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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION,
PESTICIDES
AND TOXIC
SUBSTANCES

August 17, 2009

MEMORANDUM

Subject: Efficacy Review for EPA File Symbol 777-RRU, Sting
DP Barcode: 366325

From: Tajah L. Blackburn, Ph.D., Microbiologist
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P) *[Signature]*
8/17/09

Thru: Michele Wingfield, Chief
Product Science Branch
Antimicrobials Division (7510P)

To: Velma Noble PM 31/Drusilla Copeland
Regulatory Management Branch I
Antimicrobials Division (7510P)

Applicant: Reckitt Benckiser, Inc.
399 Interpace Parkway
Parsippany, NJ 07054

Formulation from the Label:

<u>Active Ingredient(s)</u>	<u>% by wt.</u>
Alkyl (50% C14, 40% C12, 10% C16)	
Dimethyl Benzyl Ammonium Chloride.....	0.26%
<u>Other Ingredients</u>	99.74 %
Total.....	100.00 %

I BACKGROUND

The product, Sting (EPA File Symbol 777-RRU), is a new product. The applicant requested to register the product for use as a disinfectant (bactericide, virucide), sanitizer, fungistat, and deodorizer on hard, non-porous surfaces in household, institutional, commercial, food service, animal care, and hospital or medical environments. Marketing claims on the proposed label state that the product is a "one-step" disinfectant and sanitizer. Studies were conducted at Reckitt Benckiser Inc., Microbiology Laboratory, located at One Philips Parkway, in Montvale, NJ 07645; and ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121.

This data package contained a letter from the applicant to EPA (dated May 27, 2009), EPA Form 8570-35 (Data Matrix), twenty-eight studies (MRID 477640-16 through 477640-36 and 477640-44 through 477640-50), Statements of No Data Confidentiality Claims for all twenty-eight studies, and the proposed label.

Note: The laboratory reports describe studies conducted for the product, Formula 1435-014. A letter from the applicant to EPA (dated May 27, 2009) states that the product, Formula 1435-014, is the basic formulation of the product, Sting, which is the product for which registration is sought.

II USE DIRECTIONS

The product is designed for disinfecting and sanitizing hard, non-porous surfaces, including: appliance exteriors, bathtubs, changing tables, computer keyboards, computer mice, counters, cribs, diaper pails, doorknobs, exhaust fans, faucets, fixtures, floors, garbage cans, laundry baskets, light switches, non-wood cabinets, non-wood furniture, outdoor furniture, rails, remote controls, showers, sinks, sports equipment, tables, telephones, toilet exteriors, toilet seats, toys, urinal exteriors, vanity tops, and walls. The proposed label states that the product may be used on hard, non-porous surfaces including: aluminum, brass, ceramic, chrome, Corian, crystal, enamel, fiberglass fixtures, Formica, glazed ceramic, glazed porcelain, granite, laminate, linoleum, metal, plastic, Plexiglas, stainless steel, terra cotta, tin, and vinyl. Directions on the proposed label provided the following information regarding use of the product:

As a disinfectant: Pre-clean surface. Use enough fresh wipes to thoroughly wet surface. Allow to remain wet for 10 minutes. Allow surface to air dry. Toss dirty wipe away.

As a sanitizer: Pre-clean surface. Use enough fresh wipes to thoroughly wet surface. Allow to remain wet for 30 seconds. Allow surface to air dry. Toss dirty wipe away.

To prevent the growth of mold and mildew: Pre-clean surface. Wipe surface and allow to remain wet for 10 minutes. Allow surface to air dry. Repeat applications in weekly intervals or when mold and mildew growth appears.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

Antimicrobial Products for Use on Hard Surfaces Using Pre-saturated or Impregnated Towelettes

Towelette products represent a unique combination of antimicrobial chemical and applicator, pre-packaged as a unit in fixed proportions. As such, the complete product, as offered for sale, should be tested according to the directions for use to ensure the product's effectiveness in treating hard surfaces. The standard test methods available for hard surface disinfectants and sanitizers, if followed exactly, would not closely simulate the way a towelette product is used. Agency guidelines recommend that a simulated-use test be conducted by modifying the standard test methods. Agency guidelines further recommend that instead of spraying the inoculated surface of the carrier, the product should be tested by wiping the surface of the carrier with the saturated towelette, and then subculturing the slides after a specified holding time. Performance standards of the standard test methods must be met. These Agency standards are presented in EPA Pesticide Assessment Guidelines, Subdivision G, §91-2(h), Pre-saturated or impregnated towelettes; and the April 12, 2001 EPA Memorandum, Draft Interim Guidance for Non-Residual Sanitization of Hard Inanimate Food Contact Surfaces Using Pre-Saturated Towelettes.

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 as Fungicides (Against Pathogenic Fungi, Using the AOAC Germicidal Spray Products as Disinfectants Method)

The effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data using an appropriate test. The AOAC Germicidal Spray Products as Disinfectants Method contains procedures for testing fungicidal activity. 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.

Note: As an interim policy, EPA is accepting studies with dried carrier counts that are at least 10^4 for *Trichophyton mentagrophytes*, *Aspergillus niger*, and *Candida albicans*. EPA recognizes laboratories are experiencing problems in maintaining dried carrier counts at the 10^6 level. This interim policy will be in effect until EPA determines that the laboratories are able to achieve consistent carrier counts at the 10^6 level.

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.

Fungistats

The effectiveness of fungistat may be supported by efficacy data derived using the EPA Hard Surface Mildew Fungistatic Test Method. All ten treated tiles must be free of fungal growth after 7 days. To be considered a valid test, untreated control tiles must be at least 50% covered with fungal growth after 7 days. Agency standards are presented in the Pesticide Assessment Guidelines, Subdivision G, Section 93-30, Product Performance, November 1982.

Sanitizers (For Non-Food Contact Surfaces)

The effectiveness of sanitizers for non-food contact surfaces must be supported by data that show that the product will substantially reduce the numbers of test bacteria on a treated surface. The test surface(s) should represent the type(s) of surfaces recommended for treatment on the label, i.e., porous or non-porous. Products that are represented as "one-step sanitizers" should be tested with an appropriate organic soil load, such as 5 percent serum. Tests should be performed with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old against *Staphylococcus aureus* (ATCC 6538) and either *Klebsiella pneumoniae* (aberrant, ATCC 4352) or *Enterobacter aerogenes* (ATCC 13048 or 15038). Results must show a bacterial reduction of at least 99.9 percent over the parallel control within 5 minutes.

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.

IV COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. MRID 477640-16 “Hospital Type Disinfectant Efficacy Testing In The Presence of Organic Soil for Single Use Premoistened Towelettes,” Test Organism: *Staphylococcus aureus* (ATCC 6538), for Formula 1435-014, by Kyle T. Smith. Study conducted at Reckitt Benckiser Inc. Study completion date – May 1, 2009. Amended final report date – May 26, 2009. Original Protocol date—October 27, 2008. Master Schedule No. 2008-0213.

This study was conducted against *Staphylococcus aureus* (ATCC 6538). Three lots (Lot Nos. 1453-081A, 1453-081B, and 1453-082) of the product, Formula 1435-014, were tested using the AOAC Germicidal Spray Products as Disinfectants Method (modified) as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. Each of the product lots tested was at least 60 days old at the time of testing. The product was received ready-to-use, as a pre-saturated towelette. A culture of the challenge microorganism was prepared in accordance with the published AOAC method, with the following exceptions: (1) the final culture was incubated for 48±2; and (2) the final culture was filtered through a coarse filtration medium after incubation (which differs from the AOAC method specification to shake the final culture and allow the culture to stand for 10 minutes). Horse serum was added to the inoculum to achieve a 5% organic soil load. Sixty (60) glass slide carriers (20 mm x 25 mm) per product lot were inoculated with 0.01 mL of a 48±2 hour old suspension of test organism. The culture was spread evenly over each carrier surface. The carriers were dried for 40-42 minutes at 32.6-36.8°C (which differs from the AOAC method specification of 30-40 minutes at 37°C). Each carrier was wiped back and forth twice, for a total of four passes. One towelette was used to treat 10 carriers. The area of the towelette used for wiping was rotated so as to expose a maximum amount of its surface during wiping. The carriers were allowed to remain wet for 10 minutes at 22.7-23.2°C. Following the exposure period, individual carriers were transferred to 20 mL of Letheen Broth to neutralize. [It is unknown whether tubes containing neutralizer were shaken thoroughly after addition of the carriers as specified in the AOAC method.] All subcultures were incubated for at least 67 hours at 32.7-34.7°C (which differs from the AOAC method specification of 48 hours at 37°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for inoculum count, dried recovery count, test system verification (i.e., purity and identity), sterility, and neutralizer efficacy.

Note: Testing was conducted according to Good Laboratory Practice (GLP) standards with the following exception: “The stability of the test substance under the conditions of the test site was not known at the time of the efficacy study. This deviation did not affect the integrity of the study because the 2-year stability at 25°C and the 3-month accelerated stability at 40°C were initiated prior to the start of the efficacy study.”

Note: The initial laboratory report was amended to identify the product lots that were at least 60 days old at the time of testing, and add a protocol deviation.

Note: Protocol deviations/amendments reported in the study were documented.

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable. Note that confirmatory data were provided in the study assigned MRID

477640-17; coarse filtration (an in-house methodology) of the cultures was not used for the confirmation study.

2. MRID 477640-17 “Disinfectant Efficacy Testing In The Presence of Organic Soil for Single Use Premoistened Towelettes,” Test Organism: *Staphylococcus aureus* (ATCC 6538), for Formula 1435-014, by Kyle T. Smith. Study conducted at Reckitt Benckiser Inc. Study completion date – May 1, 2009. Amended final report date – May 26, 2009. Master Schedule No. 2009-0001.

This confirmatory study was conducted against *Staphylococcus aureus* (ATCC 6538). Three lots (Lot Nos. 1453-081A, 1453-081B, and 1453-082) of the product, Formula 1435-014, were tested using the AOAC Germicidal Spray Products as Disinfectants Method (modified) as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. Each of the product lots tested was at least 60 days old at the time of testing. The product was received ready-to-use, as a pre-saturated towelette. A culture of the challenge microorganism was prepared in accordance with the published AOAC method, with the following exception: the culture was incubated for 48±2 hours. Horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass slide carriers (20 mm x 25 mm) per product lot were inoculated with 0.01 mL of a 48±2 hour old suspension of test organism. The culture was spread evenly over each carrier surface. The carriers were dried for 40 minutes at 34.3-34.9°C (which differs from the AOAC method specification of 30-40 minutes at 37°C). Each carrier was wiped back and forth twice, for a total of four passes. One towelette was used to treat 10 carriers. The area of the towelette used for wiping was rotated so as to expose a maximum amount of its surface during wiping. The carriers were allowed to remain wet for 10 minutes at 21.7-21.9°C. Following the exposure period, individual carriers were transferred to 20 mL of Lethen Broth to neutralize. [It is unknown whether tubes containing neutralizer were shaken thoroughly after addition of the carriers as specified in the AOAC method.] All subcultures were incubated for ~65 hours at 33.3-35.2°C (which differs from the AOAC method specification of 48 hours at 37°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for inoculum count, dried recovery count, test system verification (i.e., purity and identity), viability, sterility, and neutralizer efficacy.

Note: Testing was conducted according to Good Laboratory Practice (GLP) standards with the following exception: “The stability of the test substance under the conditions of the test site was not known at the time of the efficacy study. This deviation did not affect the integrity of the study because the 2-year stability at 25°C and the 3-month accelerated stability at 40°C were initiated prior to the start of the efficacy study.”

Note: The initial laboratory report was amended to identify the product lots that were at least 60 days old at the time of testing, add a protocol deviation, and add the name of the test system to the viability control result table (omitting any test system name abbreviations).

Note: Protocol deviations/amendments reported in the study were documented.

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable.

3. MRID 477640-18 “Hospital Type Disinfectant Efficacy Testing In The Presence of Organic Soil for Single Use Premoistened Towelettes,” Test Organism: *Pseudomonas aeruginosa* (ATCC 15442), for Formula 1435-014, by Kyle T. Smith. Study conducted at Reckitt Benckiser Inc. Study completion date – April 17, 2009. Amended final report date – May 26, 2009. Master Schedule No. 2008-0227.

This study was conducted against *Pseudomonas aeruginosa* (ATCC 15442). Three lots (Lot Nos. 1453-081A, 1453-081B, and 1453-082) of the product, Formula 1435-014, were tested using the AOAC Germicidal Spray Products as Disinfectants Method (modified) as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. Each of the product lots tested was at least 60 days old at the time of testing. The product was received ready-to-use, as a pre-saturated towelette. A culture of the challenge microorganism was prepared in accordance with the published AOAC method, with the following exception: the final culture was incubated for 48±2 hours. Horse serum was added to the inoculum to achieve a 5% organic soil load. Sixty (60) glass slide carriers (20 mm x 25 mm) per product lot were inoculated with 0.01 mL of a 48±2 hour old suspension of test organism. The culture was spread evenly over each carrier surface. The carriers were dried for 40-42 minutes at 32.2-37.5°C (which differs from the AOAC method specification of 30-40 minutes at 37°C). Each carrier was wiped back and forth twice, for a total of four passes. One towelette was used to treat 10 carriers. The area of the towelette used for wiping was rotated so as to expose a maximum amount of its surface during wiping. The carriers were allowed to remain wet for 10 minutes at 22.5-23.0°C. Following the exposure period, individual carriers were transferred to 20 mL of Lethen Broth to neutralize. [It is unknown whether tubes containing neutralizer were shaken thoroughly after addition of the carriers as specified in the AOAC method.] All subcultures were incubated for ~68 hours at 33.1-35.0°C (which differs from the AOAC method specification of 48 hours at 37°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for inoculum count, dried recovery count, test system verification (i.e., purity and identity), viability, sterility, and neutralizer efficacy.

Note: Testing was conducted according to Good Laboratory Practice (GLP) standards with the following exception: “The stability of the test substance under the conditions of the test site was not known at the time of the efficacy study. This deviation did not affect the integrity of the study because the 2-year stability at 25°C and the 3-month accelerated stability at 40°C were initiated prior to the start of the efficacy study.”

Note: The initial laboratory report was amended to identify the product lots that were at least 60 days old at the time of testing, add a protocol deviation, and add the name of the test system to the viability control result table (omitting any test system name abbreviations).

Note: Protocol deviations/amendments reported in the study were documented.

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable.

4. MRID 477640-19 “Hospital Type Disinfectant Efficacy Testing In The Presence of Organic Soil for Single Use Premoistened Towelettes,” Test Organism: *Salmonella enterica* (ATCC 10708), for Formula 1435-014, by Kyle T. Smith. Study conducted at Reckitt Benckiser Inc. Study completion date – April 17, 2009. Amended final report date – May 26, 2009. Master Schedule No. 2008-0232.

This study was conducted against *Salmonella enterica* (ATCC 10708). Three lots (Lot Nos. 1453-081A, 1453-081B, and 1453-082) of the product, Formula 1435-014, were tested using the AOAC Germicidal Spray Products as Disinfectants Method (modified) as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. Each of the product lots tested was at least 60 days old at the time of testing. The product was received ready-to-use, as a pre-saturated towelette. A culture of the challenge microorganism was prepared in accordance with the published AOAC method, with the following exception: the culture was incubated for 48±2 hours. Horse serum was added to the inoculum to achieve a 5% organic soil load. Sixty (60) glass slide carriers (20 mm x 25 mm) per product lot were inoculated with 0.01 mL of a 48±2 hour old suspension of test organism. The culture was spread evenly over each carrier surface. The carriers were dried for 40–42 minutes at 34.1–34.6°C (which differs from the AOAC method specification of 30–40 minutes at 37°C). Each carrier was wiped back and forth twice, for a total of four passes. One towelette was used to treat 10 carriers. The area of the towelette used for wiping was rotated so as to expose a maximum amount of its surface during wiping. The carriers were allowed to remain wet for 10 minutes at 24.1–25.7°C. Following the exposure period, individual carriers were transferred to 20 mL of Lethen Broth to neutralize. [It is unknown whether tubes containing neutralizer were shaken thoroughly after addition of the carriers as specified in the AOAC method.] All subcultures were incubated for at least 48 hours at 33.6–35.4°C (which differs from the AOAC method specification of 48 hours at 37°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for inoculum count, dried recovery count, test system verification (i.e., purity and identity), viability, sterility, and neutralizer efficacy.

Note: Testing was conducted according to Good Laboratory Practice (GLP) standards with the following exception: “The stability of the test substance under the conditions of the test site was not known at the time of the efficacy study. This deviation did not affect the integrity of the study because the 2-year stability at 25°C and the 3-month accelerated stability at 40°C were initiated prior to the start of the efficacy study.”

Note: The laboratory reported a failed study set up on December 4, 2008. In the study, one or more of the dried recovery control replicates for each product lot did not achieve a recovery level of $\geq 10^4$ organisms per carrier. In addition, four test replicates for one product lot (i.e., Lot No. 1453-081B) contained growth that was concluded to be a contaminant. The laboratory did not accept the assay. These data were not used to evaluate efficacy of the product. Testing was repeated on January 14, 2009. See page 15 and Appendix B of the laboratory report.

Note: The initial laboratory report was amended to identify the product lots that were at least 60 days old at the time of testing, add a protocol deviation, and add the name of the test system to the viability control result table (omitting any test system name abbreviations).

Note: Protocol deviations/amendments reported in the study were documented.

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable.

5. MRID 477640-20 “Virucidal Efficacy of Pre-Saturated Towelettes for Hard Surface Disinfection, U.S. EPA and Health Canada Submission,” Virus: Human immunodeficiency virus type 1, for Formula 1435-014, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – December 19, 2008. Project Number A07033.

This study was conducted against Human immunodeficiency virus type 1 (Strain HTLV-III_B; obtained from Advanced Biotechnologies, Inc., Columbia, MD), using MT-2 cells (human T-cell leukemia cells; obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH; maintained in-house) as the host system. Three lots (Lot Nos. 1453-081A, 1453-081B, and 1453-082) of the product, Formula 1435-014, were tested according to ATS Labs Protocol No. REK01101308.HIV (copy provided). The product was received ready-to-use, as a pre-saturated towelette. The stock virus culture was adjusted to contain 50% human whole blood as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the undersides of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 22.0°C at 12.8% relative humidity. Five replicates per product lot were tested. Using sterile gloves, each towelette was folded in half lengthwise two times. The far end was folded up once and the outside edges were pulled upward forming a “U” shape. Each carrier was wiped back and forth twice with an individual saturated towelette, for a total of four passes. The carriers were allowed to remain wet for 30 seconds at 20.0°C. Following exposure, 2.00 mL of test medium was added to each Petri dish, and the dishes were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in RPMI-1640 supplemented with 10% (v/v) heat-inactivated fetal bovine serum, 2.0 mM L-glutamine, and 50 µg/mL gentamicin. Following titration, the 10⁻² dilution of each replicate was passed through an additional individual Sephadex column to further aid in removing the cytotoxic effects of the product to the host system. MT-2 cells in multi-well culture dishes were inoculated in quadruplicate with 0.2 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO₂. The cultures were scored periodically for 8 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were documented.

6. MRID 477640-21 “Virucidal Efficacy of Pre-Saturated Towelettes for Hard Surface Disinfection, U.S. EPA and Health Canada Submission,” Virus: Respiratory syncytial virus, for Formula 1435-014, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – January 13, 2009. Project Number A07100.

This study was conducted against Respiratory syncytial virus (Strain Long; ATCC VR-26), using Hep-2 cells (human larynx carcinoma cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division, Minnetonka, MN; maintained in-house) as the host system. Three lots (Lot Nos. 1453-081A, 1453-081B, and 1453-082) of the product, Formula 1435-014, were tested according to ATS Labs Protocol No. REK01101608.RSV (copy provided). The product was received ready-to-use, as a pre-saturated towelette. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the undersides of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 52% relative humidity. Five replicates per product lot were tested. Using sterile gloves, each towelette was folded in half lengthwise two times. The far end was folded up once and the outside edges were pulled upward forming a “U” shape. Each carrier was wiped back and forth twice with an individual saturated towelette, for a total of four passes. The carriers were allowed to remain wet for 10 minutes at 20.0°C. Following exposure, 2.00 mL of test medium was added to each Petri dish, and the dishes were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Eagle's Minimum Essential Medium supplemented with 2% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL Fungizone, 10 µg/mL vancomycin, 2 mM L-glutamine, and 10 mM HEPES. Hep-2 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO₂. The cultures were scored periodically for 10 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

7. MRID 477640-22 “Virucidal Efficacy of Pre-Saturated Towelettes for Hard Surface Disinfection,” Virus: Herpes simplex virus type 1, for Formula 1435-014, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – February 13, 2009. Project Number A07246.

This study was conducted against Herpes simplex virus type 1 (Strain F(1); ATCC VR-733), using RK cells (rabbit kidney cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division, Minneapolis, MN; maintained in-house) as the host system. Two lots (Lot Nos. 1453-081A and 1453-081B) of the product, Formula 1435-014, were tested according to ATS Labs Protocol No. REK01011309.HSV1 (copy provided). The product was received ready-to-use, as a pre-saturated towelette. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the undersides of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 50% relative humidity. Using sterile gloves, each towelette was folded in half lengthwise two times. The far end was folded up once and the outside edges were pulled upward forming a “U” shape. Each carrier was wiped back and forth twice with an individual

saturated towelette, for a total of four passes. The carriers were allowed to remain wet for 10 minutes at 20.0°C. Following exposure, 2.00 mL of test medium was added to each Petri dish, and the dishes were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 5% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. RK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO₂. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

8. MRID 477640-23 "Virucidal Efficacy of Pre-Saturated Towelettes for Hard Surface Disinfection," Virus: Influenza A virus (Strain A/New Caledonia/20/99), for Formula 1435-014, by Mary J. Miller. Study conducted at ATS Labs. Study completion date -- February 26, 2009. Project Number A07245.

This study was conducted against Influenza A virus (Strain A/New Caledonia/20/99; obtained from the Centers for Disease Control), using RMK cells (Rhesus monkey kidney cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division, Minneapolis, MN; maintained in-house) as the host system. Two lots (Lot Nos. 1453-081A and 1453-081B) of the product, Formula 1435-014, were tested according to ATS Labs Protocol No. REK01010809.FLUA.2 (copy provided). The product was received ready-to-use, as a pre-saturated towelette. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the undersides of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 50% relative humidity. Using sterile gloves, each towelette was folded in half lengthwise two times. The far end was folded up once and the outside edges were pulled upward forming a "U" shape. Each carrier was wiped back and forth twice with an individual saturated towelette, for a total of four passes. The carriers were allowed to remain wet for 10 minutes at 20.0°C. Following exposure, 2.00 mL of test medium was added to each Petri dish, and the dishes were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 1% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO₂. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note: The laboratory reported a failed study set up on January 22, 2009. In the study, test virus infectivity was observed in the cytotoxicity control for Lot# 1453-081B. The study was terminated after the initial reading of the cell cultures. The laboratory did not accept the assay. These data were not used to evaluate efficacy of the product. Testing

was repeated on February 10, 2009. See page 8 and Attachment I of the laboratory report.

Note: Protocol deviations/amendments reported in the study were documented.

9. MRID 477640-24 “Virucidal Efficacy of Pre-Saturated Towelettes for Hard Surface Disinfection,” Virus: Avian influenza A virus, for Formula 1435-014, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – March 4, 2009. Project Number A07398.

This study was conducted against Avian influenza A (H5N1) virus (Strain VNH5N1-PR8/CDC-RG CDC#2006719965; obtained from the Centers for Disease Control, Atlanta, GA), using RMK cells (Rhesus monkey kidney cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division, Minneapolis, MD; maintained in-house) as the host system. Two lots (Lot Nos. 1453-081A and 1453-081B) of the product, Formula 1435-014, were tested according to ATS Labs Protocol No. REK01020909.AFLU (copy provided). The product was received ready-to-use, as a pre-saturated towelette. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the undersides of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 22.0°C at 16.2% relative humidity. Using sterile gloves, each towelette was folded in half lengthwise two times. The far end was folded up once and the outside edges were pulled upward forming a “U” shape. Each carrier was wiped back and forth twice with an individual saturated towelette, for a total of four passes. The carriers were allowed to remain wet for 10 minutes at 22.0°C. Following exposure, 2.00 mL of test medium was added to each Petri dish, and the dishes were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 1% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO₂. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

10. MRID 477640-25 “Virucidal Efficacy of Pre-Saturated Towelettes for Hard Surface Disinfection,” Virus: Human coronavirus, for Formula 1435-014, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – April 2, 2009. Project Number A07444.

This study was conducted against Human coronavirus (Strain 229E; ATCC VR-740), using WI-38 cells (ATCC CCL-75; propagated in-house) as the host system. Two lots (Lot Nos. 1453-081A and 1453-081B) of the product, Formula 1435-014, were tested according to ATS Labs Protocol No. REK01022609.COR (copy provided). The product was received ready-to-use, as a pre-saturated towelette. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the undersides of

separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 50% relative humidity. Using sterile gloves, each towelette was folded in half lengthwise two times, and folded up widthwise once. Then, the sides were pulled upward forming a "U" shape. Each carrier was wiped back and forth twice with an individual saturated towelette, for a total of four passes. The carriers were allowed to remain wet for 10 minutes at 20.0°C. Following exposure, 2.00 mL of test medium was added to each Petri dish, and the dishes were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 2% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. WI-38 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO₂. The cultures were scored periodically for 10 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

11. MRID 477640-26 "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (Modification for Pre-Saturated Towelette Product Application)," Test Organism: *Streptococcus pyogenes* (ATCC 19615), for Formula 1435-014, by Becky Lien. Study conducted at ATS Labs. Study completion date – December 19, 2008. Project Number A07042.

This study was conducted against *Streptococcus pyogenes* (ATCC 19615). Two lots (Lot Nos. 1453-081A and 1453-081B) of the product, Formula 1435-014, were tested. The laboratory report referenced the Sanitizer Test from DIS/TSS-10 and the Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (ASTM E1153). The product was received ready-to-use, as a pre-saturated towelette. Fetal bovine serum was added to the inoculum to achieve a 5% organic soil load. Five sterile glass carriers per product lot were inoculated with 20.0 µL of a 48±4-hour old suspension of test organism (which differs from the 18-24 hour old suspension specified in DIS/TSS-10). The inoculum was spread to within 1/8 inch of the edges of each carrier. The carriers were dried for 30 minutes at 26.9°C at 64% relative humidity (which differs from the 40-minute drying time specified in DIS/TSS-10). Each carrier was wiped back and forth twice with an individual saturated towelette, for a total of four passes. One towelette was used to treat five carriers. Each carrier remained in contact with the product for 30 seconds at 20°C. Following exposure, each carrier was placed into a sterile vessel containing 20 mL of Lethen Broth with 0.07% Lecithin and 0.5% Tween 80. The vessels were rotated vigorously on an even plane for ~50 rotations to suspend the surviving organisms. Within 30 minutes of the addition of the neutralizer, 1.00 mL and 0.10 mL of the 10⁰ dilutions were plated in duplicate on tryptic soy agar with 5% sheep's blood. All subcultures were incubated for ~45.5 hours at 35-37°C in CO₂, and then stored for 1 day at 2-8°C prior to examination. Following incubation and storage, the subcultures were visually enumerated. Controls included those for inoculum count, carrier quantitation, purity, sterility, and neutralization confirmation.

Note: Protocol deviations/amendments reported in the study were documented.

12. MRID 477640-27 “Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (Modification for Pre-Saturated Towelette Product Application),” Test Organism: *Listeria monocytogenes* (ATCC 19111), for Formula 1435-014, by Anne Stemper. Study conducted at ATS Labs. Study completion date – January 22, 2009. Project Number A07028.

This study was conducted against *Listeria monocytogenes* (ATCC 19111). Two lots (Lot Nos. 1453-081A and 1453-081B) of the product, Formula 1435-014, were tested. The laboratory report referenced the Sanitizer Test from DIS/TSS-10 and the Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (ASTM E1153). The product was received ready-to-use, as a pre-saturated towelette. Fetal bovine serum was added to the inoculum to achieve a 5% organic soil load. Five sterile glass carriers (1 inch x 1 inch) per product lot were inoculated with 20.0 µL of a 48±4-hour old suspension of test organism (which differs from the 18-24 hour old suspension specified in DIS/TSS-10). The inoculum was spread to within 1/8 inch of the edges of each carrier. The carriers were dried for 20 minutes at 36.0°C at 40% relative humidity (which differs from the 40-minute drying time specified in DIS/TSS-10). Each carrier was wiped back and forth twice with an individual saturated towelette, for a total of four passes. One towelette was used to treat five carriers. Each carrier remained in contact with the product for 30 seconds at 19°C. Following exposure, each carrier was placed into a sterile vessel containing 20 mL of Lethen Broth with 0.07% Lecithin and 0.5% Tween 80. The vessels were rotated vigorously on an even plane for ~50 rotations to suspend the surviving organisms. Within 30 minutes of the addition of the neutralizer, 1.00 mL of the 10⁰ and 10⁻¹ dilutions were plated in duplicate on tryptic soy agar with 5% sheep's blood. All subcultures were incubated for 48±4 hours at 35-37°C, and then stored for 1 day at 2-8°C prior to examination. Following incubation and storage, the subcultures were visually enumerated. Controls included those for inoculum count, carrier quantitation, purity, sterility, and neutralization confirmation.

Note: Protocol deviations/amendments reported in the study were documented.

13. MRID 477640-28 “Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (Modification for Pre-Saturated Towelette Product Application),” Test Organism: *Campylobacter jejuni* (ATCC 29428), for Formula 1435-014, by Anne Stemper. Study conducted at ATS Labs. Study completion date – January 16, 2009. Project Number A07027.

This study was conducted against *Campylobacter jejuni* (ATCC 29428). Two lots (Lot Nos. 1453-081A and 1453-081B) of the product, Formula 1435-014, were tested. The laboratory report referenced the Sanitizer Test from DIS/TSS-10 and the Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (ASTM E1153). The product was received ready-to-use, as a pre-saturated towelette. Fetal bovine serum was added to the inoculum to achieve a 5% organic soil load. Five sterile glass carriers (1 inch x 1 inch) per product lot were inoculated with 20.0 µL of a 4-day old suspension of test organism (which differs from the 18-24 hour old suspension specified in DIS/TSS-10). The inoculum was spread to within 1/8 inch of the

edges of each carrier. The carriers were dried for 20 minutes at 26.3°C at 65% relative humidity (which differs from the 40-minute drying time specified in DIS/TSS-10). Each carrier was wiped back and forth twice with an individual saturated towelette, for a total of four passes. One towelette was used to treat five carriers. The towelette was rotated in between carriers to expose the maximum amount of product to the carrier surface. Each carrier remained in contact with the product for 30 seconds at 22°C. Following exposure, each carrier was placed into a sterile vessel containing 20 mL of Lethen Broth with 0.07% Lecithin and 0.5% Tween 80. The vessels were rotated vigorously on an even plane for ~50 rotations to suspend the surviving organisms. Within 30 minutes of the addition of the neutralizer, 1.00 mL and 0.10 mL of the 10⁰ dilutions were plated in duplicate on tryptic soy agar with 5% sheep's blood. All subcultures were incubated for 5 days at 35-37°C in CO₂, and then stored for 3 days at 2-8°C prior to examination. Following incubation and storage, the subcultures were visually enumerated. Controls included those for inoculum count, carrier quantitation, purity, sterility, and neutralization confirmation.

Note: Protocol deviations/amendments reported in the study were documented.

14. MRID 477640-29 “Sanitization Study: Quantitative Reduction of Bacteria on a Non-Food Contact Surface,” Test Organism: *Salmonella enterica* (ATCC 10708), for Formula 1435-014, by Kyle T. Smith. Study conducted at Reckitt Benckiser Inc. Study completion date – April 17, 2009. First amended final report date – May 8, 2009. Second amended final report date – May 26, 2009. Master Schedule No. 2008-0220.

This study was conducted against *Salmonella enterica* (ATCC 10708). Three lots (Lot Nos. 1453-081A, 1453-081B, and 1453-082) of the product, Formula 1435-014, were tested. The laboratory report referenced the Sanitizer Test from DIS/TSS-10 and the Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (ASTM E1153; modified). The product was received ready-to-use, as a pre-saturated towelette. Horse serum was added to the inoculum to achieve a 5% organic soil load. Five sterile glass slide carriers (20 mm x 25 mm, which is approximately equal to the one square inch area specified in DIS/TSS-10) per product lot were inoculated with 0.02 mL of a 24±2 hour old suspension of test organism (which differs from the 18-24 hour old suspension specified in DIS/TSS-10). The inoculum was spread evenly over each carrier surface. The carriers were dried for 40 minutes at 33.2-34.7°C (which complies with the 40-minute drying time specified in DIS/TSS-10). Each carrier was wiped back and forth twice, for a total of four passes. One towelette was used to treat five carriers. The area of the towelette used for wiping was rotated so as to expose a maximum amount of its surface during wiping. Each carrier remained in contact with the product for 30 seconds at 22.3-22.5°C. Following exposure, each carrier was transferred to a tube containing 20 mL of Lethen Broth to neutralize. The tubes were “vortex” mixed to suspend the surviving organisms. Either 1 mL or 0.1 mL aliquots of selected dilutions were plated on tryptic soy agar. All subcultures were incubated for at least 48 hours at 32.8-34.5°C. Following incubation, the colonies were counted. Controls included a non-active control and those for inoculum count, dried recovery count, test system verification (i.e., purity and identity), viability, sterility, and neutralizer efficacy.

Note: Testing was conducted according to Good Laboratory Practice (GLP) standards with two exceptions: (1) "The Non-Active control reference substance, 0.01% Triton X-100, Reagent No. 4662, was prepared in the laboratory for use in this sanitization assay. The solution was prepared using a stock of Triton X-100 (CAS 9002-93-1), and sterile deionized water. The sample was kept in the GLP locked storage cabinet, and its usage was tracked via a Chain of Custody test substance form. The identity, strength, purity, composition, solubility and stability were not determined for this sample." (2) "The stability of the test substance under the conditions of the test site was not known at the time of the efficacy study. This deviation did not affect the integrity of the study because the 2-year stability at 25°C and the 3-month accelerated stability at 40°C were initiated prior to the start of the efficacy study."

Note: The initial laboratory report was amended to add an additional compliance deviation to the GLP compliance statement, add information regarding the non-active control substance, and correct the pagination of the report.

Note: The initial laboratory report was amended a second time to identify the product lots that were at least 60 days old at the time of testing, add a protocol deviation, and properly shade a blank box in the result table.

Note: Protocol deviations/amendments reported in the study were documented.

15. MRID 477640-30 "Sanitization Study: Quantitative Reduction of Bacteria on a Non-Food Contact Surface," Test Organism: Methicillin Resistant *Staphylococcus aureus* (MRSA) (ATCC BAA-1556), for Formula 1435-014, by Kyle T. Smith. Study conducted at Reckitt Benckiser Inc. Study completion date – May 1, 2009. Amended final report date – May 26, 2009. Master Schedule No. 2008-0229.

This study was conducted against Methicillin Resistant *Staphylococcus aureus* (MRSA) (ATCC BAA-1556; USA 300 clone of Community Acquired MRSA). Three lots (Lot Nos. 1453-081A, 1453-081B, and 1453-082) of the product, Formula 1435-014, were tested. The laboratory report referenced the Sanitizer Test from DIS/TSS-10. The product was received ready-to-use, as a pre-saturated towelette. Horse serum was added to the inoculum to achieve a 5% organic soil load. Five sterile glass slide carriers (20 mm x 25 mm, which is approximately equal to the one square inch area specified in DIS/TSS-10) per product lot were inoculated with 0.01 mL of a 24±2 hour old suspension of test organism (which differs from the 18-24 hour old suspension specified in DIS/TSS-10). The inoculum was spread evenly over each carrier surface. The carriers were dried for 40 minutes at 32.2-32.7°C (which complies with the 40-minute drying time specified in DIS/TSS-10). Each carrier was wiped back and forth twice, for a total of four passes. One towelette was used to treat five carriers. The area of the towelette used for wiping was rotated so as to expose a maximum amount of its surface during wiping. Each carrier remained in contact with the product for 30 seconds at 20.1-20.3°C. Following exposure, each carrier was transferred to a tube containing 20 mL of Lethen Broth to neutralize. The tubes were "vortex" mixed to suspend the surviving organisms. Either 1 mL or 0.1 mL aliquots of selected dilutions were plated on tryptic soy agar. All subcultures were incubated for at least 46 hours at 32.2-35.2°C. Following incubation, the colonies were counted. Controls included a non-active control and those for inoculum count, dried recovery count, test system verification (i.e., purity and identity), viability, sterility, neutralizer efficacy, and antibiotic resistance.

Note: Testing was conducted according to Good Laboratory Practice (GLP) standards with two exceptions: (1) "On 02/19/2009, the 0.5 McFarland Standard used to visually compare the inoculum prepared for use in the Verification of Resistance Profile assay was expired by 2 days. (Expiration Date: 02/17/2009)." (2) "The Non-Active control reference substances, 0.01% Triton X-100, Reagent Numbers 4662 and 4739, were prepared in the laboratory for use in this sanitization assay. Each solution was prepared using a stock of Triton X-100 (CAS 9002-93-1), and sterile deionized water. Each sample was kept in the GLP locked storage cabinet, and its usage was tracked via a Chain of Custody test substance form. The identity, strength, purity, composition, solubility and stability were not determined for these samples." (3) "The stability of the test substance under the conditions of the test site was not known at the time of the efficacy study. This deviation did not affect the integrity of the study because the 2-year stability at 25°C and the 3-month accelerated stability at 40°C were initiated prior to the start of the efficacy study."

Note: The laboratory reported a failed study set up on December 3, 2008. In the study, the test organism did not demonstrate an acceptable resistance profile to oxacillin. The laboratory did not accept the assay. These data were not used to evaluate efficacy of the product. Testing was repeated on February 18, 2009 with a new stock of the test organism. See page 20 and Appendix D of the laboratory report.

Note: The initial laboratory report was amended to identify the product lots that were at least 60 days old at the time of testing, add a protocol deviation, add the name of the test system to the viability control result table (omitting any test system name abbreviations), properly format the test system name, properly include the acceptance criteria of the antibiotic resistance control, and define a critical abbreviation.

Note: Antibiotic resistance of Methicillin Resistant *Staphylococcus aureus* (ATCC BAA-1556) was verified on a representative culture. The methodology followed was taken from the instructions provided with the BD BBL™ Sensi-Disc™ Antimicrobial Susceptibility Test Discs used in the assay. *Staphylococcus aureus* (ATCC 25923) was the control organism. The measured zone of inhibition (i.e., 7.46-8.53 mm) confirmed antibiotic resistance of Methicillin Resistant *Staphylococcus aureus* (ATCC BAA-1556) to oxacillin. See pages 16 and 29 of the laboratory report.

Note: Protocol deviations/amendments reported in the study were documented.

16. MRID 477640-31 "Sanitization Study: Quantitative Reduction of Bacteria on a Non-Food Contact Surface," Test Organism: Methicillin Resistant *Staphylococcus aureus* (MRSA) (ATCC 33591), for Formula 1435-014, by Kyle T. Smith. Study conducted at Reckitt Benckiser Inc. Study completion date – May 1, 2009. Amended final report date – May 26, 2009. Master Schedule No. 2008-0230.

This study was conducted against Methicillin Resistant *Staphylococcus aureus* (MRSA) (ATCC 33591; considered a hospital-acquired strain of MRSA). Three lots (Lot Nos. 1453-081A, 1453-081B, and 1453-082) of the product, Formula 1435-014, were tested. The laboratory report referenced the Sanitizer Test from DIS/TSS-10. The product was received ready-to-use, as a pre-saturated towelette. Horse serum was added to the inoculum to achieve a 5% organic soil load. Five sterile glass slide carriers

(20 mm x 25mm, which is approximately equal to the one square inch area specified in DIS/TSS-10) per product lot were inoculated with 0.02 mL of a 24±2 hour old suspension of test organism (which differs from the 18-24 hour old suspension specified in DIS/TSS-10). The inoculum was spread evenly over each carrier surface. The carriers were dried for 40 minutes at 34.5-35.8°C (which complies with the 40-minute drying time specified in DIS/TSS-10). Each carrier was wiped back and forth twice, for a total of four passes. One towelette was used to treat five carriers. The area of the towelette used for wiping was rotated so as to expose a maximum amount of its surface during wiping. Each carrier remained in contact with the product for 30 seconds at 22.9-23.3°C. Following exposure, each carrier was transferred to a tube containing 20 mL of Lethen Broth to neutralize. The tubes were "vortex" mixed to suspend the surviving organisms. Either 1 mL or 0.1 mL aliquots of selected dilutions were plated on tryptic soy agar. All subcultures were incubated for at least 50 hours at 33.7-36.4°C. Following incubation, the colonies were counted. Controls included a non-active control and those for inoculum count, dried recovery count, test system verification (i.e., purity and identity), viability, sterility, neutralizer efficacy, and antibiotic resistance.

Note: Testing was conducted according to Good Laboratory Practice (GLP) standards with the following exceptions: (1) "The Non-Active control reference substance, 0.01% Triton X-100, Reagent No. 4662, was prepared in the laboratory for use in this sanitization assay. The solution was prepared using a stock of Triton X-100 (CAS 9002-93-1), and sterile deionized water. The sample was kept in the GLP locked storage cabinet, and its usage was tracked via a Chain of Custody test substance form. The identity, strength, purity, composition, solubility and stability were not determined for this sample." (2) "The stability of the test substance under the conditions of the test site was not known at the time of the efficacy study. This deviation did not affect the integrity of the study because the 2-year stability at 25°C and the 3-month accelerated stability at 40°C were initiated prior to the start of the efficacy study."

Note: The initial laboratory report was amended to identify the product lots that were at least 60 days old at the time of testing, add a protocol deviation, add the name of the test system to the viability control result table and the inoculum count result table (omitting any test system name abbreviations), properly format the test system name, properly include the acceptance criteria of the antibiotic resistance control, and define a critical abbreviation.

Note: Antibiotic resistance of Methicillin Resistant *Staphylococcus aureus* (ATCC 33591) was verified on a representative culture. The methodology followed was taken from the instructions provided with the BD BBL™ Sensi-Disc™ Antimicrobial Susceptibility Test Discs used in the assay. *Staphylococcus aureus* (ATCC 25923) was the control organism. The measured zone of inhibition (i.e., 0 mm) confirmed antibiotic resistance of Methicillin Resistant *Staphylococcus aureus* (ATCC 33591) to oxacillin. See pages 15 and 26 of the laboratory report.

Note: Protocol deviations/amendments reported in the study were documented.

17. MRID 477640-32 "Sanitization Study: Quantitative Reduction of Bacteria on a Non-Food Contact Surface," Test Organism: Methicillin Resistant *Staphylococcus aureus* (MRSA) (ATCC 33592), for Formula 1435-014, by Kyle T. Smith. Study conducted at Reckitt Benckiser Inc. Study completion date – May 1, 2009. Amended final report date – May 26, 2009. Master Schedule No. 2008-0231.

This study was conducted against Methicillin Resistant *Staphylococcus aureus* (MRSA) (ATCC 33592). Three lots (Lot Nos. 1453-081A, 1453-081B, and 1453-082) of the product, Formula 1435-014, were tested. The laboratory report referenced the Sanitizer Test from DIS/TSS-10. The product was received ready-to-use, as a pre-saturated towelette. Horse serum was added to the inoculum to achieve a 5% organic soil load. Five sterile glass slide carriers (20 mm x 25 mm, which is approximately equal to the one square inch area specified in DIS/TSS-10) per product lot were inoculated with 0.01 mL of a 24±2 hour old suspension of test organism (which differs from the 18-24 hour old suspension specified in DIS/TSS-10). The inoculum was spread evenly over each carrier surface. The carriers were dried for 41 minutes at 34.5-35.8°C (which differs from the 40-minute drying time specified in DIS/TSS-10). Each carrier was wiped back and forth twice, for a total of four passes. One towelette was used to treat five carriers. The area of the towelette used for wiping was rotated so as to expose a maximum amount of its surface during wiping. Each carrier remained in contact with the product for 30 seconds at 22.9-23.2°C. Following exposure, each carrier was transferred to a tube containing 20 mL of Lethen Broth to neutralize. The tubes were "vortex" mixed to suspend the surviving organisms. Either 1 mL or 0.1 mL aliquots of selected dilutions were plated on tryptic soy agar. All subcultures were incubated for at least 46 hours at 33.7-36.4°C. Following incubation, the colonies were counted. Controls included a non-active control and those for inoculum count, dried recovery count, test system verification (i.e., purity and identity), viability, sterility, neutralizer efficacy, and antibiotic resistance.

Note: Testing was conducted according to Good Laboratory Practice (GLP) standards with the following exceptions: (1) "The Non-Active control reference substance, 0.01% Triton X-100, Reagent No. 4662, was prepared in the laboratory for use in this sanitization assay. The solution was prepared using a stock of Triton X-100 (CAS 9002-93-1), and sterile deionized water. The sample was kept in the GLP locked storage cabinet, and its usage was tracked via a Chain of Custody test substance form. The identity, strength, purity, composition, solubility and stability were not determined for this sample." (2) "The stability of the test substance under the conditions of the test site was not known at the time of the efficacy study. This deviation did not affect the integrity of the study because the 2-year stability at 25°C and the 3-month accelerated stability at 40°C were initiated prior to the start of the efficacy study."

Note: The initial laboratory report was amended to identify the product lots that were at least 60 days old at the time of testing, add a protocol deviation, add the name of the test system to the viability control result table and the inoculum count result table (omitting any test system name abbreviations), properly format the test system name, properly include the acceptance criteria of the antibiotic resistance control, and define a critical abbreviation.

Note: Antibiotic resistance of Methicillin Resistant *Staphylococcus aureus* (ATCC 33592) was verified on a representative culture. The methodology followed was taken

from the instructions provided with the BD BBL™ Sensi-Disc™ Antimicrobial Susceptibility Test Discs used in the assay. *Staphylococcus aureus* (ATCC 25923) was the control organism. The measured zone of inhibition (i.e., 0 mm) confirmed antibiotic resistance of Methicillin Resistant *Staphylococcus aureus* (ATCC 33592) to oxacillin. See pages 15 and 26 of the laboratory report.

Note: Protocol deviations/amendments reported in the study were documented.

18. MRID 477640-33 “Sanitization Study: Quantitative Reduction of Bacteria on a Non-Food Contact Surface,” Test Organisms: *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048), for Formula 1435-014, by Kyle T. Smith. Study conducted at Reckitt Benckiser Inc. Study completion date – April 17, 2009. First amended final report date – May 8, 2009. Second amended final report date – May 26, 2009. Master Schedule No. 2008-0239.

This study was conducted against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048). Three lots (Lot Nos. 1453-081A, 1453-081B, and 1453-082) of the product, Formula 1435-014, were tested. The laboratory report referenced the Sanitizer Test from DIS/TSS-10. Each of the product lots tested was at least 60 days old at the time of testing. The product was received ready-to-use, as a pre-saturated towelette. Horse serum was added to each inoculum to achieve a 5% organic soil load. Five sterile glass slide carriers (20 mm x 25 mm, which is approximately equal to the one square inch area specified in DIS/TSS-10) per product lot per test organism were inoculated with 0.01-0.03 mL of a 24±2 hour old suspension of test organism (which differs from the 18-24 hour old suspension specified in DIS/TSS-10). The inoculum was spread evenly over each carrier surface. The carriers were dried for 40-41 minutes at 34.4-35.2°C (which differs from the 40-minute drying time specified in DIS/TSS-10). Each carrier was wiped back and forth twice, for a total of four passes. One towelette was used to treat five carriers. The area of the towelette used for wiping was rotated so as to expose a maximum amount of its surface during wiping. Each carrier remained in contact with the product for 30 seconds at 21.6-23.1°C. Following exposure, each carrier was transferred to a tube containing 20 mL of Lethen Broth to neutralize. The tubes were “vortex” mixed to suspend the surviving organisms. Either 1 mL or 0.1 mL aliquots of selected dilutions were plated on tryptic soy agar. All *Staphylococcus aureus* subcultures were incubated for at least 68 hours at 25.1-36.8°C. All *Enterobacter aerogenes* subcultures were incubated for at least 50 hours at 29.8-30.6°C. Following incubation, the colonies were counted. Controls included non-active controls and those for inoculum count, dried recovery count, test system verification (i.e., purity and identity), viability, sterility, and neutralizer efficacy.

Note: Testing was conducted according to Good Laboratory Practice (GLP) standards with the following exception: (1) “The Non-Active control reference substance, 0.01% Triton X-100, Reagent No. 4662, was prepared in the laboratory for use in this sanitization assay. The solution was prepared using a stock of Triton X-100 (CAS 9002-93-1), and sterile deionized water. The sample was kept in the GLP locked storage cabinet, and its usage was tracked via a Chain of Custody test substance form. The identity, strength, purity, composition, solubility and stability were not determined for this sample.” (2) The stability of the test substance under the conditions of the test site was not known at the time of the efficacy study. This deviation did not affect the integrity of

the study because the 2-year stability at 25°C and the 3-month accelerated stability at 40°C were initiated prior to the start of the efficacy study.”

Note: The initial laboratory report was amended to add an additional compliance deviation to the GLP Compliance Statement, add information about the non-active control substance, and correct the pagination.

Note: The initial laboratory report was further amended to identify the product lots that were at least 60 days old at the time of testing, add a protocol deviation, add the name of the test systems to the viability control result table (omitting any test system name abbreviations), and properly shade a blank box in the result table.

Note: Protocol deviations/amendments reported in the study were documented.

19. MRID 477640-34 “Sanitization Study: Quantitative Reduction of Bacteria on a Non-Food Contact Surface,” Test Organism: *Escherichia coli* O157:H7 (ATCC 43888), for Formula 1435-014, by Ann Marie De Luca. Study conducted at Reckitt Benckiser Inc. Study completion date – May 4, 2009. Amended final report date – May 26, 2009. Master Schedule No. 2009-0002.

This study was conducted against *Escherichia coli* O157:H7 (ATCC 43888). Three lots (Lot Nos. 1453-081A, 1453-081B, and 1453-082) of the product, Formula 1435-014, were tested. The laboratory report referenced the Sanitizer Test from DIS/TSS-10. The product was received ready-to-use, as a pre-saturated towelette. Horse serum was added to the inoculum to achieve a 5% organic soil load. Five sterile glass slide carriers (20 mm x 25 mm, which is approximately equal to the one square inch area specified in DIS/TSS-10) per product lot were inoculated with 0.01 mL of a 48±2 hour old suspension of test organism (which differs from the 18-24 hour old suspension specified in DIS/TSS-10). The inoculum was spread evenly over each carrier surface. The carriers were dried for 42 minutes at 34.4-34.9°C (which differs from the 40-minute drying time specified in DIS/TSS-10). Each carrier was wiped back and forth twice, for a total of four passes. One towelette was used to treat five carriers. The area of the towelette used for wiping was rotated so as to expose a maximum amount of its surface during wiping. Each carrier remained in contact with the product for 30 seconds at 22.5-22.7°C. Following exposure, each carrier was transferred to a tube containing 20 mL of Lethen Broth to neutralize. The tubes were “vortex” mixed to suspend the surviving organisms. Either 1 mL or 0.1 mL aliquots of selected dilutions were plated on tryptic soy agar. All subcultures were incubated for at least 46 hours at 33.9-35.0°C. Following incubation, the colonies were counted. Controls included a non-active control and those for inoculum count, dried recovery count, test system verification (i.e., purity and identity), viability, sterility, and neutralizer efficacy.

Note: Testing was conducted according to Good Laboratory Practice (GLP) standards with the following exceptions: (1) “The Non-Active control reference substance, 0.01% Triton X-100, Reagent No. 4662, was prepared in the laboratory for use in this sanitization assay. The solution was prepared using a stock of Triton X-100 (CAS 9002-93-1), and sterile deionized water. The sample was kept in the GLP locked storage cabinet, and its usage was tracked via a Chain of Custody test substance form. The identity, strength, purity, composition, solubility and stability were not determined for this sample.” (2) “The stability of the test substance under the conditions of the test site was

not known at the time of the efficacy study. This deviation did not affect the integrity of the study because the 2-year stability at 25°C and the 3-month accelerated stability at 40°C were initiated prior to the start of the efficacy study.”

Note: The initial laboratory report was amended to identify the product lots that were at least 60 days old at the time of testing, add a protocol deviation, add the name of the test system to the viability control result table (omitting any test system name abbreviations), and remove duplicated sections of the protocol.

Note: Protocol deviations/amendments reported in the study were documented.

20. MRID 477640-35 “Hospital Type Disinfectant Efficacy Testing In The Presence of Organic Soil,” Test Organisms: *Staphylococcus aureus* (ATCC 6538), *Salmonella enterica* (ATCC 10708), and *Pseudomonas aeruginosa* (ATCC 15442), for Formula 1435-014, by Kyle T. Smith. Study conducted at Reckitt Benckiser Inc. Study completion date – May 5, 2009. Master Schedule No. 2009-0046.

Note: Intentionally formulated, low-dose towelettes were used in this study to demonstrate efficacy at the lower certified limit with towelettes containing the least amount of expressed liquid.

This confirmatory study was conducted against *Staphylococcus aureus* (ATCC 6538), *Salmonella enterica* (ATCC 10708), and *Pseudomonas aeruginosa* (ATCC 15442). One lot (Lot No. 1532-157) of the product, Formula 1435-014, was tested using the AOAC Germicidal Spray Products as Disinfectants Method (modified) as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. The product was received ready-to-use, as a pre-saturated towelette. Cultures of the challenge microorganisms were prepared in accordance with the published AOAC method, with the following exceptions: (1) the *Pseudomonas aeruginosa* culture was incubated for 48-54 hours (which differs from the AOAC method specification of 18-24 hours); and (2) the *Staphylococcus aureus* and *Salmonella enterica* cultures were incubated for 48±2 hours. Horse serum was added to each inoculum to achieve a 5% organic soil load. Thirty (30) glass slide carriers (20 mm x 25 mm) per product lot per test organism were inoculated with 0.01 mL of a 48±2 hour old suspension of test organism. For each test organism, three different towelettes were tested against 10 inoculated carriers. The culture was spread evenly over each carrier surface. The carriers were dried for 40-42 minutes at 35.0-35.6°C (which differs from the AOAC method specification of 30-40 minutes at 37°C). Each carrier was wiped back and forth twice, for a total of four passes. One towelette was used to treat 10 carriers. The area of the towelette used for wiping was rotated so as to expose a maximum amount of its surface during wiping. The carriers were allowed to remain wet for 10 minutes at 22.3-22.5°C. Following the exposure period, individual carriers were transferred to 20 mL of Lethen Broth to neutralize. The tubes containing neutralizer were gently agitated after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for ~46 hours at 35.3-36.6°C (which differs from the AOAC method specification of 48 hours at 37°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for inoculum count, dried recovery count, test system verification (i.e., purity and identity), viability, sterility, and neutralizer efficacy.

Note: Testing was conducted according to Good Laboratory Practice (GLP) standards with the following exception: "The stability of the test substance under the conditions of the test site was not known at the time of the efficacy study. This deviation did not affect the integrity of the study because the 2-year stability at 25°C and the 3-month accelerated stability at 40°C were initiated prior to the start of the efficacy study."

Note: The laboratory reported a failed study set up on March 27, 2009. In the study, contamination was observed. The laboratory did not accept the assay. These data were not used to evaluate efficacy of the product. Testing was repeated on April 6, 2009. See page 17 and Appendix C of the laboratory report.

Note: Protocol deviations/amendments reported in the study were documented.

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable.

**21. MRID 477640-36 "Fungistatic Activity In The Presence of Organic Soil,"
Test Organism: *Aspergillus niger* (ATCC 6275), for Formula 1435-014, by
Kyle T. Smith. Study conducted at Reckitt Benckiser Inc. Study
completion date – May 1, 2009. Amended final report date – May 26, 2009.
Master Schedule No. 2008-0218.**

This study was conducted against *Aspergillus niger* (ATCC 6275). Two lots (Lot Nos. 1453-081A and 1453-081B) of the product, Formula 1435-014, were tested using the EPA Hard Surface Mildew Fungistatic Test Method (modified). The product was received ready-to-use as a pre-saturated towelette. A culture of the challenge microorganism was prepared using growth on agar plates. [Details of the conidial suspension preparation were not provided.] A 1 mL aliquot of a standardized conidial suspension was added to 20 mL of Czapek Dox Broth. Horse serum was added to the suspension to achieve a 5% organic soil load. Sterile 25 mm x 25 mm glazed ceramic tiles (10 per treatment) were used (which meets the EPA method specification of 25 mm square tiles with a glazed surface). Each carrier was wiped back and forth twice, for a total of four passes. One towelette was used to treat 10 tiles. The area of the towelette used for wiping was rotated so as to expose a maximum amount of its surface during wiping. The tiles were allowed to remain wet for 3 minutes at 22.6-22.9°C. At the 3-minute contact time, the tiles were placed in a vertical position to allow excess liquid to drain. Following treatment, the tiles were dried for 40 minutes at 34.9-35.3°C (which differs from the EPA method specification of 37°C). Following the drying period, the surfaces of each test tile and each untreated control tile were inoculated with 0.01 mL of the conidial suspension. The conidial suspension was spread evenly over each carrier surface. The tiles were returned to 34.6-35.3°C and dried for 40 minutes (which differs from the EPA method specification of 37°C). Each tile (treated side up) was placed in an individual Petri dish containing 2% water agar. The plates were incubated for at least 7 days at 23.7-25.7°C at 95.60% relative humidity (which differs from the EPA method specification of 25°C and a minimum of 95% relative humidity). Following incubation, the tiles were examined for the presence or absence of fungal growth. The absence of growth was confirmed by inspecting negative (no growth) tiles under at least 15X magnification. Controls included those for stock spore suspension count, adjusted spore suspension count, test spore suspension count, test system verification (i.e., purity and identity), and sterility.

Note: Testing was conducted according to Good Laboratory Practice (GLP) standards with the following exception: "The stability of the test substance under the conditions of the test site was not known at the time of the efficacy study. This deviation did not affect the integrity of the study because the 2-year stability at 25°C and the 3-month accelerated stability at 40°C were initiated prior to the start of the efficacy study."

Note: The initial laboratory report was amended to identify the product lots that were at least 60 days old at the time of testing, and add a protocol deviation.

Note: Protocol deviations/amendments reported in the study were documented.

22. MRID 477640-44 "Fungicidal Efficacy of a Disinfectant In the Presence of Organic Soil," Test Organism: *Aspergillus niger* (ATCC 6275), for Formula 1435-014, by Kyle T. Smith. Study conducted at Reckitt Benckiser Inc. Study completion date – April 29, 2009. Master Schedule No. 2008-0221.

Note: The laboratory report was submitted for informational purposes only. The results obtained in the study failed to support the intended claim.

This study was conducted against *Aspergillus niger* (ATCC 6275). Three lots (Lot Nos. 1453-081A, 1453-081B, and 1453-082) of the product, Formula 1435-014, were tested using the AOAC Germicidal Spray Products as Disinfectants Method (modified) as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. The product was received ready-to-use, as a pre-saturated towelette. A culture of the challenge microorganism was prepared in accordance with the published AOAC method, with the following exceptions: (1) the culture was incubated for 7 days (which differs from the AOAC method specification of incubation at 10-15 days at 25-30°C (for *Trichophyton mentagrophytes*)); and (2) the culture was not standardized (which differs from the AOAC method specification of diluting the stock suspension using physiological NaCl solution so that it contains 5×10^6 conidia/mL (for *Trichophyton mentagrophytes*)). Horse serum was added to the conidial suspension to achieve a 5% organic soil load. Ten (10) glass slide carriers (20 mm x 25 mm) per product lot were inoculated with 0.01 mL of a 7-day old suspension of test organism. The conidial suspension was uniformly spread over each carrier surface. The carriers were dried for 41 minutes at 32.8-34.3°C (which differs from the AOAC method specification of 30-40 minutes at 37°C). Each carrier was wiped back and forth twice, for a total of four passes. One towelette was used to treat 10 carriers. The area of the towelette used for wiping was rotated so as to expose a maximum amount of its surface during wiping. The carriers were allowed to remain wet for 10 minutes at 22.5-22.7°C. Following the exposure period, individual carriers were transferred to 20 mL of Lethen Broth to neutralize. The tubes containing neutralizer were gently agitated after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for at least 5 days at 30.2-30.7°C (which differs from the AOAC method specification of 7 days at 25-30°C (for *Trichophyton mentagrophytes*)). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for inoculum count, dried recovery count, test system verification (i.e., purity and identity), viability, sterility, and neutralizer efficacy.

Note: The study referenced in the laboratory report was not conducted according to Good Laboratory Practice (GLP) standards. The laboratory report was submitted for informational purposes only. The results obtained in the study failed to support the intended claim. Because the claim was not supported by the raw data, neither the Study Director nor the Quality Assurance Unit reviewed the data or report for compliance.

Note: Protocol deviations/amendments reported in the study were documented.

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable.

23. MRID 477640-45 “Fungicidal Efficacy of a Disinfectant In the Presence of Organic Soil,” Test Organism: *Trichophyton mentagrophytes* (ATCC 9533), for Formula 1435-014, by Kyle T. Smith. Study conducted at Reckitt Benckiser Inc. Study completion date – April 29, 2009. Master Schedule No. 2008-0233.

Note: The laboratory report was submitted for informational purposes only. The results obtained in the study failed to support the intended claim.

This study was conducted against *Trichophyton mentagrophytes* (ATCC 9533). Three lots (Lot Nos. 1453-081A, 1453-081B, and 1453-082) of the product, Formula 1435-014, were tested using the AOAC Germicidal Spray Products as Disinfectants Method (modified) as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. The product was received ready-to-use, as a pre-saturated towelette. A culture of the challenge microorganism was prepared in accordance with the published AOAC method, with the following exception: the culture was not standardized (which differs from the AOAC method specification of diluting the stock suspension using physiological NaCl solution so that it contains 5×10^6 conidia/mL (for *Trichophyton mentagrophytes*)). Horse serum was added to the conidial suspension to achieve a 5% organic soil load. Ten (10) glass slide carriers (20 mm x 25 mm) per product lot were inoculated with 0.01 mL of a 14-day old suspension of test organism. The conidial suspension was uniformly spread over each carrier surface. The carriers were dried for 42 minutes at 36.0-36.7°C (which differs from the AOAC method specification of 30-40 minutes at 37°C). Each carrier was wiped back and forth twice, for a total of four passes. One towelette was used to treat 10 carriers. The area of the towelette used for wiping was rotated so as to expose a maximum amount of its surface during wiping. The carriers were allowed to remain wet for 10 minutes at 23.2-23.6°C. Following the exposure period, individual carriers were transferred to 20 mL of Lethen Broth to neutralize. The tubes containing neutralizer were gently agitated after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for at least 9 days at 18.1-32.3°C (which differs from the AOAC method specification of 7 days at 25-30°C (for *Trichophyton mentagrophytes*)). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for inoculum count, dried recovery count, test system verification (i.e., purity and identity), viability, sterility, and neutralizer efficacy.

Note: The study referenced in the laboratory report was not conducted according to Good Laboratory Practice (GLP) standards. The laboratory report was submitted for informational purposes only. The results obtained in the study failed to support the

intended claim. Because the claim was not supported by the raw data, neither the Study Director nor the Quality Assurance Unit reviewed the data or report for compliance.

Note: Protocol deviations/amendments reported in the study were documented.

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable.

24. MRID 477640-46 "Inactivation of Coronavirus in the Presence of Organic Soil for Single Use Premoistened Towelettes," for Formula 1435-014, by Ziyan Chu. Study conducted at Reckitt Benckiser Inc. Study completion date – April 29, 2009. Master Schedule No. 2009-0012.

Note: The laboratory report was submitted for informational purposes only. The results obtained in the study failed to support the intended claim.

This study was conducted against Human coronavirus (Strain 229E; ATCC VR-740), using WI-38 cells (human lung fibroblast cells; obtained from ViroMed Laboratories, Minneapolis, MN) as the host system. Three lots (Lot Nos. 1453-081A, 1453-081B, and 1453-082) of the product, Formula 1435-014, were tested. ATSM E1053 was referenced. The product was received ready-to-use, as a pre-saturated towelette. Testing was conducted on February 10, 2009, February 17, 2009, and February 25, 2009. The stock virus culture was adjusted to contain at least 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the undersides of separate sterile polystyrene Petri dishes. The virus films were dried for 16-55 minutes at 24.8-25.6°C at 7.587-21.34% relative humidity. Five replicates per product lot were tested. Each carrier was wiped back and forth twice with an individual saturated towelette, for a total of four passes. The carriers were allowed to remain wet for 30 seconds at 19.6-20.3°C. Following exposure, 2.0 mL of test medium was added to each Petri dish, and the dishes were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium (MEM) supplemented with gentamicin and 2% serum. WI-38 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated for 8-9 days at 36.2-37.5°C in 1.093-7.758% CO₂. For testing conducted on February 10, 2009, plates were re-fed with MEM supplemented with gentamicin and 5% serum. Following incubation, the cultures were observed for cytotoxicity or characteristic cytopathic effects (i.e., granulation and vacuolation of cells, degeneration of cells). Controls included those for host cell viability, virus count, cytotoxicity, and neutralization effectiveness. Viral and cytotoxicity titers were calculated by the method of Reed and Muench.

Note: The study referenced in the laboratory report was not conducted according to Good Laboratory Practice (GLP) standards. The laboratory report was submitted for informational purposes only. The results obtained in the study failed to support the intended claim. Because the claim was not supported by the raw data, neither the Study Director nor the Quality Assurance Unit reviewed the data or report for compliance.

Note: Protocol deviations/amendments reported in the study were documented.

**25. MRID 477640-47 "Fungistatic Activity In The Presence of Organic Soil,"
Test Organism: *Trichophyton mentagrophytes* (ATCC 9533), for Formula
1435-014, by Kyle T. Smith. Study conducted at Reckitt Benckiser Inc.
Study completion date – April 29, 2009. Master Schedule No. 2009-0013.**

Note: The laboratory report was submitted for informational purposes only. The results obtained in the study failed to support the intended claim.

This study was conducted against *Trichophyton mentagrophytes* (ATCC 9533). Two lots (Lot Nos. 1453-081A and 1453-081B) of the product, Formula 1435-014, were tested using the EPA Hard Surface Mildew Fungistatic Test Method (modified). The product was received ready-to-use as a pre-saturated towelette. A culture of the challenge microorganism was prepared using growth on agar plates. [Details of the conidial suspension preparation were not provided.] A 1 mL aliquot of a standardized conidial suspension was added to 20 mL of Czapek Dox Broth. Horse serum was added to the suspension to achieve a 5% organic soil load. Sterile 25 mm x 25 mm glazed ceramic tiles (10 per treatment) were used (which meets the EPA method specification of 25 mm square tiles with a glazed surface). Each carrier was wiped back and forth twice, for a total of four passes. One towelette was used to treat 10 tiles. The area of the towelette used for wiping was rotated so as to expose a maximum amount of its surface during wiping. The tiles were allowed to remain wet for 3 minutes at 22.9-23.2°C. At the 3-minute contact time, the tiles were placed in a vertical position to allow excess liquid to drain. Following treatment, the tiles were dried for 40 minutes at 33.9-34.4°C (which differs from the EPA method specification of 37°C). Following the drying period, the surfaces of each test tile and each untreated control tile were inoculated with 0.01 mL of the conidial suspension. The conidial suspension was spread evenly over each carrier surface. The tiles were returned to 33.8-34.4°C and dried for 40 minutes (which differs from the EPA method specification of 37°C). Each tile (treated side up) was placed in an individual Petri dish containing 2% water agar. The plates were incubated for at least 7 days at 11.8-28.2°C at 95.60% relative humidity (which differs from the EPA method specification of 25°C and a minimum of 95% relative humidity). Following incubation, the tiles were examined for the presence or absence of fungal growth. The absence of growth was confirmed by inspecting negative (no growth) tiles under at least 15X magnification. Controls included those for stock spore suspension count, test spore suspension count, test system verification (i.e., purity and identity), and sterility.

Note: The study referenced in the laboratory report was not conducted according to Good Laboratory Practice (GLP) standards. The laboratory report was submitted for informational purposes only. The results obtained in the study failed to support the intended claim. Because the claim was not supported by the raw data, neither the Study Director nor the Quality Assurance Unit reviewed the data or report for compliance.

Note: Protocol deviations/amendments reported in the study were documented.

26. MRID 477640-48 “Virucidal Efficacy of Pre-Saturated Towelettes for Hard Surface Disinfection, U.S. EPA and Health Canada Submission,” Virus: Influenza A (H1N1) virus, for Formula 1435-014, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – January 7, 2009. Project Number A07070.

Note: The laboratory report was submitted for informational purposes only. The results obtained in the study failed to support the intended claim.

This study was conducted against Influenza A (H1N1) virus (Strain A/Malaya/302/54; ATCC VR-98), using RMK cells (Rhesus monkey kidney cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Three lots (Lot Nos. 1453-081A, 1453-081B, and 1453-082) of the product, Formula 1435-014, were tested according to ATS Labs Protocol No. REK01101608.FLUA.2 (copy provided). The product was received ready-to-use, as a pre-saturated towelette. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the undersides of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 59% relative humidity. Five replicates per product lot were tested. Using sterile gloves, each towelette was folded in half lengthwise one time. The far end was folded up once and then folded three more times in the same direction for a total of four folds. The outside edges were pulled upward forming a “U” shape. Each carrier was wiped back and forth twice with an individual saturated towelette, for a total of four passes. The carriers were allowed to remain wet for 30 seconds at 20.0°C. Following exposure, 2.00 mL of test medium was added to each Petri dish, and the dishes were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 1% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO₂. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

27. MRID 477640-49 “Virucidal Efficacy of Pre-Saturated Towelettes for Hard Surface Disinfection,” Virus: Influenza A (H1N1) virus, for Formula 1435-014, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – February 16, 2009. Project Number A07244.

Note: The laboratory report was submitted for informational purposes only. The results obtained in the study failed to support the intended claim.

This study was conducted against Influenza A (H1N1) virus (Strain A/Malaya/302/54; ATCC VR-98), using RMK cells (Rhesus monkey kidney cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division, Minneapolis, MN; maintained in-house) as the host system. Two lots (Lot Nos. 1453-081A and 1453-081B) of the product, Formula 1435-014, were tested according to ATS Labs Protocol No. REK01010809.FLUA.1 (copy provided). The product was received ready-to-use, as a pre-saturated towelette. The stock virus culture was adjusted to contain 5% fetal

bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the undersides of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 50% relative humidity. Using sterile gloves, each towelette was folded in half lengthwise two times. The far end was folded up once and the outside edges were pulled upward forming a "U" shape. Each carrier was wiped back and forth twice with an individual saturated towelette, for a total of four passes. The carriers were allowed to remain wet for 10 minutes at 20.0°C. Following exposure, 2.00 mL of test medium was added to each Petri dish, and the dishes were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 1% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO₂. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

**28. MRID 477640-50 "Virucidal Efficacy of Pre-Saturated Towelettes for Hard Surface Disinfection, U.S. EPA and Health Canada Submission,"
Virus: Influenza A virus (Strain A/New Caledonia/20/99), for Formula 1435-014, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – January 13, 2009. Project Number A07096.**

Note: The laboratory report was submitted for informational purposes only. The results obtained in the study failed to support the intended claim.

This study was conducted against Influenza A virus (Strain A/New Caledonia/20/99; obtained from the Centers for Disease Control), using RMK cells (Rhesus monkey kidney cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division, Minneapolis, MN; maintained in-house) as the host system. Three lots (Lot Nos. 1453-081A, 1453-081B, and 1453-082) of the product, Formula 1435-014, were tested according to ATS Labs Protocol No. REK01101608.FLUA.1 (copy provided). The product was received ready-to-use, as a pre-saturated towelette. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the undersides of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 52% relative humidity. Five replicates per product lot were tested. Using sterile gloves, each towelette was folded in half lengthwise two times. The far end was folded up once and the outside edges were pulled upward forming a "U" shape. Each carrier was wiped back and forth twice with an individual saturated towelette, for a total of four passes. The carriers were allowed to remain wet for 30 seconds at 20.0°C. Following exposure, 2.00 mL of test medium was added to each Petri dish, and the dishes were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 1% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO₂. The

cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

V RESULTS

A. Results Supporting Efficacy, as Identified by Reckitt Benckiser Inc.

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested			Dried Recovery Count (CFU/Carrier)
		Lot No. 1453-081A	Lot No. 1453-081B	Lot No. 1453-082	
477640-16	<i>Staphylococcus aureus</i>	1/60	0/60	1/60	9.2 x 10 ⁵ to 2.66 x 10 ⁶
477640-17	<i>Staphylococcus aureus</i>	0/10	0/10	0/10	1.09 x 10 ⁵ to 1.48 x 10 ⁶
477640-18	<i>Pseudomonas aeruginosa</i>	0/60	1/60	0/60	9.1 x 10 ⁴ to 2.86 x 10 ⁶
477640-19	<i>Salmonella enterica</i>	1/60	0/60	0/60	1.16 x 10 ⁵ to 8.3 x 10 ⁵

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested		Dried Recovery Count (CFU/Carrier)
			Lot No. 1532-157	
477640-35	<i>Staphylococcus aureus</i>	Sample 16, Towelette #8	0/10	1.26 x 10 ⁶ to 2.24 x 10 ⁶
		Sample 15, Towelette #13	0/10	
		Sample 15, Towelette #9	0/10	
	<i>Salmonella enterica</i>	Sample 17, Towelette #10	0/10	5.2 x 10 ⁵ to 7.6 x 10 ⁵
		Sample 17, Towelette #20	0/10	
		Sample 17, Towelette #21	0/10	
	<i>Pseudomonas aeruginosa</i>	Sample 18, Towelette #15	0/10	5.7 x 10 ⁵ to 1.59 x 10 ⁶
		Sample 18, Towelette #18	0/10	
		Sample 18, Towelette #19	0/10	

MRID Number	Organism	Results			Dried Virus Count	
			Lot No. 1453-081A	Lot No. 1453-081B		Lot No. 1453-082
477640-20	Human immunodeficiency virus type 1	10 ⁻¹ to 10 ⁻⁷ dilutions	Complete inactivation			10 ^{4.7} TCID ₅₀ / 0.2 mL
		TCID ₅₀ /0.2 mL	≤10 ^{0.5}	≤10 ^{0.5}	≤10 ^{0.5}	
477640-21	Respiratory syncytial virus	10 ⁻¹ to 10 ⁻⁶ dilutions	Complete inactivation			10 ^{4.7} TCID ₅₀ /

MRID Number	Organism	Results			Dried Virus Count	
			Lot No. 1453-081A	Lot No. 1453-081B		Lot No. 1453-082
		TCID ₅₀ /0.1 mL	≤10 ^{0.5}	≤10 ^{0.5}	≤10 ^{0.5}	0.1 mL
477640-22	Herpes simplex virus type 1	10 ⁻¹ to 10 ⁻⁷ dilutions	Complete inactivation		---	10 ^{5.0} TCID ₅₀ /0.1 mL
		TCID ₅₀ /0.1 mL	≤10 ^{0.5}	≤10 ^{0.5}	---	
477640-23	Influenza A virus (Strain A/New Caledonia/20/99)	10 ⁻¹ to 10 ⁻⁷ dilutions	Complete inactivation		---	10 ^{4.75} TCID ₅₀ /0.1 mL
		TCID ₅₀ /0.1 mL	≤10 ^{0.5}	≤10 ^{0.5}	---	
477640-24	Avian influenza A (H5N1) virus	10 ⁻¹ to 10 ⁻⁷ dilutions	Complete inactivation		---	10 ^{4.5} TCID ₅₀ /0.1 mL
		TCID ₅₀ /0.1 mL	≤10 ^{0.5}	≤10 ^{0.5}	---	
477640-25	Human coronavirus	10 ⁻¹ to 10 ⁻⁶ dilutions	Complete inactivation*		---	10 ^{5.25} TCID ₅₀ /0.1 mL
		TCID ₅₀ /0.1 mL	≤10 ^{0.5}	≤10 ^{0.5}	---	

*Note that cytotoxicity was observed in the 10⁻¹ dilutions for the cytotoxicity and neutralization controls.

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested		Control Tiles
		Lot No. 1453-081A	Lot No. 1453-081B	
477640-36	<i>Aspergillus niger</i>	0/10	0/10	10/10*

* At least 50% fungal growth on each untreated control tile was observed.

MRID Number	Organism	Lot No.	Total No. Surviving	Parallel Count	Percent Reduction
			(CFU/carrier)		
477640-26	<i>Streptococcus pyogenes</i>	1453-081A*	$<2.00 \times 10^1$	6.46×10^4	>99.9
		1455-081B*	$<2.00 \times 10^1$	6.46×10^4	>99.9
477640-27	<i>Listeria monocytogenes</i>	1453-081A*	$<2.00 \times 10^1$	3.72×10^5	>99.9
		1453-081B*	$<2.00 \times 10^1$	3.72×10^5	>99.9
477640-28	<i>Campylobacter jejuni</i>	1453-081A*	$<2.00 \times 10^1$	3.39×10^5	>99.9
		1453-081B*	$<2.00 \times 10^1$	3.39×10^5	>99.9
477640-29	<i>Salmonella enterica</i>	1453-081A**	1	3.72×10^5	>99.9
		1453-081B**	1.82×10^0	3.72×10^5	>99.9
		1453-082**	1.82×10^0	3.72×10^5	>99.9
477640-30	Methicillin Resistant <i>Staphylococcus aureus</i> (ATCC BAA-1556)	1453-081A	3.31×10^0	6.16×10^6	>99.9
		1453-081B	3.31×10^0	6.16×10^6	>99.9
		1453-082	3.31×10^0	6.16×10^6	>99.9
477640-31	Methicillin Resistant <i>Staphylococcus aureus</i> (ATCC 33591)	1453-081A†	1.82×10^0	7.76×10^5	>99.9
		1453-081B†	6.02×10^0	7.76×10^5	>99.9
		1453-082†	1.82×10^0	7.76×10^5	>99.9
477640-32	Methicillin Resistant <i>Staphylococcus aureus</i> (ATCC 33592)	1453-081A††	1	1.38×10^5	>99.9
		1453-081B††	1.82×10^0	1.38×10^5	>99.9
		1453-082††	1	1.38×10^5	>99.9
477640-33	<i>Staphylococcus aureus</i>	1453-081A	1.82×10^0	1.32×10^7	>99.9
		1453-081B	6.02×10^0	1.32×10^7	>99.9
		1453-082	1.10×10^1	1.32×10^7	>99.9
	<i>Enterobacter aerogenes</i>	1453-081A	1.20×10^1	6.46×10^6	>99.9
		1453-081B	6.02×10^0	6.46×10^6	>99.9
		1453-082	1.82×10^0	6.46×10^6	>99.9
477640-34	<i>Escherichia coli</i> O157:H7	1453-081A	1.0×10^0	2.82×10^6	>99.9
		1453-081B	1.0×10^0	2.82×10^6	>99.9
		1453-082	1.0×10^0	2.82×10^6	>99.9

*The carrier quantitation control did not demonstrate an average of at least 7.5×10^5 surviving organisms, which is the criterion set forth in ASTM 1153.

**The zero-time control demonstrated an average of at least 7.5×10^5 surviving organisms, which is the criterion set forth in ASTM 1153; however, the non-active control did not.

†The non-active control demonstrated an average of at least 7.5×10^5 surviving organisms, which is the criterion set forth in ASTM 1153; however, the zero-time control did not.

††Neither the non-active control nor the zero-time control demonstrated an average of at least 7.5×10^5 surviving organisms, which is the criterion set forth in ASTM 1153.

B. Results Failing to Support Efficacy, as Identified by Reckitt Benckiser Inc.;
Submitted for Informational Purposes

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested			Dried Recovery Count (CFU/Carrier)
		Lot No. 1453-081A	Lot No. 1453-081B	Lot No. 1453-082	
477640-44	<i>Aspergillus niger</i>	10/10	6/10 (page 19)	8/10	5.3×10^4 to 3.5×10^5
477640-45	<i>Trichophyton mentagrophytes</i>	10/10	10/10	10/10	1.6×10^5 to 2.5×10^5

MRID Number	Organism	Results				Dried Virus Count (ID ₅₀)
			Lot No. 1453-081A	Lot No. 1453-081B	Lot No. 1453-082	
477640-46	Human coronavirus Test Date: 2/10/09	10 ⁻¹ to 10 ⁻⁵ dilutions	Complete inactivation	Complete inactivation	---	10 ^{3.5}
		ID ₅₀	≤10 ^{0.50}	≤10 ^{0.50}	---	
		Log Reduction	†	†	---	
477640-46	Human coronavirus Test Date: 2/17/2009	10 ⁻¹ to 10 ⁻⁵ dilutions	---	---	Complete inactivation	10 ^{2.67}
		ID ₅₀	---	---	≤10 ^{0.50}	
		Log Reduction	---	---	†	
477640-46	Human coronavirus Test Date: 2/25/2009	ID ₅₀	---	Complete inactivation	---	10 ^{2.67}
		10 ⁻¹ to 10 ⁻⁵ dilutions	---	≤10 ^{0.50}	---	
		Log Reduction	---	†	---	

†Inconclusive due to unsuccessful neutralization effectiveness control results

MRID Number	Organism	Results				Dried Virus Count
			Lot No. 1453-081A	Lot No. 1453-081B	Lot No. 1453-082	
477640-48	Influenza A (H1N1) virus	10 ⁻¹ dilution	Infectivity			10 ^{5.8} TCID ₅₀ / 0.1 mL
		10 ⁻² dilution	Infectivity	Complete inactivation		
		10 ⁻³ to 10 ⁻⁷ dilutions	Complete inactivation			
		TCID ₅₀ /0.1 mL	≤10 ^{1.3}	≤10 ^{0.75}	≤10 ^{0.55}	
		Log Reduction	≥4.50 log ₁₀	≥5.05 log ₁₀	≥5.25 log ₁₀	
477640-49	Influenza A (H1N1) virus	10 ⁻¹ to 10 ⁻³ dilutions	Infectivity	Infectivity	---	10 ^{6.5} TCID ₅₀ /

MRID Number	Organism	Results			Dried Virus Count	
			Lot No. 1453-081A	Lot No. 1453-081B		Lot No. 1453-082
		10 ⁻⁴ dilution	Complete inactivation	Infectivity	---	0.1 mL
		10 ⁻⁵ to 10 ⁻⁷ dilutions	Complete inactivation		---	
		TCID ₅₀ /0.1 mL	≤10 ^{3.25}	≤10 ^{3.5}	---	
		Log Reduction	≥3.25 log ₁₀	≥3.0 log ₁₀		
477640-50	Influenza A virus (Strain A/New Caledonia/20/99)	10 ⁻¹ dilution	Complete inactivation	Infectivity		10 ^{4.6} TCID ₅₀ /0.1 mL
		10 ⁻² to 10 ⁻⁶ dilution	Complete inactivation			
		TCID ₅₀ /0.1 mL	≤10 ^{0.5}	≤10 ^{0.70}	≤10 ^{0.70}	
		Log Reduction	≥4.1 log ₁₀	≥3.9 log ₁₀	≥3.9 log ₁₀	

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested		Control Tiles
		Lot No. 1453-081A	Lot No. 1453-081B	
477640-47	<i>Trichophyton mentagrophytes</i>	0/10 Inconclusive	0/10 Inconclusive	0/10*

* At least 50% fungal growth on each untreated control tile was not observed.

VI CONCLUSIONS

A. Conclusions Regarding Use of the Product as a Disinfectant

1. The submitted efficacy data support the use of the product, Sting (also known as Formula 1435-014), 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 10-minute contact time:

Staphylococcus aureus
Pseudomonas aeruginosa
Salmonella enterica

MRID 477640-16, -17, and -35
 MRID 477640-18 and -35
 MRID 477640-19 and -35

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. At least one of the product lots tested was at least 60 days old at the time of testing. Neutralizer efficacy testing showed positive growth of the microorganisms. When reported, viability controls were positive for growth. Sterility controls did not show growth. Test system verification controls confirmed the identity of the challenge microorganisms. [Note that the study assigned MRID 477640-35 demonstrated efficacy of intentionally formulated, low-dose towelettes

(i.e., lower certified limit of the active ingredient; towelettes containing the least amount of expressed liquid).]

Note: The "Comments on the Submitted Efficacy Studies" section of this report identifies AOAC method deviations with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable.

2. The submitted efficacy data do not support the use of the product, Sting (also known as Formula 1435-014), 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 10-minute contact time:

<i>Aspergillus niger</i>	MRID 477640-44
<i>Trichophyton mentagrophytes</i>	MRID 477640-45

Acceptable killing was not observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralizer efficacy testing showed positive growth of the microorganisms. Viability controls were positive for growth. Test system verification controls confirmed the identity of the challenge microorganisms. Sterility controls did not show growth.

Note: The "Comments on the Submitted Efficacy Studies" section of this report identifies AOAC method deviations with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable.

3. The submitted efficacy data support the use of the product, Sting (also known as Formula 1435-014), as a disinfectant with virucidal activity against the following microorganisms on hard, non-porous surfaces in the presence of at least a 5% organic soil load for a 10-minute contact time (30-second contact time against Human immunodeficiency virus type 1):

Human immunodeficiency virus type 1	MRID 477640-20
Respiratory syncytial virus	MRID 477640-21
Herpes simplex virus type 1	MRID 477640-22
Influenza A virus (Strain A/New Caledonia/20/99)	MRID 477640-23
Avian influenza (H5N1) virus	MRID 477640-24
Human coronavirus	MRID 477640-25

Recoverable virus titers of at least 10^4 were achieved. Cytotoxicity was not observed. Complete inactivation (no growth) was indicated in all dilutions tested.

4. The submitted efficacy data (MRID 477640-46) do not support the use of the product, Sting (also known as Formula 1435-014), as a disinfectant with virucidal activity against Human coronavirus on hard, non-porous surfaces in the presence of at least a 5% organic soil load for a 30-second contact time. The reduction in virus titer per product lot was inconclusive due to unsuccessful results of the neutralization effectiveness control.

5. The submitted efficacy data do not support the use of the product, Sting (also known as Formula 1435-014), as a disinfectant with virucidal activity against the following organisms on hard, non-porous surfaces in the presence of a 5% organic soil load:

Influenza A (H1N1) virus (Strain A/Malaya/302/54)	MRID 477640-48
Influenza A (H1N1) virus (Strain A/Malaya/302/54)	MRID 477640-49
Influenza A virus (Strain A/New Caledonia/20/99)	MRID 477640-50

Recoverable virus titers of at least 10^4 were achieved. In studies against Influenza A (H1N1) virus for a 30-second contact time, test virus infectivity was detected in the 10^{-1} dilutions of various (but not all) replicates of each of the three product lots. In studies against Influenza A (H1N1) virus for a 10-minute contact time, test virus infectivity was detected in the 10^{-1} to 10^{-3} dilutions of both product lots. In studies against Influenza A virus (Strain A/New Caledonia/20/99) for a 30-second contact time, test virus infectivity was detected in the 10^{-1} dilutions of at least one replicate of two product lots.

B. Conclusions Regarding Use of the Product as a Fungistat

1. The submitted efficacy data (MRID 477640-36) support the use of the product, Sting (also known as Formula 1435-014), as a fungistat against *Aspergillus niger* in the presence of a 5% organic soil load for a 3-minute contact time. No growth was observed on the treated tiles 7 days after treatment. Testing was conducted on 2 product lots. Untreated control tiles exhibited sufficient growth on at least 50% of each untreated tile surface. Test system verification controls confirmed the identity of the challenge microorganisms. Sterility controls did not show growth.

2. The submitted efficacy data (MRID 477640-47) do not support the use of the product, Sting (also known as Formula 1435-014), as a fungistat against *Trichophyton mentagrophytes* in the presence of a 5% organic soil load for a 3-minute contact time. No growth was observed on the treated tiles 7 days after treatment; however, the untreated control tiles did not exhibit sufficient growth. Test system verification controls confirmed the identity of the challenge microorganisms. Sterility controls did not show growth.

C. Conclusions Regarding Use of the Product as a Sanitizer

1. The submitted efficacy data support the use of the product, Sting (also known as Formula 1435-014), as a sanitizer against the following microorganisms on hard, non-porous, non-food contact surfaces in the presence of a 5% organic soil load for a 30-second contact time:

Methicillin Resistant <i>Staphylococcus aureus</i> (ATCC BAA-1556)	MRID 477640-30
<i>Staphylococcus aureus</i>	MRID 477640-33
<i>Enterobacter aerogenes</i>	MRID 477640-33
<i>Escherichia coli</i> O157:H7	MRID 477640-34

Bacterial reductions of at least 99.9 percent over the parallel control were observed within 5 minutes (i.e., 30 seconds specifically). In testing against *Staphylococcus aureus* and *Enterobacter aerogenes*, at least one of the product lots tested was at least 60 days old at the time of testing. The parallel controls and dried recovery controls demonstrated an average of at least 7.5×10^5 surviving organisms, which is the criterion set forth in ASTM 1153. Neutralizer efficacy testing showed positive growth of the microorganisms. Viability controls were positive for growth. Test system verification controls confirmed the identity of the challenge microorganisms. Sterility controls did not show growth.

2. The submitted efficacy data do not support the use of the product, Sting (also known as Formula 1435-014), as a sanitizer against the following microorganisms on hard, non-porous, non-food contact surfaces in the presence of a 5% organic soil load for a 30-second contact time:

<i>Streptococcus pyogenes</i>	MRID 477640-26
<i>Listeria monocytogenes</i>	MRID 477640-27
<i>Campylobacter jejuni</i>	MRID 477640-28
Methicillin Resistant <i>Staphylococcus aureus</i> (ATCC 33592)	MRID 477640-32

Although a bacterial reduction of at least 99.9 percent over the parallel control was observed within 5 minutes (i.e., 30 seconds specifically), the carrier quantitation control (or non-active control and dried recovery control for the study against Methicillin Resistant *Staphylococcus aureus* (ATCC 33592)) did not demonstrate an average of at least 7.5×10^5 surviving organisms, which is the criterion set forth in ASTM 1153. In the studies conducted by ATS Labs, neutralization confirmation testing met the acceptance criterion of growth within $1 \log_{10}$ of the numbers control. In the study conducted by Reckitt Benckiser Inc., neutralizer efficacy testing showed positive growth of the microorganisms. When reported, viability controls were positive for growth. When reported, purity controls were reported as pure. When reported, test system verification controls confirmed the identity of the challenge microorganisms. Sterility controls did not show growth. The registrant must provide a rationale for the lower carrier count controls.

3. The submitted efficacy data do not support the use of the product, Sting (also known as Formula 1435-014), as a sanitizer against the following microorganisms on hard, non-porous, non-food contact surfaces in the presence of a 5% organic soil load for a 30-second contact time:

<i>Salmonella enterica</i>	MRID 477640-29
Methicillin Resistant <i>Staphylococcus aureus</i> (ATCC 33591)	MRID 477640-31

Although a bacterial reduction of at least 99.9 percent over the parallel control was observed within 5 minutes (i.e., 30 seconds specifically), both the non-active control and the zero-time control did not demonstrate an average of at least 7.5×10^5 surviving organisms, which is the criterion set forth in ASTM 1153. [That is, one of the two controls demonstrated an average of at least 7.5×10^5 surviving organisms, and one of the two controls did not.] Neutralizer efficacy testing showed positive growth of the microorganisms. When reported, viability controls were positive for growth. Test system verification controls confirmed the identity of the challenge microorganisms. Sterility controls did not show growth. The registrant must provide a rationale for the lower carrier count controls.

VII RECOMMENDATIONS

A. Recommendations Regarding Disinfectant Claims

1. The proposed label claims that the product, Sting, is an effective disinfectant against the following microorganisms on pre-cleaned, hard, non-porous surfaces for a 10-minute contact time (30-second contact time against Human immunodeficiency type 1 virus):

Staphylococcus aureus
Pseudomonas aeruginosa
Salmonella enterica
Respiratory syncytial virus
Herpes simplex virus type 1
Human immunodeficiency virus type 1
Influenza A virus (Strain New Caledonia/20/99)
Avian influenza (H5N1) virus
Human coronavirus

These claims are acceptable as they are supported by the submitted data.

2. The proposed label claims that the product, Sting, is an effective disinfectant against *E. coli*. [See page 7, 15th item from page bottom, of the proposed label.] Data were not provided to support this claim. This claim must be deleted from the proposed label.

B. Recommendations Regarding Fungistat Claims

1. The proposed label claims that the product, Sting, is an effective fungistat against *Aspergillus niger* on pre-cleaned, hard, non-porous surfaces for a 10-minute contact time. This claim is acceptable as it is supported by the submitted data.

C. Recommendations Regarding Sanitizer Claims

1. The proposed label claims that the product, Sting, is an effective sanitizer against the following microorganisms on pre-cleaned, hard, non-porous, non-food contact surfaces for a 30-second contact time:

Staphylococcus aureus
Enterobacter aerogenes
Escherichia coli O157:H7
Staphylococcus aureus MRSA, Community Acquired MRSA (ATCC BAA-1556)

These claims are acceptable as they are supported by the submitted data. Note that "(E. coli)" must be corrected to read "(E. coli O157:H7)" on page 6 of the proposed label.

2. The proposed label claims that the product, Sting, is an effective sanitizer against the following microorganisms on pre-cleaned, hard, non-porous, non-food contact surfaces for a 30-second contact time:

Streptococcus pyogenes

Listeria monocytogenes
Campylobacter jejuni
Staphylococcus aureus MRSA (ATCC 33592)

Data provided do not support these claims. As noted in the "Conclusions" section of this report, the carrier quantitation control (or non-active control and dried recovery control for the study against Methicillin Resistant *Staphylococcus aureus* (ATCC 33592)) did not demonstrate an average of at least 7.5×10^5 surviving organisms, which is the criterion set forth in ASTM 1153. The registrant must provide a rationale for the lower carrier count controls, before the Agency can consider this claim.

3. The proposed label claims that the product, Sting, is an effective sanitizer against the following microorganisms on pre-cleaned, hard, non-porous, non-food contact surfaces for a 30-second contact time:

Salmonella enterica
Staphylococcus aureus MRSA, Hospital Acquired MRSA (ATCC 33591)

Data provided do not support these claims. As noted in the "Conclusions" section of this report, both the non-active control and the zero-time control did not demonstrate an average of at least 7.5×10^5 surviving organisms, which is the criterion set forth in ASTM 1153. [i.e., one of the two controls demonstrated an average of at least 7.5×10^5 surviving organisms, and one of the two controls did not.] The registrant must provide a rationale for the lower carrier count controls, before the Agency can consider this claim.

D. Miscellaneous Recommendations Regarding Label Claims

1. The proposed label claims that the product is an effective one-step cleaner. [See page 4 of the proposed label.] This claim must be deleted.

2. The "Advisory Statements" section on page 10 of the proposed label states that the product is not recommended for use on glass, mirrors, and polished wood. This is contradicted on page 12 of the proposed label, under the "Surface Types" section. This inconsistency must be addressed.

3. The following revisions to the proposed label must be made:

- On page 5 of the proposed label, remove the claim "removes allergens at the source". This claim has not been demonstrated.
- ✎ - On page 5 of the proposed label, remove the claim "removes X% of allergens". This claim has not been demonstrated.
- On pages 12 and 13 of the proposed label, change "enamel tile" to read "baked enamel tile." Enamel is a porous surface.
- On page 12 of the proposed label, change "outdoor furniture" to read "outdoor furniture (except cushions and woodframes)."

- On page 13 of the proposed label, change “ceramic” to read “glazed ceramic.” Ceramic is a porous surface.
 - On page 13 of the proposed label, change “granite” to read “sealed granite.” Granite is a porous surface.
 - On page 13 of the proposed label, change “terra cotta” to read “sealed terra cotta.” Terra cotta is a porous surface.
4. The following revisions to the proposed label are recommended:
- Under the “Ingredients Statement” of the proposed label, change “40%, C12” to read “40% C12.”
 - Under the “For pouch packaging” section on page 9 of the proposed label, change “then done” to read “when done.”
 - The directions to disinfect and to sanitize on page 10 of the proposed label state to pre-clean surfaces. Efficacy was demonstrated in the presence of a 5% organic soil load. The “Pre-clean surface” instruction could be revised to read “Pre-clean heavily soiled surfaces,” if desired.
 - On page 12 of the proposed label, delete “small surfaces” from the list of surfaces.
 - On page 13 of the proposed label, change “Plexiglass” to read “Plexiglas.”
 - On page 13 of the proposed label, the “Special Instructions for Cleaning and Decontamination Against HIV-1” specify a 10-minute contact time. Efficacy was demonstrated for a 30-second contact time. The contact time could be revised, if desired.
 - On page 14 of the proposed label under the “Terminal Sterilant Statement,” change “or otherwise enters” to read “or otherwise enter.”
 - On page 14 of the proposed label under the third “Storage and Disposal” section of the product label, change “or for recycling” to read “or offer for recycling.”
 - On page 14 of the proposed label under the fourth and last “Storage and Disposal” section on the product label, change “– refill container >>” to read “– non-refillable container >>.”

E. Other Recommendations

1. The following revisions to EPA Form 8570-35 (Data Matrix) are recommended:

- On page 1 of the Data Matrix, change “Enforcement of Analytical Method” to read “Enforcement Analytical Method.”

- On page 3 of the Data Matrix, change "Particle Coefficient" to read "Partition Coefficient."
- On page 4 of the Data Matrix, change "*S. auereus*" to read "*S. aureus*."