UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION,
PESTICIDES
AND TOXIC
SUBSTANCES

April 11, 2009

MEMORANDUM

Subject: **E**fficacy Review for EPA File Symbol No. 777-RNI, Gattuso GP
DP Barcode: 362066

From: Tajah L. Blackburn, Ph.D., Microbiologist
Efficacy Evaluation Team
Product Science Branch
Antimicrobials **D**ivision (7510P)
4/7/09

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

To: Tracy Lantz PM 34/ Stacey Grisby
Regulatory Management Branch II
Antimicrobials Division (7510P)

Applicant: Reckitt Benckiser, Inc.
Morris Corporate Center IV
399 Interspace Parkway
Parsippany, NJ 07054-0225

Formulations from Label

Active Ingredient(s)	% by wt.
Citric Acid	3.5%
Other Ingredients	96.5%
Total	100.0%

I BACKGROUND

The product, Gattuso GP (EPA File Symbol 777-RNI), is a new product. The applicant requested to register the ready-to-use product for use as a disinfectant (bactericide, virucide), sanitizer, and deodorizer on hard, non-porous surfaces in household, institutional, commercial, and hospital or medical environments. The studies in this data package were submitted to the Agency in response to a letter from the Agency to the applicant (dated November 5, 2008). Studies were conducted at Reckitt Benckiser Inc., Microbiology Laboratory, located at One Phillips Parkway, in Montvale, NJ 07645.

This data package contained a letter from the applicant to EPA (dated February 5, 2009), EPA Form 8570-1 (Application for Pesticide), EPA Form 8570-4 (Confidential Statement of Formula; for the basic formulation and three alternate formulations), EPA Form 8570-34 (Certification with Respect to Citation of Data), EPA Form 8570-35 (Data Matrix), nine studies (MRID 476702-12 through 476702-20), Statements of No Data Confidentiality Claims for all nine studies, and the proposed label.

Note: EPA Form 8570-4 (Confidential Statement of Formula) contains Confidential Business Information. Data or information claimed by the applicant to be FIFRA confidential has not been included in this report.

Note: The laboratory reports describe studies conducted for the products, Formula Number 2262-144A and Formula Number 1333-117A. Information in the data package indicates that Formula Number 1262-144A is the basic formulation of the product, Gattuso GP, and Formula Number 1333-117A is an alternate formulation of the product, Gattuso GP.

Note: Per the registrant's letter, dated February 5, 2009, a re-cap of numerous discussions with the Agency was provided. Briefly,

- An Agency email, dated August 19, 2008 (Adam Heyward and Mary Pisculli) (as it relates to efficacy data), it was included that "RB had conducted hospital confirmatory efficacy data on the revised formulation."

- An Agency email, dated November 14, 2008 (Adam Heyward and Hal Ambuter) and RB email, dated November 17, 2008 (Christine Dellanno and Adam Heyward) (as it related to efficacy data), it was determined that the (1) "Agency will accept MRID Nos. 474557-18, -19, -20, and -31 which utilizes coarse filtration step provided that RB conduct and submit hospital confirmatory testing without utilizing a coarse filtration (See Volume 21); (2) "Agency will accept MRID Nos. 474557-29 and -30 provided RB amend the reports to address the dried recovery carrier counts (See Volumes 17 and 18)." Briefly, "Volume 17 provides dried recovery carrier counts that do not exceed the minimum 1.0×10^4 dried recovery carrier count as in the AOAC Disinfectant Assays but not the 7.5×10^5 average per the ASTM E1153-03 method. RB would like to request that this study be considered for acceptance because the Non-Food Contact Sanitization Test Methods were developed for *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Enterobacter* aerogenes organisms but other organisms were not considered then developing the dried recovery counts at 7.5×10^5. The data for Volume 17 which was performed for MRID No. 474557-29 still provided a

99.9% reduction and have met the DIS/TSS-10 and the Subdivision G 91-2(j) requirements for a non-food contact sanitizer." Furthermore, "Volume 18 provides the dried recovery carrier counts that exceeds the 7.5 x 10^5 average as per the EPA request for MRID No, 474557-30 against Enterobacter aerogenes. This study along with MRID [No.] 474557-30 concludes that Gattuso GP is an effective product as a sanitizer for non-food contact surfaces." Lastly, "do to time and resource restraints, RB was not able to conduct the Agency requested fungistat testing utilizing a 95% humidity conditions. Therefore, at this time, we have removed all references pertaining to the removal of *Aspergillus niger*."

II USE DIRECTIONS

The product is designed for disinfecting and sanitizing hard, non-porous surfaces, including: bathtubs, cabinets, counter tops, faucets, fixtures, floors, shower curtains, shower stalls, showers, sinks, toilet bowl exteriors, urinals, and vanity tops. The proposed label indicates that the product may be used on hard, non-porous surfaces including: enamel, glass, glazed ceramic, glazed porcelain, glazed tile, laminated plastic, linoleum, metal (e.g., chrome, stainless steel), and vinyl. Directions on the proposed label provide the following information regarding use of the product: Pre-clean surfaces. Spray surfaces until thoroughly wet. To disinfect, let stand for 5 minutes. To sanitize, let stand for 30 seconds. Wipe off with a clean, damp cloth or sponge.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments

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

Disinfectants for Use in Hospital or Medical Environments; Confirmatory Efficacy Data Requirements

Under certain circumstances, an applicant is permitted to rely on previously submitted efficacy data to support an application or amendment for registration of a product and to submit only minimal confirmatory efficacy data on his own product to demonstrate his ability to produce an effective formation. This includes a minor formulation change (e.g., a change in an inert ingredient) in a registered product. Confirmatory data must be developed on the applicant=s own finished product. For hospital disinfectants, 10 carriers on each of 2 samples representing 2 different product lots must be tested against *Salmonella enterica* (ATCC 10708; formerly *Salmonella choleraesuis*), *Staphylococcus aureus* (ATCC 6538), and *Pseudomonas aeruginosa*

(ATCC 15442) using either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants Method. Killing on all carriers is required.

Virucides

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

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. [Testing requirements in EPA DIS/TSS-10 may be used.] 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.

IV SYNOPSIS OF SUBMITTED EFFICACY DATA

Note: Eight of the nine studies in this data package were previously provided to and reviewed by the Agency. The eight reports have been amended to remove formic acid as an active ingredient, and correct minor report details.

1. MRID 476702-12 "Disinfectant Efficacy Testing In The Presence of Organic Soil," Test Organism: *Staphylococcus aureus* (ATCC 6538), for Formula Number 1262-144A, by Kyle T. Smith. Study conducted at Reckitt Benckiser Inc. Study completion date – March 11, 2008. Amended final report date – December 16, 2008. Master Schedule No. 2007-0111.

Note: This study was previously assigned MRID 474557-18 (based on a comparison of the master schedule numbers).

This study was conducted against *Staphylococcus aureus* (ATCC 6538). Three lots (Lot Nos. 1333-030, 1333-056, and 1333-058) of the product, Formula Number 1262-144A, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. At least one of the product lots tested (i.e., Lot No. 1333-030) was at least 60 days old at the time of testing. The product was received ready-to-use, as a trigger spray. 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 48±2 hours at a target temperature of 35±2.5°C (which differs from the AOAC method specification of 48 hours for all bacterial cultures except *Pseudomonas aeruginosa*); and (2) the final culture transfer was coarse filtered. Horse serum was added to the culture to achieve a 5% organic soil load. Sixty (60) glass slide carriers (20 mm x 25 mm) were inoculated with 0.01 mL of a 48±2 hour old suspension of the test organism. Inoculum was uniformly spread over the surface of the carriers. The carriers were dried for 40-65 minutes at 31.6-37.5°C (which differs from the AOAC method specification of 30-40 minutes at 37°C). For each lot of product, separate carriers were sprayed (2-3 pumps) with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 5 minutes at 21.9-22.6°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth to neutralize. Subcultures then were gently agitated or shaken. All subcultures were incubated for at least 68 hours at 32.7-37.9°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 carrier count, test system verification (i.e., purity, identity), sterility, and neutralizer efficacy.

Note: The initial laboratory report was amended to remove formic acid as an active ingredient, correct page breaks, include page 24, and correct the name of the Study Sponsor.

Note: The study was conducted according to the GLP standards with the following exception: An expired reagent (Gram Crystal Violet, C#1202) was used in the Gram stain procedure. The laboratory report stated: "It has been concluded that although an expired reagent was used in the Verification of the Test System Assay, this did not affect

the results of each Gram Stain. In addition, sufficient information was provided from the other Test System Verification components to determine that the organism used in each assay was the test system, *Staphylococcus aureus*.

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

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable, with the exception of the coarse filtration culture preparation step. Note that a letter from the applicant to EPA (dated February 5, 2009) states that – based on an Agency email dated November 14, 2008 – the Agency will accept MRID 474557-18 (now re-submitted as MRID 476702-12) which utilizes a "coarse filtration" step provided that the applicant conducts and submits hospital confirmatory testing without utilizing a "coarse filtration" step. **The applicant conducted such testing; however, the laboratory report identified as Volume 21 was rejected by the Agency and is not yet available for review.**

2. MRID 476702-13 "Disinfectant Efficacy Testing In The Presence of Organic Soil," Test Organism: *Salmonella enterica* (ATCC 10708), for Formula Number 1262-144A, by Kyle T. Smith. Study conducted at Reckitt Benckiser Inc. Study completion date – March 11, 2008. Amended final report date – January 27, 2009. Master Schedule No. 2007-0116.

Note: This study was previously assigned MRID 474557-19.

This study was conducted against *Salmonella enterica* (ATCC 10708). Three lots (Lot Nos. 1333-036, 1333-056, and 1333-058) of the product, Formula Number 1262-144A, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. Each of the three product lots tested was at least 60 days old at the time of testing. The product was received ready-to-use, as a trigger spray. 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 48±2 hours at a target temperature of 35±2.5°C (which differs from the AOAC method specification of 48 hours for all bacterial cultures except *Pseudomonas aeruginosa*); and (2) the final culture transfer was coarse filtered. Horse serum was added to the culture to achieve a 5% organic soil load. Sixty (60) glass slide carriers (20 mm x 25 mm) were inoculated with 0.01 mL of a 48±2 hour old suspension of the test organism. Inoculum was uniformly spread over the surface of the carriers. The carriers were dried for 40-42 minutes at 33.5-35.1°C (which differs from the AOAC method specification of 30-40 minutes at 37°C). For each lot of product, separate carriers were sprayed (2-3 pumps) with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 5 minutes at 21.6-21.9°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth to neutralize. Subcultures then were gently agitated or shaken. All subcultures were incubated for at least 69 hours at 33.9-35.8°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 carrier count, test system verification (i.e., purity, identity), sterility, and neutralizer efficacy.

Note: The initial laboratory report was amended to remove formic acid as an active ingredient and correct two references to the product.

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

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable, with the exception of the coarse filtration culture preparation step. Note that a letter from the applicant to EPA (dated February 5, 2009) states that – based on an Agency email dated November 14, 2008 – the Agency will accept MRID 474557-19 (now re-submitted as MRID 476702-13) which utilizes a "coarse filtration" step provided that the applicant conducts and submits hospital confirmatory testing without utilizing a "coarse filtration" step. **The applicant conducted such testing; however, the laboratory report identified as Volume 21 was rejected by the Agency and is not yet available for review.**

3. MRID 476702-14 "Disinfectant Efficacy Testing In The Presence of Organic Soil," Test Organism: *Pseudomonas aeruginosa* (ATCC 15442), for Formula Number 1262-144A, by Kyle T. Smith. Study conducted at Reckitt Benckiser Inc. Study completion date – March 11, 2008. Amended final report date – January 27, 2009. Master Schedule No. 2007-0117.

Note: This study was previously assigned MRID 474557-20.

This study was conducted against *Pseudomonas aeruginosa* (ATCC 15442). Three lots (Lot Nos. 1333-030, 1333-056, and 1333-058) of the product, Formula Number 1262-144A, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. Each of the three product lots tested was at least 60 days old at the time of testing. The product was received ready-to-use, as a trigger spray. 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 48±2 hours at a target temperature of 35±2.5°C (which differs from the AOAC method specification of 18-24 hours); and (2) the final culture transfer was coarse filtered. Horse serum was added to the culture to achieve a 5% organic soil load. Sixty (60) glass slide carriers (20 mm x 25 mm) were inoculated with 0.01 mL of a 48±2 hour old suspension of the test organism. Inoculum was uniformly spread over the surface of the carriers. The carriers were dried for 39-41 minutes at 32.7-34.3°C (which differs from the AOAC method specification of 30-40 minutes at 37°C). For each lot of product, separate carriers were sprayed (2-3 pumps) with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 5 minutes at 21.6-21.9°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth to neutralize. Subcultures then were agitated or shaken. All subcultures were incubated for at least 69 hours at 33.5-35.4°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 carrier count, test system verification (i.e., purity, identity), sterility, and neutralizer efficacy.

Note: The initial laboratory report was amended to remove formic acid as an active ingredient and correct three references to the product.

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

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable, with the exception of the coarse filtration culture preparation step. Note that a letter from the applicant to EPA (dated February 5, 2009) states that -- based on an Agency email dated November 14, 2008 -- the Agency will accept MRID 474557-20 (now re-submitted as MRID 476702-14) which utilizes a "coarse filtration" step provided that the applicant conducts and submits hospital confirmatory testing without utilizing a "coarse filtration" step. **The applicant conducted such testing; however, the laboratory report identified as Volume 21 was rejected by the Agency and is not yet available for review.**

4. MRID 476702-15 "Inactivation of Influenza A Virus (H1N1/Avian Flu) in the Presence of Organic Matter" for Formula Number 1262-144A, by Kyle T. Smith. Study conducted at Reckitt Benckiser Inc. Study completion date -- March 11, 2008. Amended final report date -- January 27, 2009. Master Schedule No. 2007-0129.

Note: This study was previously assigned MRID 474557-21.

This study was conducted against Influenza A virus (H1N1/Avian Flu) (Strain A/Malaya/302/54; ATCC VR-98), using Rhesus monkey kidney cells (RMK cells; obtained from ViroMed Laboratories, Minneapolis, MN) as the host system. Two lots (Lot Nos. 1333-030 and 1333-056) of the product, Formula Number 1262-144A, were tested according to Reckitt Benckiser Inc.'s protocol, "Inactivation of Influenza A virus (H1N1/Avian flu) in the Presence of Organic Matter," dated November 12, 2007 (copy provided). The product was received ready-to-use, as a trigger spray. The stock virus culture contained at least a 5% organic soil load. Films of virus were prepared by spreading 0.3 mL of virus inoculum uniformly over pre-marked undersides of separate sterile polystyrene Petri dishes. The virus films were dried for 43-59 minutes at 25.5°C at 20.44-23.38% humidity. Five replicates per product lot were tested. For each lot of product, separate dried virus films were sprayed (2-3 pumps) with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 19.2-20.3°C. Following exposure, the plates were neutralized with 2.0 mL of fetal bovine serum. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with gentamicin. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.2 mL of the dilutions. The plates were re-fed using 1-2 mL of Minimum Essential Medium supplemented with gentamicin and 1% serum. The cultures were incubated for 7 days at 35.8-36.2°C in 1.941-8.271% CO_2. Following incubation, the cultures were examined for the presence or absence of cytopathic effects (i.e., cellular detachment, degeneration of the cell sheet, giant cell/syncytial formation) and cytotoxicity. Controls included those for host cell viability, dried virus count, cytotoxicity, and neutralization effectiveness. Viral and cytotoxicity titers were calculated by the method of Reed and Muench.

Note: The initial laboratory report was amended to remove formic acid as an active ingredient, correct the name of the Study Director, and clarify a statement referring to actual test parameters.

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

5. MRID 476702-16 "Inactivation of Poliovirus Type 1 in the Presence of Organic Matter" for Formula Number 1262-144A, by Kyle T. Smith. Study conducted at Reckitt Benckiser Inc. Study completion date – March 18, 2008. Amended final report date – December 16, 2008. Master Schedule No. 2007-0139.

Note: This study was previously assigned MRID 474557-24.

This study was conducted against Poliovirus type 1 (Strain Chat; ATCC VR-192), using Vero cells (obtained from ViroMed Laboratories, Minneapolis, MN) as the host system. Three lots (Lot Nos. 1333-030, 1333-056, and 1333-058) of the product, Formula Number 1262-144A, were tested according to Reckitt Benckiser Inc.'s protocol, "Inactivation of Poliovirus type 1 in the Presence of Organic Matter," dated December 10, 2007 (copy provided). The product was received ready-to-use, as a trigger spray. Testing was conducted on December 12, 2007 and January 11, 2008. 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 0.3 mL of virus inoculum uniformly over pre-marked undersides of separate sterile polystyrene Petri dishes. The virus films were dried for 44-72 minutes at 25.2-25.6° at 27.48-31.80% humidity. Five replicates per product lot were tested. For each lot of product, separate dried virus films were sprayed (2-3 pumps) with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 5 minutes at 19.8-20.8°C. Following exposure, the plates were neutralized with 2.0 mL of fetal bovine serum. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with gentamicin. Vero cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated for 5 days at 36.5-37.0°C in 3.340-7.464% CO_2. Following incubation, the cultures were examined for the presence or absence of cytopathic effects (i.e., refractile rounding and sloughing) and cytotoxicity. Controls included those for host cell viability, dried virus count, cytotoxicity, and neutralization effectiveness. Viral and cytotoxicity titers were calculated by the method of Reed and Muench.

Note: The initial laboratory report was amended to remove formic acid as an active ingredient and correct the name of the Study Director.

Note: The laboratory reported a failed study set up on December 12, 2007. In that study, a recoverable virus titer of at least 10^4 was not achieved. The laboratory did not accept the assay. These data were not used to evaluate efficacy of the product. Testing was repeated on January 11, 2008. See page 12 and Attachment 3 of the laboratory study.

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

6. MRID 476702-17 "Sanitization Study: Quantitative Reduction of Bacteria on a Non-Food Contact Surface," Test Organism: *Salmonella enterica* (ATCC 10708), for Formula Number 1262-144A, by Kyle T. Smith. Study conducted at Reckitt Benckiser Inc. Study completion date – March 11, 2008. Amended final report date – December 16, 2008. Master Schedule No. 2007-0134.

Note: This study was previously assigned MRID 474557-29.

This study was conducted against *Salmonella enterica* (ATCC 10708). Three lots (Lot Nos. 1333-030, 1333-056, and 1333-058) of the product, Formula Number 1262-144A, were tested. The laboratory report referenced a modification of ASTM E1153-87 (Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces). The product was received ready-to-use, as a trigger spray. Testing was conducted on November 29, 2007 and December 4, 2007. Horse serum was added to the culture to achieve a 5% organic soil load. Five sterile glass slide carriers (20 mm x 25 mm) per product lot were inoculated with 0.02 mL of a 24±2 hour old suspension of the test organism. The culture was spread evenly over the surface of the carriers. The carriers were dried for 40 minutes at 35.4-35.8°C. For each lot of product, separate carriers were sprayed (2-3 pumps) with the product at a distance of 6-8 inches from the carrier surface. Each carrier remained in contact with the product for 30 seconds at 21.9°C. Following exposure, individual carriers were transferred to 20 mL of D/E Broth to neutralize. All subcultures were incubated for at least 50 hours at 34.3-36.2°C. Following incubation, the colonies were counted. Controls included a non-active control and those for inoculum count, dried recovery carrier count, test system verification (i.e., purity, identity), sterility, and neutralizer efficacy.

Note: The initial laboratory report was amended to remove formic acid as an active ingredient.

Note: The laboratory reported a failed study set up on November 29, 2007. In that study, the non-active control and dried recovery carrier control values were below the required minimum range of 1×10^4 CFU/carrier. In addition, it was determined that 10 mL of the neutralizing medium (i.e., D/E Broth) was insufficient in neutralizing the product. The laboratory did not accept the assay. These data were not used to evaluate efficacy of the product. Testing was repeated on December 4, 2007. See page 15 and Appendix B of the laboratory report.

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

7. MRID 476702-18 "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 Number 1262-144A, by Kyle T. Smith. Study conducted at Reckitt Benckiser Inc. Study completion date – March 11, 2008. Amended final report date – December 16, 2008. Master Schedule No. 2007-0135.

Note: This study was previously assigned MRID 474557-30.

This study was conducted against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC t3048). Three lots (Lot Nos. t333-030, 1333-056, and t333-058) of the product, Formula Number 1262-t44A, were tested. The laboratory report referenced a modification of ASTM E t153-87 (Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces). Each of the three product lots tested was at least 60 days old at the time of testing. The product was received ready-to-use, as a trigger spray. Horse serum was added to each culture to achieve a 5% organic soil load. Five sterile glass slide carriers (20 mm x 25 mm) per product lot per microorganism were inoculated with 0.03 mL of a 24 hour old suspension of a test organism. The culture was spread evenly over the surface of the carriers. The carriers were dried for 51-55 minutes at 35. t-35.4°C. For each lot of product, separate carriers were sprayed (2-3 pumps) with the product at a distance of 6-8 inches from the carrier surface. Each carrier was exposed to the product for 30 seconds at 21.9-22.3°C. Following exposure, individual carriers were transferred to 20 mL of D/E Broth to neutralize. All subcultures were incubated for at least 68 hours at 29.4-36.2°C. Following incubation, the colonies were counted. Controls included a non-active control and those for inoculum count, dried recovery carrier count, test system verification (i.e., purity, identity), sterility, and neutralizer efficacy.

Note: The initial laboratory report was amended to remove formic acid as an active ingredient and correct the study title on the quality assurance unit statement page.

8. MRID 476702-19 "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 Number 1333-117A, by Kyle T. Smith. Study conducted at Reckitt Benckiser Inc. Study completion date – April 3, 2008. Amended final report date – December 16, 2008. Master Schedule No. 2008-0044.

Note: This study was previously assigned MRID 474557-31.

This confirmatory study was conducted against *Staphylococcus aureus* (ATCC 6538), *Salmonella enterica* (ATCC 10708), and *Pseudomonas aeruginosa* (ATCC t5442). Three lots (Lot Nos. t333-173, 1333-175, and 1333-176) of the product, Formula Number 1333-t t7A, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. The product was received ready-to-use, as a trigger spray. Cultures of the challenge microorganisms were prepared in accordance with the published AOAC method, with the following exceptions: (1) the *Staphylococcus aureus* and *Salmonella enterica* cultures were incubated for 48±2 hours at a target temperature of 35±2.5°C (which differs from the AOAC method specification of 48 hours for all bacterial cultures

except *Pseudomonas aeruginosa*); (2) the *Pseudomonas aeruginosa* culture was incubated for 48±2 hours at a target temperature of 35±2.5°C (which differs from the AOAC method specification of 18-24 hours); and (3) the final culture transfers were coarse filtered. Horse serum was added to the cultures to achieve a 5% organic soil load. Ten (10) glass slide carriers (20 mm x 25 mm) were inoculated with 0.01 mL of a 48±2 hour old suspension of a test organism. Inoculum was uniformly spread over the surface of the carriers. The carriers were dried for 40-42 minutes at 33.9-34.8°C (which differs from the AOAC method specification of 30-40 minutes at 37°C). For each lot of product, separate carriers were sprayed (2-3 pumps) with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 5 minutes at 22.1-22.9°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth to neutralize. Subcultures then were agitated or shaken. All subcultures were incubated for at least 67 hours at 33.5-35.5°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 carrier count, test system verification (i.e., purity, identity), sterility, and neutralizer efficacy.

Note: The initial laboratory report was amended to remove formic acid as an active ingredient.

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

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable, with the exception of the coarse filtration culture preparation step. Note that a letter from the applicant to EPA (dated February 5, 2009) states that – based on an Agency email dated November 14, 2008 – the Agency will accept MRID 474557-31 which utilizes a "coarse filtration" step provided that the applicant conducts and submits hospital confirmatory testing without utilizing a "coarse filtration" step. **The applicant conducted such testing; however, the laboratory report identified as Volume 21 was rejected by the Agency and is not yet available for review.**

> **9. MRID 476702-20 "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 Number 1333-117A, by Kyle T. Smith. Study conducted at Reckitt Benckiser Inc. Study completion date – December 16, 2008. Master Schedule No. 2008-0191.**

This confirmatory study was conducted against *Staphylococcus aureus* (ATCC 6538), *Salmonella enterica* (ATCC 10708), and *Pseudomonas aeruginosa* (ATCC 15442). Three lots (Lot Nos. 1453-108, 1453-110, and 1453-111) of the product, Formula Number 1333-117A, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. [The tested product formulation did not contain formic acid.] The product was received ready-to-use, as a trigger spray. Cultures of the challenge microorganisms were prepared in accordance with the published AOAC method, with the following exceptions: (1) the *Staphylococcus aureus* and *Salmonella enterica* cultures were incubated for 48±2 hours at a target temperature of 35±2.5°C (which differs from the

AOAC method specification of 48 hours for all bacterial cultures except *Pseudomonas aeruginosa*); (2) the *Pseudomonas aeruginosa* culture was incubated for 48±2 hours at a target temperature of 35±2.5°C (which differs from the AOAC method specification of 18-24 hours); and (3) the final culture transfers were coarse filtered. Horse serum was added to the cultures to achieve a 5% organic soil load. Ten (10) glass slide carriers (20 mm x 25 mm) were inoculated with 0.01 mL of a 48±2 hour old suspension of a test organism. Inoculum was uniformly spread over the surface of the carriers. The carriers were dried for 41-42 minutes at 32.9-34.6°C (which differs from the AOAC method specification of 30-40 minutes at 37°C). For each lot of product, separate carriers were sprayed (2-3 pumps) with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 5 minutes at 22.3-23.0°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth to neutralize. Subcultures then were agitated or shaken. All subcultures were incubated for at least 47 hours at 33.2-34.9°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 carrier count, test system verification (i.e., purity, identity), sterility, and neutralizer efficacy.

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

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

V RESULTS

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested				Dried Recovery Carrier Count (CFU/ Carrier)
		Lot No. 1333-030	Lot No. 1333-036	Lot No. 1333-056	Lot No. 1333-058	
476702-12	*Staphylococcus aureus*	0/60	---	0/60	0/60	1.36×10^6 to 5.5×10^6
476702-13	*Salmonella enterica*	---	1/60	0/60	0/60	4.1×10^4 to 2.61×10^5
476702-14	*Pseudomonas aeruginosa*	1/60	---	0/60	0/60	1.12×10^6 to 8.3×10^6

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested			Dried Recovery Carrier Count (CFU/ Carrier)
		Lot No. 1333-173	Lot No. 1333-175	Lot No. 1333-176	
Confirmatory Data					
476702-19	*Staphylococcus aureus*	0/10	0/10	0/10	8.7×10^5 to 1.12×10^6
476702-19	*Salmonella enterica*	0/10	0/10	0/10	1.61×10^5 to 6.0×10^5
476702-19	*Pseudomonas aeruginosa*	0/10	0/10	0/10	5.0×10^5 to 1.28×10^6
		Lot No. 1453-108	**Lot No. 1453-110**	**Lot No. 1453-111**	
Confirmatory Data; Product Formulated without Formic Acid					
476702-20	*Staphylococcus aureus*	0/10	0/10	0/10	3.8×10^5 to 4.2×10^6
476702-20	*Salmonella enterica*	0/10	0/10	0/10	5.0×10^5 to 7.6×10^5
476702-20	*Pseudomonas aeruginosa*	0/10	0/10	0/10	3.1×10^6 to 4.9×10^6

MRID Number	Organism	Results			Dried Virus Control
			Lot No. 1333-030	Lot No. 1333-056	Lot No. 1333-058	
476702-15	Influenza A virus (H1N1/Avian Flu)	10^{-1} to 10^{-2} dilutions	Cytotoxicity		----	$10^{7.17}$ ID_{50}/ 0.2 mL
		10^{-3} to 10^{-5} dilutions	Complete inactivation		----	
		ID_{50}/0.2 mL	$\leq 10^{2.50}$	$\leq 10^{2.50}$	----	
		Log reduction	≥ 4.67 $\log_{10}$		----	
476702-16	Poliovirus type 1	10^{-2} to 10^{-5} dilutions	Complete inactivation			$10^{7.33}$ ID_{50}/ 0.1 mL
		ID_{50}/0.1 mL	$\leq 10^{1.50}$	$\leq 10^{1.50}$	$\leq 10^{1.50}$	

MRID Number	Organism	Lot No.	Geometric Mean Surviving	Parallel Control	Percent Reduction
			(CFU/ carrier)		
476702-17	*Salmonella enterica*	1333-030	1.0×10^1	3.98×10^4	>99.9
		1333-056	1.0×10^1	3.98×10^4	>99.9
		1333-058	1.0×10^1	3.98×10^4	>99.9
476702-18	*Staphylococcus aureus*	1333-030	1.0×10^1	2.34×10^7	>99.9
		1333-056	1.0×10^1	2.34×10^7	>99.9
		1333-058	1.0×10^1	2.34×10^7	>99.9
	Enterobacter aerogenes	1333-030	1.32×10^1	1.65×10^5	>99.9
		1333-056	1.51×10^1	1.65×10^5	>99.9
		1333-058	1.51×10^1	1.65×10^5	>99.9

VI CONCLUSIONS

1. The submitted efficacy data (MRID 476702-12 through 476702-14) do not yet support the use of the product, Gattuso GP (also referred to as Formula Number 1262-144A), as a disinfectant with bactericidal activity against *Staphylococcus aureus*, *Salmonella enterica*, and *Pseudomonas aeruginosa* on hard, non-porous surfaces in the presence of a 5% organic soil load for a 5-minute contact time. **The Agency has stated that it would accept these three efficacy studies which utilize a "coarse filtration" step provided that the applicant conducts and submits hospital confirmatory testing without utilizing a "coarse filtration" step.** The **applicant conducted such testing; however, the laboratory report identified as Volume 21 was rejected by the Agency and is not yet available for review.** Killing was observed in the subcultures of at least 59 of the 60 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. Test system verification confirmed that the cultures were acceptable for use in the studies. Sterility controls did not show growth. Neutralizer efficacy testing showed positive growth of the microorganisms.

Note: The "Synopsis of 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, with the exception of the coarse filtration culture preparation step.

2. The submitted confirmatory efficacy data (MRID 476702-19 and -20) do not yet demonstrate that the product, Gattuso GP formulated without formic acid (also referred to as Formula Number 1333-117A), is an effective disinfectant against *Staphylococcus aureus*, *Salmonella enterica*, and *Pseudomonas aeruginosa* on hard, non-porous surfaces in the presence of a 5% organic soil load for a 5-minute contact time. **Results from confirmatory testing which does not use a "coarse filtration" step must be submitted to the Agency.** Complete killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Test system verification confirmed that the cultures were acceptable for use in the studies. Sterility controls did not show growth. Neutralizer efficacy testing showed positive growth of the microorganisms.

Note: The "Synopsis of 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, with the exception of the coarse filtration culture preparation step.

3. The submitted efficacy data support the use of the product, Gattuso GP (also referred to as Formula Number 1262-144A), 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 the contact time specified:

Influenza A virus (H1N1/Avian Flu)	30 seconds	MRID 476702-15
Poliovirus type 1	5 minutes	MRID 476702-16

Recoverable virus titers of at least 10^4 were achieved. In studies against Influenza A virus (H1N1/Avian Flu), cytoloxicity was observed in the 10^{-1} and 10^{-2} dilutions. Complete inactivation (no growth) was indicated in all higher dilutions tested. At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level. In studies against Poliovirus type 1, cytotoxicity was not observed. Complete inactivation (no growth) was indicated in all dilutions tested.

Note: It is unclear why the applicant re-submitted these two studies. Five virucidal studies were submitted in the initial data package.

4. The submitted efficacy data do support the use of the product, Gattuso GP (also referred to as Formula Number 1262-144A), 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:

Enterobacter aerogenes	MRID 476702-18
Salmonella enterica	MRID 476702-17
Staphylococcus aureus	MRID 476702-18

Bacterial reductions of at least 99.9 percent over the parallel controls were observed within 5 minutes (actually 30 seconds). At least one of the product lots tested against *Staphylococcus aureus* and *Enterobacter aerogenes* was at least 60 days old at the time of testing. Neutralizer efficacy testing showed positive growth of the microorganisms. Sterility controls did not show growth. In studies against *Salmonella enterica*, the dried recovery carrier count and the non-active control count failed to demonstrate an average of at least 7.5 x 10^5 surviving organisms, which is the criterion set forth in ASTM 1153. In studies against *Enterobacter aerogenes*, the non-active control count failed to demonstrate an average of at least 7.5 x 10^5 surviving organisms, which is the criterion set forth in ASTM 1153. **A letter from the applicant to EPA (dated February 5, 2009) states that – based on an Agency email dated November 14, 2008 – the Agency will accept MRID 474557-29 and 474557-30 (now re-submitted as MRID 476702-17 and 476702-18) provided that the reports are amended to address the dried recovery carrier counts. The amended reports do not address the dried recovery counts.** However, the letter from the applicant to EPA (dated February 5, 2009) requests that the Agency accept the study against *Salmonella enterica* because the dried recovery carrier count and the non-active control counts exceed 1 x 10^4 CFU/carrier, which is the criterion set forth in the AOAC disinfectant assays (even though the counts do not exceed the 7.5 x 10^5 criterion set forth in ASTM 1153) and that the Agency accept the study against *Enterobacter aerogenes* because the dried recovery carrier count exceeds the 7.5 x 10^5 criterion set forth in ASTM 1153. The Agency will accept the dried carrier counts as detailed in this current submission. In future submissions, the registrant should attempt to achieve the control carrier counts as documented in the method.

VII RECOMMENDATIONS

1. The proposed label claims that the product, Gattuso GP, is an effective disinfectant against *Staphylococcus aureus*, *Salmonella enterica*, and *Pseudomonas aeruginosa* on pre-cleaned, hard, non-porous surfaces for a 5-minute contact time. These claims are not yet supported by the submitted data. As stated in the "Conclusions" section of this

report, results from confirmatory testing which does not use a "coarse filtration" step must be submitted to the Agency.

2. The proposed label claims that the product, Gattuso GP, is an effective disinfectant against Poliovirus type t, Respiratory syncytial virus, and Rotavirus on pre-cleaned, hard, non-porous surfaces for a 5-minute contact time. These claims are acceptable as they are supported by the submitted data in this data package and/or the data package assigned D354310. However, virucidal claims cannot be extended to the product until disinfectant claims are accepted by the Agency.

3. The proposed label claims that the product, Gattuso GP, is an effective disinfectant against Influenza A virus (Hong Kong) and Influenza A virus (H tN1/Avian Flu) on pre-cleaned, hard, non-porous surfaces for a 30-second contact time. These claims are acceptable as they are supported by the submitted data in this data package and/or the data package assigned D3543 t0. However, virucidal claims cannot be extended to the product until disinfectant claims are accepted by the Agency

4. The proposed label claims that the product, Gattuso GP, is an effective sanitizer against *Enterobacter aerogenes*, *Salmonella enterica*, and *Staphylococcus aureus* on pre-cleaned, hard, non-porous, non-food contact surfaces for a 30-second contact time. These claims are supported by the submitted data.

5. The following changes must be made to the proposed label:

- Under the "Net Contents" listings on page t of the proposed label, change "1. t25 GLA" to read "1.125 GAL."

- Under the "Fast Acting Sanitizer" claims on page 4 of the proposed label, delete the reference to viruses.

- Under the disinfecting claims on page 5 of the proposed label, delete the following claim: "Kills t00% of bacteria and viruses."

- Under the surface types listed on page 8 of the proposed label, change "Enamal" to read "Baked enamel."

- Under the "Terminal Sterilant Statement" on page 8 of the proposed label, change "otherwise enters" to read "otherwise enter."

- On page 8 of the proposed label, the registrant must define "tough soil". Data was generated in the presence of 5% organic soil.