20 mL aliquots of Letheen Broth + 0.14% Lecithin +1.0% Tween 80 for≥30 minutes. All subcultures and controls were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, organic sterility, carrier sterility, neutralization confirmation, viability and carrier population. Efficacy data were generated at the lower certified limits, consistent with the CSF.

6. MRID 487484-20, "AOAC Germicidal Spray Method", Test Organism: *Streptococcus pyogenes* (ATCC 19615) for Hitman, by Joshua Luedtke, M.S. Study conducted at ATS Labs located at 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121. Study completion date – January 12, 2012. Project No. A12281.

This study was conducted against *Streptococcus pyogenes* (ATCC 19615) . The product was received ready-to-use. Two lots (Lot # SA1255BLE and #SA1256BLB – both ≥ 60 days old) of the product, Hitman, were tested. Cultures of the challenge microorganism were prepared in accordance with the published AOAC Methods. From a stock slant, an initial tube (10 mL) of culture broth was inoculated. The culture was termed the "initial broth suspension". From this suspension a minimal of three daily transfers were made using 1 loopful (10µL) of culture into 10 mL of culture media were performed on consecutive days prior to use in testing procedure. The appropriate growth medium was used for each test organism and subcultured using daily transfers. In all, a total of 12 daily transfers of each test organism culture were performed before use in testing. A 48-54 hour broth culture incubated at 35-37°C was prepared. The test culture was vortex mixed for 3 to 4 seconds and allowed to stand for ≥ 10 minutes prior to use. After this time, the upper portion of the culture was removed, leaving behind any clumps or debris. The removed portion was transferred to a sterile vessel and used for testing. The final test culture was thoroughly mixed. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of each broth culture to yield a 5% organic soil load. Individual glass slide carriers were each inoculated with 10.0 µL of culture using a calibrated pipettor. The inoculum was uniformly spread over the entire surface of the slide contained in the Petri dish. The dish was covered immediately, and the procedure was repeated until all slides were individually inoculated. Slides were allowed to dry for 30 minutes at 35-37°C and a relative humidity of 65%. For each lot of test substance, test carriers were sprayed, in a horizontal position, at staggered intervals with the test substance at a distance of 6-8 inches from the carrier using 3 sprays. After the exposure period of 3 minutes, each carrier was transferred using sterile forceps at staggered intervals to 20mL aliquots of Letheen Broth + 0.14% Lecithin + 1.0% Tween 80 + 0.01% Catalase. Carriers were then transferred from primary to secondary subcultures containing 20 mL aliquots of Letheen Broth + 0.14% Lecithin +1.0% Tween 80 for ≥ 30 minutes. All subcultures and controls were incubated for 48 ± 4 hours at 35-37°C. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, organic sterility, carrier sterility, neutralization confirmation, viability and carrier population. Efficacy data were generated at the lower certified limits, consistent with the CSF.

7. MRID 487484-21, "AOAC Germicidal Spray Method modified for Fungi", Test Organism: *Trichophyton mentagrophytes* (ATCC 9533) for Hitman, by Joshua Luedtke, M.S. Study conducted at ATS Labs located at 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121. Study completion date – January 11, 2012. Project No. A12254.

This study was conducted against *Trichophyton mentagrophytes* (ATCC 9533). The product was received ready-to-use. Three lots (Lot # SA1255BLE, SA1256BLB and SA1256BLD – all lots ≥ 60 days old) of the product, Hitman, were tested. A culture of *Trichophyton mentagrophytes* was prepared by inoculating 20 agar plates using a stock culture and incubating at 25-30°C for 10 days. The mycelia were removed from all plates using a sterile swab. The mycelia were transferred to a glass bottle containing beads and saline/Triton Solution (0.85% saline + 0.05% Triton X-100), and mixed thoroughly. The culture was filtered through sterile gauze to remove hyphal fragments. The conidial concentration was estimated by counting in a hemacytometer. The conidial count was 1.8×10^8 conidia/mL. The test culture was thoroughly mixed prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of each broth culture to yield a 5% organic soil load. Individual glass slide carriers were each inoculated with 10.0 µL of culture using a calibrated pipettor. The inoculum was uniformly spread over the entire surface of the slide contained in the Petri dish. The dish was covered immediately, and the procedure was repeated until all slides were individually inoculated. Slides were allowed to dry for 30 minutes at 35-37°C and a relative humidity of 40%. For each lot of test substance, test carriers were sprayed, in a horizontal position, at staggered intervals with the test substance at a distance of 6-8 inches from the carrier using 3 sprays. After the exposure period of 3 minutes, each carrier was transferred using sterile forceps at staggered intervals to 20mL aliquots of Sabouraud Dextrose Broth + 0.14% Lecithin + 1.0% Tween 80 + 0.01% Catalase. Carriers were then transferred from primary to secondary subcultures containing 20 mL aliquots of Sabouraud Dextrose Broth + 0.14% Lecithin +1.0% Tween 80 for ≥ 30 minutes. All neutralized subcultures were incubated for 10 days at 25-30°C. The agar plate subcultures were incubated for 44-76 hours at 25-30°C. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, organic sterility, carrier sterility, neutralization confirmation, viability and carrier population. Efficacy data were generated at the lower certified limits, consistent with the CSF.

8. MRID 487484-22, "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces", Virus: Influenza A (H1N1) for Hitman, by Mary J. Miller, M.T. Study conducted at ATS Labs located at 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121. Study completion date – January 6, 2012. Project No. A12317.

This study was conducted against Influenza A Virus (H1N1)(ATCC VR-1496). The product was received ready-to-use. Three lots (Lot # SA1255BLE, #SA1256BLB and Lot #SA1256BLD – all lots ≥60 days old) of the product, Hitman were tested. The Influenza A Virus (ATCC VR-1469) was obtained from the American Type Culture Collection in Manassas, VA. The stock virus was prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells were disrupted and cell debris removed by centrifugation at approximately 2000 RPM for five minutes at approximately 4°C. The supernatant was removed and stored until the day of use. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 200µL of virus inoculum uniformly over the bottoms of twenty separate 100 x 15 mm sterile glass Petri dishes.

Virus films were dried at 20.0°C. Carriers were sprayed at a distance of 6-8 inches from the surface of the carrier until thoroughly wet. Just prior to the end of the exposure time (3 minutes), the carriers were scraped with a cell scraper to resuspend the contents and at the end of the exposure time the virus-test mixture were immediately passed through individual Sephadex columns in order to detoxify the mixtures. Filtrates were tittered 10-fold serial dilutions and then once again filtered through Sephadex columns and then remaining titrations were performed. Dilutions were then assayed for infectivity and/or cytotoxicity. Controls included those for treatment of dried virus film, cytotoxicity, and assay of non-virucidal level of test substance. Efficacy data were generated at the lower certified limits, consistent with the CSF

9. MRID 487484-23, "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces", Virus: Respiratory Syncytial virus for Hitman, by Shaneen Conway, B.S. Study conducted at ATS Labs located at 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121. Study completion date – January 6, 2012. Project No. A12296.

This study was conducted against Respiratory Syncytial virus (RSV) (Strain Long; ATCC Vr-26). The product was received ready-to-use. Three lots (Lot # SA1255BLE, #SA1256BLB and SA1256BLD – all lots ≥ 60 days old) of the product, Hitman, were tested . The stock virus was prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells were disrupted and cell debris removed by centrifugation at approximately 2000 RPM for five minutes at approximately 4°C. The supernatant was removed and stored until the day of use. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. The stock virus demonstrated cytopathic effects (CPE) typical of Respiratory Syncytial virus on Hep-2 (human larynx carcinoma) cells. Films of virus were prepared by spreading 200µL of virus inoculum uniformly over the bottoms of twenty separate 100 x 15 mm sterile glass Petri dishes. Virus films were dried at 20.0°C, at a relative humidity of 50% until visibly dry. For each lot of test substance, five dried virus films were individually exposed for 3 minutes to the amount of spray released under use conditions. Carriers were sprayed at a distance of 6-8 inches from the surface of the carrier until thoroughly wet. Just prior to the end of the exposure time, the carriers were scraped with a cell scraper to resuspend the contents and at the end of the exposure time (3 minutes) the virus-test mixture were immediately passed through individual Sephadex columns in order to detoxify the mixtures. Filtrates were tittered 10-fold serial dilutions and then once again filtered through Sephadex columns and then remaining titrations were performed. Dilutions were then assayed for infectivity and/or cytotoxicity. Controls included those for treatment of dried virus film, cytotoxicity, and assay of non-virucidal level of test substance. Efficacy data were generated at the lower certified limits, consistent with the CSF.

10. MRID 487484-24, "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces", Virus: Human Coronavirus for Hitman, by Shaneen Conway, B.S. Study conducted at ATS Labs located at 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121. Study completion date – January 6, 2012. Project No. A12297.

This study was conducted against Human Coronavirus (ATCC VR-740). The product was received ready-to-use. Three lots (Lot # SA1255BLE, #SA1256BLB and #SA1256BLD – all lots ≥ 60 days old) of the product, Hitman, were tested . The stock virus was prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells were

disrupted and cell debris removed by centrifugation at approximately 2000 RPM for five minutes at approximately 4°C. The supernatant was removed and stored until the day of use. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. The stock virus demonstrated cytopathic effects (CPE) typical of Human Coronavirus on WI-38 (human lung) cells. Films of virus were prepared by spreading 200µL of virus inoculum uniformly over the bottoms of twenty separate 100 x 15 mm sterile glass Petri dishes. Virus films were dried at 20.0°C, at a relative humidity of 50% until visibly dry. For each lot of test substance, five dried virus films were individually exposed for 3 minutes to the amount of spray released under use conditions. The carriers were sprayed until thoroughly wet at a distance of 6-8 inches and held for the exposure time. Just prior to the end of the exposure time, the plates were scraped with a cell scraper to resuspend the contents and at the end of the exposure time (3 minutes) the virus-test mixture were immediately passed through individual Sephadex columns in order to detoxify the mixtures. Filtrates were tittered 10-fold serial dilutions and then once again filtered through Sephadex columns and then remaining titrations were performed. Dilutions were then assayed for infectivity and/or cytotoxicity. Controls included those for treatment of dried virus film, cytotoxicity, and assay of non-virucidal level of test substance. Efficacy data were generated at the lower certified limits, consistent with the CSF.

11. MRID 487484-25, "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Feline Calicivirus as a Surrogate Virus for Norovirus – Confirmatory Assay", for Hitman, by Mary J. Miller, M.T. Study conducted at ATS Labs located at 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121. Study completion date – January 9, 2012. Project No. A12414.

This study was conducted against Feline Calicivirus (F-9 strain) (ATCC VR-782). The product was received ready-to-use. Two lots (Lot # SA1255BLE and t# SA1256BLB) of the product, Hitman, were tested. The stock virus was prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells were disrupted and cell debris removed by centrifugation at approximately 2000 RPM for five minutes at approximately 4°C. The supernatant was removed and stored until the day of use. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. The cytopathic effects (CPE) typical of the F-9 strain of Feline Calicivirus were demonstrated on Crandel Reese feline kidney cells showing small, rounding of the cells with a slight granular look. Films of virus were prepared by spreading 200µL of virus inoculum uniformly over the bottoms of twenty separate 100 x 15 mm sterile glass Petri dishes. Virus films were dried at 20.0°C, and a relative humidity of 50% until visibly dry. For each lot of test substance, five dried virus films were individually exposed for 6 minutes to the amount of spray released under use conditions. The carriers were sprayed until thoroughly wet at a distance of 6-8 inches at 20°C and held for the exposure time. Just prior to the end of the exposure time, the plates were scraped with a cell scraper to resuspend the contents and at the end of the exposure time (6 minutes) the virus-test mixture was immediately passed through individual Sephadex columns in order to detoxify the mixtures. Filtrates were tittered 10-fold serial dilutions and then once again filtered through Sephadex columns utilizing the syringe plunger. Dilutions were then assayed for infectivity and/or cytotoxicity. Controls included those for treatment of dried virus film, cytotoxicity, and assay of non-virucidal level of test substance. Efficacy data were generated at the lower certified limits, consistent with the CSF.

12. MRID 487484-26, "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Feline Calicivirus as a Surrogate Virus for Norovirus – Confirmatory Assay", for Hitman, by Shaneen Conway, B.S. Study conducted at ATS Labs located at 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121. Study completion date – January 9, 2012. Project No. A12088.

This study was conducted against Feline Calicivirus (F-9 strain) (ATCC VR-782). The product was received ready-to-use. One lot (Lot # SA1255BLE) of the product, Hitman, was tested. The stock virus was prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells were disrupted and cell debris removed by centrifugation at approximately 2000 RPM for five minutes at approximately 4°C. The supernatant was removed and stored until the day of use. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. The cytopathic effects (CPE) typical of the F-9 strain of Feline Calicivirus were demonstrated on Crandel Reese feline kidney cells showing small, rounding of the cells with a slight granular look. Films of virus were prepared by spreading 200µL of virus inoculum uniformly over the bottoms of twenty separate 100 x 15 mm sterile glass Petri dishes. Virus films were dried at 20.0°C, and a relative humidity of 50% until visibly dry. For each lot of test substance, five dried virus films were individually exposed for 5 minutes to the amount of spray released under use conditions. The carriers were sprayed until thoroughly wet at a distance of 6-8 inches at 20°C and held for the exposure time. Just prior to the end of the exposure time, the plates were scraped with a cell scraper to resuspend the contents and at the end of the exposure time (5 minutes) the virus-test mixture was immediately passed through individual Sephadex columns un order to detoxify the mixtures. Filtrates were tittered 10-fold serial dilutions and then once again filtered through Sephadex columns utilizing the syringe plunger. Dilutions were then assayed for infectivity and/or cytotoxicity. Controls included those for treatment of dried virus film, cytotoxicity, and assay of non-virucidal level of test substance. Efficacy data were generated at the lower certified limits, consistent with the CSF.

13. MRID No. 487484-27, "Efficacy of Sanitizers Recommended for Inanaimate Non-Food Contact Surfaces" against *Enterobacter aerogenes* and *Staphylococcus aureus* by Jessica Akins. Study Completion Date—January 20, 2012. Study Identification Number—GLP1097.

The study was conducted against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048). Three lots (Lot Nos. SA1256BLB, SA1256BLB, and SA1256BLD; all lots were ≥ 60 days old) of the product, Hitman, were tested according to ATL Labs Protocol No. P1105 (copy provided). The product was received ready-to-use as a pump spray. Fetal bovine serum was added to each inoculum to achieve a 5% organic soil load. Five tserile glass carriers per product lot per microorganism were inoculated with 20 µL of 48-54 hour culture suspension of microorganisms. The inoculum was spread to within 3 mm of the edges of the carrier. The carriers were dried for 35±2 minutes at 36±1°C at 40-41% relative humidity. Each carrier wa transferred to a sterile jar and while tilted to a 45°angle, sprayed with 2 pumps of the product which thoroughly wet the carrier at a distance of 6-8 inches from the carrier surface. Each carrier remained in contact iwht the product for 15±2 seconds at room temperature. Afte exposure, 100 mL of 2X D/E Neutralizing Broth with 0.25% sodium lauryl sulfate and 0.1% catalase was added to each jar containing the treated carrier. The jars were rotated vigorously and repeated pipetting of the solution was used to help re-suspend the surviving organisms adhered to the carrier. Within 30 minutes following addition of the neutralizer, 10 mL, 1.0 mL, and 0.1 mL aliquots of the neutralized liquid were plated in duplicate

on tryptic soy agar. All plates were incubated for 48-54 hours at 36±1°C for S. aureus and 30±1°C for Enterobacter aerogenes. Controls included those for purity, sterility, carrier quantitation, inoculum count, and neutralization confirmation. Efficacy data were generated at the lower certified limits, consistent with the CSF.

14. MRID No. 487484-28, "Efficacy of Sanitizers Recommended for Inanaimate Non-Food Contact Surfaces" against Community Acquired Methicillin Resistant *Staphylococcus aureus*-CA-MRSA by Benjamin Tanner, Ph.D. Study Completion Date—January 20, 2012. Study Identification Number—GLP1098.

The study was conducted against Community Acquired Methicillin Resistant *Staphylococcus aureus*-CA-MRSA (NARSA NRS123, Genotype USA 400). Two lots (Lot Nos. SA1255BLE and SA1256BLB; both lots were ≥ 60 days old) of the product, Hitman, were tested according to ATL Labs Protocol No. P1106 (copy provided). The product was received ready-to-use as a pump spray. Fetal bovine serum was added to each inoculum to achieve a 5% organic soil load. Five sterile glass carriers per product lot per microorganism were inoculated with 20 µL of 48-54 hour culture suspension of microorganisms. The inoculum was spread to within 3 mm of the edges of the carrier. The carriers were dried for 35 minutes at 36±1°C at 39-40% relative humidity. Each carrier wa transferred to a sterile jar and while tilted to a 45°angle, sprayed with 2 pumps of the product which thoroughly wet the carrier at a distance of 6-8 inches from the carrier surface. Each carrier remained in contact iwht the product for 15±2 seconds at room temperature. Afte exposure, 100 mL of 2X D/E Neutralizing Broth with 0.25% sodium lauryl sulfate and 0.1% catalase was added to each jar containing the treated carrier. The jars were rotated vigorously and repeated pipetting of the solution was used to help re-suspend the surviving organisms adhered to the carrier. Within 30 minutes following addition of the neutralizer, 10 mL, 1.0 mL, and 0.1 mL aliquots of the neutralized liquid wer eplated in duplicate on tryptic soy agar. All plates were incubated for 48-54 hours at 36±1°C. Controls included those for purity, sterility, carrier quantitation, inoculum count, and neutralization confirmation. Efficacy data were generated at the lower certified limits, consistent with the CSF.

15. MRID No. 487484-29, "Residual Self-Sanitizing Activity of Dried Chemical Residues in Hard Non-porous surfaces, with Exposure and Wear Activity" against *Enterobacter aerogenes* and *Staphylococcus aureus* by Jessica Akins. Study Completion Date—January 20, 2012. Study Identification Number—GLP1084.

The study was conducted against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048). Three lots (Lot Nos. SA1255BLE, SA1256BLB, and SA1256BLD (lot ≥ 60 days old) of the product, Hitman, were tested according to ATL Labs Protocol No. P1092 (copy provided). The product was received ready-to-use as a pump spray. Fetal bovine serum was added to each inoculum to achieve a 5% organic soil load. Four sterile glass carriers (1" x 1") per lot of test and control substance per microorganism were inoculated with 0.01 mL of an intitial culture suspension with 5% soil and spread to within ⅛" of the carrier edge. The carriers were allowed to dry for 30 minutes at 35±2°C. For dry carriers per lot per microorganisms were treated in a staggered fashion with 3 sprays of the product per carrier from a 6-8 inch distance at 23-25°C and 45-55% humidity. Four dry control carriers per test organisms were treated with 0.01% Trition X-100 solution by spraying for 3 seconds with a Preval sprayer. After treatment, the carriers were allowed to dry overnight (17-18 hours) at room temperature and 45-55% relative humidity. The test and control carriers underwent 12

wear cycles (alternating between wet and dry) and 5 re-inoculations which were staggered between the initial 6 wear cycles. Abrasions were conducted at room temperature and 45-55% relative humdity using a 1084±1 gram abrasion boat on a Gardco Washability & Wear Tester set for a speed of 2.25-2.5 (total surface caontact time of 4-5 seconds). Liners and clothes wer replaced between each wear cycle. After a wear cycle, carriers are allowed to sit for at least 15 minutes beofre being re-inoculated. Carriers were re-inoculated with 0.1 mL of the re-inoculation culture with 5% soil and allowed to dry at ambient temperature for at least 30 minutes prior to the subsequent wear cycle. Approximately 46-47.5 hours after carrier test/control treatment, wear and re-inoculation cycling, the carriers were inoculated with the 18-24 hour old sanitization test culture. Five minutes after inoculation, carriers wree transferred to sterile tube containing 30 mL of 2X concentrated D/E Broth with 0.1% catalase. The tubes were sonicated for 20±2 seocnds, followed by agitation on an orbital shaker for 3-4 minutes at 250 rpm to suspend the surviving organisms. The neutralized solutions were serially diluted with R/O water. Duplicate pour plates of the 10-1 and 10-3 dilutions for the test carriers were plated on TSA. All subcultures were incubated for 48-54 hours at 35±2°C for *S.aureus* and 30±2°C for *Enterobacter aerogenes*. Following incubation, the subcultures were visually enumerated. Controls included those for purity, sterility, viability, and neutralization confirmation. For testing of Lot SA1256BLD (> 60 days old), the test carriers were weighed prior to treatment with the test substance and after drying to measure the weight of the carrier treatment. Efficacy data were generated at the lower certified limits, consistent with the CSF.

16. MRID No. 487484-30, "Residual Self-Sanitizing Activity of Dried Chemical Residues ib Hard Non-porous surfaces, with Exposure and Wear Activity" against Community Acquired Methicillin Resistant *Staphylococcus aureus* (CA-MRSA) by Benjamin Tanner. Study Completion Date—January 20, 2012. Study Identification Number—GLP1094.

The study was conducted against Community Acquired Methicillin Resistant *Staphylococcus aureus* (CA-MRSA) (NARSA NRS123, Genotype USA 400). Two lots (Lot Nos. SA1255BLE and SA1256BLB; both lots ≥ 60 days old) of the product, Hitman, were tested according to ATL Labs Protocol No. P1094 (copy provided). The product was received ready-to-use as a pump spray. Fetal bovine serum was added to each inoculum to achieve a 5% organic soil load. Four sterile glass carriers (1" x 1") per lot of test and control substance per microorganism were inoculated with 0.01 mL of an intitial culture suspension with 5% soil and spread to within ⅛" of the carrier edge. The carriers were allowed to dry for 30 minutes at 35±2°C and 45-55% relative humidity. Four dry carriers per lot per microorganisms were treated in a staggered fashion with 3 sprays of the product per carrier from a 6-8 inch distance at 21-23°C and 45-55% humidity. Four dry control carriers per test organisms were treated with 0.01% Trition X-100 solution by spraying for 3 seconds with a Preval sprayer. After treatment, the carriers were allowed to dry overnight (approx. 15 hours) at room temperature and 45-55% relative humidity. The test and control carriers underwent 12 wear cycles (alternating between wet and dry) and 5 re-inoculations which were staggered between the initial 6 wear cycles. Abrasions were conducted at room temperature and 45-55% relative humdity using a 1084±1 gram abrasion boat on a Gardco Washability & Wear Tester set for a speed of 2.25-2.5 (total surface caontact time of 4-5 seconds). Liners and clothes wer replaced between each wear cycle. After a wear cycle, carriers are allowed to sit for at least 15 minutes beofre being re-inoculated. Carriers were re-inoculated with 0.1 mL of the re-inoculation culture with 5% soil and allowed to dry at ambient temperature for at least 30 minutes prior to the subsequent wear cycle. Approximately 46-47.5 hours after carrier test/control treatment, wear and re-inoculation cycling, the carriers were inoculated with the 18-24 hour old sanitization test culture. Five

minutes after inoculation, carriers wree transferred to sterile tube containing 30 mL of 2X concentrated D/E Broth with 0.1% catalase. The tubes were sonicated for 20±2 seocnds, followed by agitation on an orbital shaker for 3-4 minutes at 250 rpm to suspend the surviving organisms. The neutralized solutions were serially diluted with R/O water. Duplicate pour plates of the 10^{-1} and 10^{-3} dilutions for the test carriers were plated on TSA. All subcultures were incubated for 48-54 hours at 35±2°C for S.aureus and 30±2°C for Enterobacter aerogenes. Following incubation, the subcultures were visually enumerated. Controls included those for purity, sterility, viability, and neutralization confirmation. For testing of Lot SA1256BLD (> 60 days old), the test carriers were weighed prior to treatment with the test substance and after drying to measure the weight of the carrier treatment. Efficacy data were generated at the lower certified limits, consistent with the CSF.

17. MRID No. 487484-31, "EPA Hard Surface Mildew-Fingistatic Test" against *Aspergillus niger* (ATCC 16404) for Hitman Spray, by Joshua Luedtke. Study conducted at ATS Labs. Study completion date—Janaury 11, 2012. Project Number A12264.

This study was conducted against *Aspergillus niger* (ATCC 16404). Two lots (Lot Nos. SA1255BLE and SA1256BLB) of the product, Hitman Spray, were tested according to ATS Labs Protocol No. SRC52051311.MSTAT.1 (copy provided). The product was receivced was ready-to-use as an pump spray. Ten (1" x 1") glazed ceramic tiles per product lot were treated with 3 sprays from a 6-8" distance. The carriers wree placed in avertical or near vertical position and to allow excess to drain. The test and untreated control carriers were dried at 35-37°C in Petri dishes for 50 minutes. Using a hemacytometer, a condidal suspension of the challenge microorganism was prepared in Czapek's solution at 2.78 x 10^8 condida/mL. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. An atmozier was used to apply the prepared inoculum to the test and control carriers. The inoculated carriers were dried at 35-57°C for 45 minutes until visibly dry. Each carrier was placed in a Petri dish containing Sterile Water Agar and incubated for 7 days at 25-30°C at ≥95% relative humidity. Following incubation, the carriers were examined for the presence or absence of visible growth. Controls included those for purity and sterility. Efficacy data were generated at the lower certified limits, consistent with the CSF.

18. MRID No. 487484-32, "Standard Test method for Efficacy sanitizers Recommended for Soft Non-Food Contact Surfaces (Modification for Spray product Application)" against *Enterobacter aerogenes* and *Staphylococcus aureus* by Christine Chan. Study Completion date—February 13, 2012. Project Number—A12632.

This study was conducted against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048). Three lots (Lot Nos. SA1256BLB, SA1256BLB, and SA1256BLD; all lots were ≥ 60 days old) of the product, Hitman, were tested according to ATS Protocol No. SRC52110311.NFS (copy provided). The product was received ready-to-use as a pump spray. Fetal bovine serum was added to each inoculum to achieve a 5% organic soil load. Five sterile, 1" x 1" carriers of 100% cotton and five sterile, 1" x 1" carriers of 100% polyester per product lot per microorganism were inoculated with 0.02 mL fo 48-54 hours old suspension of microorganisms. The inoculum was distributed by calibrated pipette evenly over the carrier. The carriers were dried for 20 minutes at 35-37°C at 40% relative humidity. Each carrier was transferred to a plastic Petri dish and was sprayed with the product with 3 sprays until thoroughly wet at a distance of 6-8 inches from the carrier surface. Each carrier remained

in contact with the product for 30 seconds at 21°C and 23% relative humidity. After exposure, each S. aureus carrier was placed into 200 mL of D/E Neutralizing Broth with 0.25% sodium laurly sulfate and 0.01% catalase with beads and each E. aerogenes carriers was placed into 20 mL of Letheen broth with 0.07% lecithin, 0.5% Tween 80, and 0.01% catalase with beads. The excess liquid remaining in each plastic Petri dish was transferred to the container with the carrier and neutralizing solution. The vessels were vortex mixed to suspend the surviving organisms. Within 30 minutes following the addition of neutralizer, 1.0 ml liquots of 100 and 10-1 dilutions were plated in duplicate on TSA with 5% sheep's blood. All plates were incubated at 48±4 hours. The S. aureus plates were incubated at 35-37°C and the E. aerogenes plates were incubated at 25-30°C prior to observation for visually enumeration. Controls included those for purity, sterility, carrier quantitation, inoculum count, and neutralization confirmation. Efficacy data were generated at the lower certified limits, consistent with the CSF.

V RESULTS

MRID	Organism	No. Exhibiting Growth/ Total No. Tested			Carrier Population
		Lot No. SA1255BLE	Lot No. SA1256BLB	Lot No. SA1256BLD ≥60 Days old	
487484-15	*Staphylococcus aureus*	10/31/11: 3/60 11/8/11 0/60	0/60	----	5.3×10^6
	Salmonella enterica	0/60	0/60,	----	3.6×10^4
	Pseudomonas aeruginosa	0/60	0/60	----	2.85×10^6
487484-16	*Staphylococcus aureus*			0/60	5.0×10^6
	Salmonella enterica			0/60	4.6×10^4
	Pseudomonas aeruginosa			0/60	6.8×10^6
487484-17	*Escherichia coli* O157:H7	0/10	0/10	----	1.52×10^5
487484-18	*Klebsiella pneumoniae*	0/10	0/10	----	1.23×10^6
487484-19	Community Acquired Methicillin-Resistant *Staphylococcus aureus* (MRSA)	0/10	0/10	----	1.59×10^5
487484-20	*Streptococcus pyogenes*	0/10	0/10		2.6×10^4
487484-21	*Trichophyton mentagrophytes*	0/10	0/10	0/10	8.5×10^5

MRID	Organism	Results				Dried Virus Control $TCID_{50}$/0.1 mL
			Lot No. SA1255BLE	Lot. No. SA1256BLB	Lot No. SA1256BLD	
487484-22	Influenza A Virus (H1N1)	10^{-1} dilutions	Cytotoxicity	Cytotoxicity	Cytotoxicity	$10^{5.62}$
		10^{-2} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	Complete inactivation	
		$TCID_{50}$/0.1 mL	$\leq 10^{1.50}$	$\leq 10^{1.50}$	$\leq 10^{1.50}$	
		Log Reduction	$\geq 4.12 \log_{10}$	$\geq 4.12 \log_{10}$	$\geq 4.12 \log_{10}$	
487484-23	Respiratory Syncytial Virus	10^{-1} dilutions	Cytotoxicity	Cytotoxicity	Cytotoxicity	$10^{5.50}$
		10^{-2} to 10^{-6} dilutions	Complete Inactivation	Complete Inactivation	Complete inactivation	
		$TCID_{50}$/0.1 mL	$\leq 10^{1.50}$	$\leq 10^{1.50}$	$\leq 10^{1.50}$	
		Log Reduction	$\geq 4.00 \log_{10}$	$\geq 4.00 \log_{10}$	$\geq 4.00 \log_{10}$	
487484-24	Human Coronavirus	10^{-1} dilutions	Cytotoxicity	Cytotoxicity	Cytotoxicity	$10^{5.50}$
		10^{-2} to 10^{-6} dilutions	Complete Inactivation	Complete Inactivation	Complete inactivation	
		$TCID_{50}$/100µL	$\leq 10^{1.50}$	$\leq 10^{1.50}$	$\leq 10^{1.50}$	
		Log Reduction	$\geq 4.00 \log_{10}$	$\geq 4.00 \log_{10}$	$\geq 4.00 \log_{10}$	
487484-25	Norovirus utilizing Feline Calicivirus	10^{-1} and 10^{-2} dilutions	Cytotoxicity	Cytotoxicity	----	$10^{5.75}$
		10^{-3} to 10^{-4} dilutions	Complete Inactivation	Complete inactivation	----	
		$TCID_{50}$/100µL	$\leq 10^{2.50}$	$\leq 10^{2.50}$	----	
		Log Reduction	$\geq 3.25 \log_{10}$	$\geq 3.25 \log_{10}$	----	
487484-26	Norovirus Utilizing Feline Calicivirus - Confirmatory	10^{-1} dilutions	----	----	Cytotoxicity	$10^{4.50}$
		10^{-2} to 10^{-7} dilutions	----	----	Complete inactivation	

		$TCID_{50}$/100µL	----	----	$\leq 10^{0.50}$	
		Log Reduction			≥4.00	

VI CONCLUSIONS

1. The submitted efficacy data (**MRID 487484-15**) do not support the use of the product, Hitman Spray, as a disinfectant against *Staphylococcus aureus*, on hard non-porous surfaces in the presence of a 5% organic soil load for a contact time of 3 minutes. Growth was observed in one of the tested lots (3/60). The registrant failed to disclose and confirm the "false positives." Repeat testing occurred on November 8, 2011 which produced a passing result of 0/60 for *Staphylococcus aureus*. However, the Agency is not accepting repeat testing for failed product lots.

2. The submitted efficacy data support the use of the product, Hitman Spray, as a disinfectant with bactericidal activity against the following microorganisms on hard, non-porous surfaces in the presence of a 5% organic soil load for a contact time of 3 minutes:

Pseudomonas aeruginosa	**MRID 487484-15 and -16**
Salmonella enterica	**MRID 487484-15 and -16**
Staphylococcus aureus	**MRID 487484-16**
Escherichia coli	**MRID 487484-17**
Klebsiella pneumoniae	**MRID 487484-18**
Community Acquired Methicillin-Resistant *Staphylococcus aureus* (MRSA)	**MRID 487484-19**
Streptococcus pyogenes	**MRID 487484-20**

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. In testing against *Staphylococcus aureus*, *Salmonella enterica* and *Pseudomonas aeruginosa*, at least one of the product lots tested was at least 60 days old at the time of testing. Neutralizer effectiveness showed positive growth of the microorganisms.

3. The submitted efficacy data support the use of the product, Hitman Spray, as a disinfectant with fungicidal activity against the following microorganisms on hard, non-porous surfaces in the presence of a 5% organic soil load for a contact time of 5 minutes:

Trichophyton mentagrophytes	**MRID 487484-21**

Complete killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralizer effectiveness testing showed positive growth of the microorganisms. Viability controls were positive for growth. Sterility controls were positive for growth.

4. The submitted efficacy data support the use of the product, Hitman Spray, as a disinfectant with Virucidal activity against the following microorganisms on hard, non-porous surfaces in the

presence of a 5% organic soil load for the time specified below:

Influenza A (H1N1) Virus	3 minutes	**MRID 487484-22**
Respiratory Syncytial Virus	3 minutes	**MRID 487484-23**
Human Coronavirus	3 minutes	**MRID 487484-24**
Norovirus	6 minutes	**MRID 487484-25**
Norovirus (confirmatory)	5 minutes	**MRID 487484-26**

Recoverable virus titers of at least 10^4 were achieved. Cytotoxicity was observed in the 10^{-1} dilutions. Complete inactivation (no growth) was indicated in all higher dilutions tested. At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level. In studies against Norovirus utilizing Feline Calicivirus as a surrogate, the initial and confirmatory studies were performed at ATS Labs but under the direction of different study directors. Both the initial and confirmatory studies tested two replicates per product lot. The confirmatory study tested one product lot.

5. The submitted study (MRID Nos. 487484-27 and -28) is acceptable regarding the use of the product, Hitman Spray, as a non-food contact sanitizer against *Staphylococcus aureus*, *Enterobacter aerogenes*, and Community Acquired Methicillin Resistant *Staphylococcus aureus* (CA-MRSA) at a contact time of 15 seconds in the presence of organic soil. Carrier counts were $\geq 7.5 \times 10^5$ CFU/carrier. At least a 99.9% reduction in the population was observed. At least one of the product lots tested was at least 60 days old at the time of testing for S. aureus and E. aerogenes. Neutralization confirmation testing met the acceptance criterion of growth within 1 log10 of the numbers control. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

6. The submitted efficacy data support the use of the product, Hitman Spray, as a sanitizer with residual activity against the following microorganisms on hard, non-porous, non-food contact surfaces in the presence of 5% organic soil and the following wear (wet and dry) and re-inoculation:

Staphylococcus aureus
Enterobacter aerogenes
Community Acquired Methicillin Resistant *Staphylococcus aureus*

Bacterial reduction of at least 99.9% over the parallel control was observed within 5 minutes on surfaces treated approximately 46-48 hours prior to testing. At least one of the product lots tested against *Staphylococcus aureus* and Enterobacter aerogenes was at least 60 days old at the time of testing. Neutralization confirmation testing demonstrated that the neutralizer was effective and not detrimental to the test system. Purity controls were reported as pure. Sterility controls did not show growth.

7. The submitted study (MRID No. 487484-31) is acceptable regarding the use of the product, Hitman Spray, as a hard surface mildewstat against Aspergillus niger for up to 7 days in the present of organic soil. The untreated control demonstrated ≥50% growth on each carrier after 7 days. No fungi were observed visually or under magnification on test carriers after 7 days. Purity controls were reported as pure. Sterility controls did not show growth.

8. The submitted study (MRID No. 487484-32) is acceptable regarding the use of the product, Hitman Spray, as a soft surface sanitizer against *Staphylococcus aureus* and *Enterobacter*

aerogenes with a contact time of 30 seconds in the presence of organic soil. At least one of the product lots tested against Staphylococcus aureus and Enterobacter aerogenes was at least 60 days olds at the time of testing. Neutralization confirmation testing demonstrated that the neutralizer was effective and not detrimental to the test system. Purity controls were reported as pure. Sterility controls did not show growth.

VII RECOMMENDATIONS

1. The proposed label claims that the product, Hitman Spray, is an effective disinfectant against the following microorganisms on hard, non-porous surfaces in the presence of a 5% organic soil load for a contact time of 5 minutes:

Salmonella enterica
Pseudomonas aeruginosa
Escherichia coli
Klebsiella pneumonia
Streptococcus pyogenes
Community Associated Methicillin-Resistant
Staphylococcus aureus (MRSA)
Influenza A Virus (H1N1)
Respiratory Syncytial Virus
Human Coronavirus

In the presence of failing data against *Staphylococcus aureus*, claims against additional Gram positive bacteria and viruses are unacceptable. Resolution of the failing data issue is required before the acceptability of additional claims can be granted. Claims against Gram negative bacteria *Salmonella enterica, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumonia* are acceptable.

2. The proposed claims that the product, Hitman Spray, is an effective disinfectant against Norovirus (utilizing Feline Calicivirus as a surrogate) on hard, non-porous surfaces in the presence of a 5% organic soil load for a contact time of 6 minutes. **In the presence of failing data against *Staphylococcus aureus*, claims against additional Gram positive bacteria and viruses are unacceptable. Resolution of the failing data issue is required before the acceptability of additional claims can be granted.**

3. The proposed label claims are acceptable regarding the use of the product, Hitman Spray, as a fungicidal against *Trichophyton mentagrophytes* on hard, non-porous surfaces in the presence of a 5% organic soil for a contact time of 5 minutes.

4. The proposed label claims are acceptable regarding the use of the product, Hitman Spray, as non-food contact sanitizer against *Staphylococcus aureus*, *Enterobacter aerogenes*, and Community Associated Methicillin Resistant *Staphylococcus aureus* (CA-MRSA) in the presence of organic soil at a contact time of 15 seconds for hard, non-porous surfaces.

5. The proposed label claims are acceptable regarding the use of the product, Hitman Spray, as a residual sanitizer against *Staphylococcus aureus*, *Enterobacter aerogenes*, and Community Associated Methicillin Resistant *Staphylococcus aureus* (CA-MRSA) in the presence of organic soil at a contact time of 5 minutes for up to 24 hours on hard, non-porous surfaces that experience wear and re-inoculation of bacteria.

6. The proposed label claims are acceptable regarding the use of the product, Hitman Spray, as a hard surface mildewstat against *Aspergillus niger* for up to 7 days in the presence of organic soil.

7. The proposed label claims are regarding the use of the product, Hitman Spray, as a soft surface sanitizer against *Staphylococcus aureus* and *Enterobacter aerogenes* for a contact time of 30 seconds in the presence of organic soil on fabric.

In the presence of failing data against *Staphylococcus aureus*, claims against additional Gram positive bacteria and viruses are unacceptable. Resolution of the failing data issue is required before the acceptability of additional claims can be granted. The following comments must be changed and addressed, as appropriate to the acceptability of the submitted efficacy data.

8. Once acceptable efficacy data has been provided to the Agency, claims against Coronavirus SARS must be revised to reflect the tested strain "Coronavirus".

9. ATCC designation numbers are required in one of the following locations:

- on the data matrix;
- on the master label (as optional text) with the listing of the organisms claimed; or
- as the final page of the master label (as optional text).

10. On page 3 (bottom), relocate "laminate flooring, countertops, and LCD screens to page 9 in the "use sites" location.

11. On page 5 of the proposed label, remove the quantitative value for bacteria that exceeds the 3 $\log_{10}$ reduction observed with sanitization. The disinfectant and fungicidal tests are qualitative, and the proposed quantitative reductions are potentially misleading.

12. On page 5 of the proposed label, remove the claim "Eliminates (kills) > 99.9% of germs on contact for the following reasons:

- The contact times range from 15 seconds to 6 minutes;
- the unqualified germ claim implies bacteria (disinfectant level), viruses, and fungi;
- Quantitative assessments for qualitative tests are unacceptable.

13. On page 5 of the proposed label, remove the claim "Advanced, Breakthrough, Leading Edge, and Progressive" as they imply heightened efficacy.

14. On page 5 of the proposed label, remove the term "fast-acting" as the Agency has not determined the contact time consistent with the term "fast".

15. On page 8 of the proposed label, provide additional information regarding the WETTASK System.

16. On page 10 of the proposed label, the 24 Hour protection logo should be clarified to reflect for sanitization.

17. Once acceptable efficacy data has been provided to the Agency, change Community "Acquired" to Community "Associated" for Methicillin Resistant *Staphylococcus aureus*.