UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION,
PESTICIDES
AND TOXIC
SUBSTANCES

August 28, 2010

MEMORANDUM

Subject: Efficacy Review for EPA Reg. No. 75266-1, Activate 5.25% Institutional Bleach; DP Barcode: 379982

From: Tajah Blackburn, PhD., Microbiologist
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)

To: Wanda Henson PM32/Tom Luminello
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Applicant: Deardorff Fitzsimmons Corporation
PO Box 539
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Formulation from the Label:

Active Ingredient(s)	% by wt.
Sodium Hypochlorite	5.25%
Other Ingredients	94.75%
Total	100.00%

I BACKGROUND

The product, Activate 5.25% Institutional Bleach (EPA Reg. No. 75266-1), is an EPA-approved disinfectant for use on hard, non-porous surfaces in institutional, food service, animal care, and hospital or medical environments. The product is for use only with the Activate Bleach Dilution Sprayer, which automatically dilutes the product to a 10% solution. The applicant requested to amend the registration of this product to (1) modify the directions for use, and (2) add new disinfectant claims. Label directions indicate that the product is effective as a "one-step" disinfectant. Studies were conducted at Antimicrobial Test Laboratories, located at 3000 Joe DiMaggio Boulevard, Suite #32, in Round Rock, TX 78665; BioScience Laboratories, Inc., located at 300 N. Willson Avenue in Bozeman, MT 59715; and MICROBIOTEST, located at 105 Carpenter Drive in Sterling, VA 20164.

This data package contained a letter from the applicant's representative to EPA (dated June 15, 2010), EPA Form 8570-1 (Application for Pesticide), EPA Form 8570-34 (Certification with Respect to Citation of Data), EPA Form 8570-35 (End-Use Data Matrix), twelve studies (MRID 481310-01 through 481310-12), Statements of No Data Confidentiality Claims for all twelve studies, and the proposed label.

II USE DIRECTIONS

The product is designed for disinfecting hard, non-porous surfaces, including: cabinets, cages, ceilings, countertops, feeding and watering equipment, instruments, kennels, utensils, and walls. The proposed label indicates that the product may be used on hard, non-porous surfaces including: ceramic tile, chrome, enamel, glass, laminated plastic, porcelain, sealed fiberglass, and stainless steel. The Activate Bleach Dilution Sprayer automatically dilutes and mixes the product to a ready-to-use 10% solution. Directions on the proposed label provide the following information regarding use of the product as a disinfectant: [Remove gross dirt and debris.] Spray product 6-8 inches from surface until surface is thoroughly wet. Let stand for 2 minutes (30 seconds against HIV-1 and HBV). Wipe with a clean cloth or paper towel and allow to air dry.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments

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

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

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

Virucides

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

Virucides – Novel Virus Protocol Standards

To ensure that a virus protocol has been adequately validated, data should be provided from at least 2 independent laboratories for each product tested (i.e., 2 product lots per laboratory).

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 481310-01 "AOAC Germicidal Spray Products as Disinfectants Test Method," Test Organism: *Staphylococcus aureus* (ATCC 6538), for Activate 5.25% Institutional Bleach, by Jason Williams. Study conducted at Antimicrobial Test Laboratories. Study completion date – April 26, 2010. Study Identification Number GLP1017.

This study was conducted against *Staphylococcus aureus* (ATCC 6538). Three lots (Lot Nos. 928t-t, 9287-1, and 9229-1) of the product, Activate 5.25% Institutional Bleach, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 31 March 2009. At least one of the product lots tested (i.e., Lot No. 9229-t) was at least 60 days old at the time of testing. Applicant-provided custom spray bottles were used to treat carriers with t:t0 use solutions (prepared using 400 ppm AOAC synthetic hard water (titration results not provided)). A culture of the challenge microorganism was prepared in accordance with the published AOAC method. Sterile equine serum was added to the culture to achieve a 7.5% organic soil load. Sixty (60) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 0.0t mL of a 48-54 hour old suspension of test organism. Inoculum was spread over a 1 inch x 1 inch area of each carrier. The carriers were dried for 40 minutes at 36±t°C. For each lot of product, separate carriers were sprayed (3-5 pumps) until thoroughly wet with the use solution at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 1 minute at 23±2°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.t% sodium thiosulfate to neutralize. The tubes containing neutralizer were shaken after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 36±t°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier enumeration, sterility, viability, and neutralization confirmation.

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

2. MRID 481310-02 "AOAC Germicidal Spray Products as Disinfectants Test Method," Test Organism: *Staphylococcus aureus* (ATCC 6538), for Activate 5.25% Institutional Bleach, by Jason Williams. Study conducted at Antimicrobial Test Laboratories. Study completion date – April 26, 2010. Study Identification Number GLP1024.

This study was conducted against *Staphylococcus aureus* (ATCC 6538). One lot (Lot No. 9229-t) of the product, Activate 5.25% Institutional Bleach, was tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 31 March 2009. The product lot tested (i.e., Lot No. 9229-t) was at least 60 days old at the time of testing. An applicant-provided custom spray bottle was used to treat carriers with a t:10 use solution (prepared using 400 ppm AOAC synthetic hard water (titration results not provided)). A culture of the challenge microorganism was prepared in accordance with the published AOAC method. Sterile equine serum was added to the culture to achieve a 7.5% organic soil load. Sixty (60) glass slide carriers (18 mm x 36 mm) were inoculated with 0.01 mL of a 48-54 hour old

suspension of test organism. Inoculum was spread over a 1 inch x 1 inch area of each carrier. The carriers were dried for 39 minutes at 36±1°C. For the single lot of product, separate carriers were sprayed (3 pumps) with the use solution at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 2 minutes at 23.6°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.1% sodium thiosulfate to neutralize. The tubes containing neutralizer were shaken after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 49.25 hours at 36±1°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier enumeration, sterility, viability, and neutralization confirmation.

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

3. MRID 481310-03 "AOAC Germicidal Spray Products as Disinfectants Test Method," Test Organism: *Salmonella enterica* (ATCC 10708), for Activate 5.25% Institutional Bleach, by Jason Williams. Study conducted at Antimicrobial Test Laboratories. Study completion date – April 26, 2010. Study Identification Number GLP1029.

This study was conducted against *Salmonella enterica* (ATCC 10708). Three lots (Lot Nos. 9299-1, 9358-1, and 9364-1) of the product, Activate 5.25% Institutional Bleach, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 31 March 2009. At least one product lot tested (i.e., Lot No. 9299-1) was at least 60 days old at the time of testing. Applicant-provided custom spray bottles were used to treat carriers with 1:10 use solutions (prepared using 400 ppm AOAC synthetic hard water (titration results not provided)). A culture of the challenge microorganism was prepared in accordance with the published AOAC method. Sterile equine serum was added to the culture to achieve a 7.5% organic soil load. Sixty (60) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 0.01 mL of a 48-54 hour old suspension of test organism. Inoculum was spread over a 1 inch x 1 inch area of each carrier. The carriers were dried for 40 minutes at 36±1°C. For each lot of product, separate carriers were sprayed (3 pumps) with the use solution at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 2 minutes at 23±2°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.1% sodium thiosulfate to neutralize. The tubes containing neutralizer were shaken after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 36±1°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier enumeration, sterility, viability, and neutralization confirmation.

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

4. MRID 481310-04 "Pre-efficacy Testing Total Chlorine Iodometric Titration," for Activate 5.25% Institutional Bleach, by Jason Williams. Study conducted at Antimicrobial Test Laboratories. Study completion date – April 26, 2010. Study Identification Number GLP1026.

[Note: This study is not an efficacy study and was not reviewed.]

5. MRID 481310-05 "AOAC Germicidal Spray Products as Disinfectants Test Method," Test Organism: *Pseudomonas aeruginosa* (ATCC 15442), for Activate 5.25% Institutional Bleach, by Jason Williams. Study conducted at Antimicrobial Test Laboratories. Study completion date – April 26, 2010. Study Identification Number GLP1027.

This study was conducted against *Pseudomonas aeruginosa* (ATCC 15442). Three lots (Lot Nos. 9299-1, 9358-1, and 9364-1) of the product, Activate 5.25% Institutional Bleach, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 31 March 2009. At least one product lot tested (i.e., Lot No. 9299-1) was at least 60 days old at the time of testing. Applicant-provided custom spray bottles were used to treat carriers with 1:10 use solutions (prepared using 400 ppm AOAC synthetic hard water (titration results not provided)). A culture of the challenge microorganism was prepared in accordance with the published AOAC method, with the following exception: the *Pseudomonas aeruginosa* culture was incubated for 48-54 hours at 36±1°C (which differs from the AOAC method specification of 18-24 hours). Sterile equine serum was added to the culture to achieve a 7.5% organic soil load. Sixty (60) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 0.01 mL of a 48-54 hour old suspension of test organism. Inoculum was spread over a 1 inch x 1 inch area of each carrier. The carriers were dried for 40 minutes at 36±1°C. For each lot of product, separate carriers were sprayed (3 pumps) with the use solution at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 2 minutes at 23±2°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.1% sodium thiosulfate to neutralize. The tubes containing neutralizer were shaken after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 36±1°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier enumeration, sterility, viability, and neutralization confirmation.

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. [The prepared culture of *Pseudomonas aeruginosa* used was more than twice the age specified in the AOAC method, and the cells may have been less viable than appropriate for a valid study.]

6. MRID 481310-06 "AOAC Germicidal Spray Products as Disinfectants Test Method," Test Organism: *Acinetobacter baumannii* (ATCC 19606), for Activate 5.25% Institutional Bleach, by Jason Williams. Study conducted at Antimicrobial Test Laboratories. Study completion date – April 26, 2010. Study Identification Number GLP1033.

This study was conducted against *Acinetobacter baumannii* (ATCC 19606). Two lots (Lot Nos. 9358-1 and 9364-1) of the product, Activate 5.25% Institutional Bleach, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 31 March 2009. Applicant-provided custom spray bottles were used to treat carriers with 1:10 use solutions (prepared using 400 ppm AOAC synthetic hard water (titration results not provided)). A culture of the challenge microorganism was prepared in accordance with the published AOAC method. Sterile equine serum was added to the culture to achieve a 7.5% organic soil load. Ten (10) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 0.01 mL of a 48-54 hour old suspension of test organism. Inoculum was spread over a 1 inch x 1 inch area of each carrier. The carriers were dried for 20 minutes at 36±1°C. For each lot of product, separate carriers were sprayed (3 pumps) with the use solution at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 2 minutes at 23.9°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.1% sodium thiosulfate to neutralize. The tubes containing neutralizer were shaken after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 36±1°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier enumeration, sterility, viability, and neutralization confirmation.

Note: Previous efficacy testing attempts resulted in insufficient carrier enumeration counts of $\leq 2.1 \times 10^3$ CFU/carrier, undetectable counts, and/or failure to meet the experimental success criteria. No positive test carriers were noted in these testing attempts. See page 9 of the laboratory study.

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

7. MRID 481310-07 "AOAC Germicidal Spray Products as Disinfectants Test Method," Test Organism: Methicillin-Resistant *Staphylococcus aureus* (ATCC 33591), for Activate 5.25% Institutional Bleach, by Jason Williams. Study conducted at Antimicrobial Test Laboratories. Study completion date – April 26, 2010. Study Identification Number GLP1034.

This study was conducted against Methicillin-Resistant *Staphylococcus aureus* (ATCC 33591). Two lots (Lot Nos. 9358-1 and 9364-1) of the product, Activate 5.25% Institutional Bleach, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 31 March 2009. Applicant-provided custom spray bottles were used to treat carriers with 1:10 use solutions (prepared using 400 ppm AOAC synthetic hard water (titration results not provided)). A culture of the challenge microorganism was prepared in accordance with the published AOAC method. Sterile equine serum was added to the culture to achieve a 7.5% organic soil load. Ten (10) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 0.01 mL of a 48-54 hour old suspension of test organism. Inoculum

was spread over a 1 inch x t inch area of each carrier. The carriers were dried for 20 minutes at 36± t°C. For each lot of product, separate carriers were sprayed (3 pumps) with the use solution at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 2 minutes at 22.7-23.1°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.1% sodium thiosulfate to neutralize. The tubes containing neutralizer were shaken after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 36±1°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier enumeration, sterility, viability, neutralization confirmation, and antibiotic resistance.

Note: A previous efficacy testing attempt resulted in insufficient carrier enumeration counts of < t.0 x 10^4 CFU/carrier. No positive test carriers were noted in this testing attempt. See page 9 of the laboratory study.

Note: Antibiotic resistance of Methicillin-Resistant *Staphylococcus aureus* (ATCC 33591) was verified on a representative culture. An individual Mueller Hinton Agar was streaked with the prepared culture. A control agar was prepared using *Staphylococcus aureus* (ATCC 6538) as a control organism. An antibiotic disk was added to the center of each plate. The plates were incubated and, following incubation, the zones of inhibition were compared. The comparison confirmed antibiotic resistance of Methicillin-Resistant *Staphylococcus aureus* (ATCC 33591) to oxacillin. See page 15 of the laboratory report.

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

8. MRID 481310-08 "AOAC Germicidal Spray Products as Disinfectants Test Method," Test Organism: *Streptococcus pyogenes* (ATCC 19615), for Activate 5.25% Institutional Bleach, by Jason Williams. Study conducted at Antimicrobial Test Laboratories. Study completion date – April 26, 2010. Study Identification Number GLP1035.

This study was conducted against *Streptococcus pyogenes* (ATCC 196t5). Two lots (Lot Nos. 9358-1 and 9364-1) of the product, Activate 5.25% Institutional Bleach, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 3t March 2009. Applicant-provided custom spray bottles were used to treat carriers with 1: t0 use solutions (prepared using 400 ppm AOAC synthetic hard water (titration results not provided)). A culture of the challenge microorganism was prepared in accordance with the published AOAC method. Sterile equine serum was added to the culture to achieve a 7.5% organic soil load. Ten (10) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 0.01 mL of a 48-54 hour old suspension of test organism. Inoculum was spread over a 1 inch x 1 inch area of each carrier. The carriers were dried for 20 minutes at 36± t°C. For each lot of product, separate carriers were sprayed (3 pumps) with the use solution at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 2 minutes at 23.8-23.9°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.1% sodium thiosulfate to neutralize. The tubes containing neutralizer were shaken after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 36± t°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 carrier enumeration, sterility, viability, and neutralization confirmation.

Note: Previous efficacy testing attempts resulted in insufficient carrier enumeration counts of $\leq 3.14 \times 10^4$ CFU/carrier, undetectable counts, and/or failure to meet the experimental success criteria. No positive test carriers were noted in these testing attempts. See page 9 of the laboratory study.

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

9. MRID 481310-09 "AOAC Germicidal Spray Products as Disinfectants Test Method," Test Organism: Vancomycin-Resistant *Enterococcus faecalis* (ATCC 51299), for Activate 5.25% Institutional Bleach, by Jason Williams. Study conducted at Antimicrobial Test Laboratories. Study completion date – April 26, 2010. Study Identification Number GLP1036.

This study was conducted against Vancomycin-Resistant *Enterococcus faecalis* (ATCC 51299). Two lots (Lot Nos. 9358-1 and 9364-1) of the product, Activate 5.25% Institutional Bleach, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 31 March 2009. Applicant-provided custom spray bottles were used to treat carriers with 1:10 use solutions (prepared using 400 ppm AOAC synthetic hard water (titration results not provided)). A culture of the challenge microorganism was prepared in accordance with the published AOAC method. Sterile equine serum was added to the culture to achieve a 5.0% organic soil load. Ten (10) glass slide carriers (18 mm x 36 mm) per product lot were inoculated with 0.01 mL of a 48-54 hour old suspension of test organism. Inoculum was spread over a 1 inch x 1 inch area of each carrier. The carriers were dried for 20 minutes at 36±1°C. For each lot of product, separate carriers were sprayed (3 pumps) with the use solution at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 2 minutes at 23.8-23.9°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.1% sodium thiosulfate to neutralize. The tubes containing neutralizer were shaken after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 36±1°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier enumeration, sterility, viability, neutralization confirmation, and antibiotic resistance.

Note: Previous efficacy testing attempts resulted in insufficient carrier enumeration counts of $<3.0 \times 10^3$ CFU/carrier, undetectable counts, and/or failure to meet the experimental success criteria. See page 9 of the laboratory study.

Note: Antibiotic resistance of Vancomycin-Resistant *Enterococcus faecalis* (ATCC 51299) was verified on a representative culture. An individual Tryptic Soy Agar was streaked with the prepared culture. A control agar was prepared using *Enterococcus faecalis* (ATCC 19433) as a control organism. An antibiotic disk was added to the center of each plate. The plates were incubated and, following incubation, the zones of inhibition were compared. The comparison confirmed antibiotic resistance of

Vancomycin-Resistant *Enterococcus faecalis* (ATCC 51299) to vancomycin. See page 15 of the laboratory report.

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

10. MRID 481310-10 "Initial Virucidal Effectiveness Test, Duck Hepatitis B Virus (Surrogate for Human Hepatitis B virus)" for Activate 5.25% Institutional Bleach, by Tien V. Mai. Study conducted at MICROBIOTEST. Study completion date – November 30, 2009. Laboratory Project Identification Number 713-101.

This study, under the direction of Study Director Tien V. Mai, was conducted against Duck hepatitis B virus (strain not specified; obtained from HepadnaVirus Testing, Inc.), using primary duck hepatocytes (ducklings obtained from Metzer Farms) as the host system. Two lots (Lot Nos. 9281-1 and 9287-1) of the product, Activate 5.25% Institutional Bleach, were tested according to a MicroBioTest Protocol titled "Initial Virucidal Effectiveness Test, Duck Hepatitis B virus (Surrogate for Human Hepatitis B virus)," dated October 23, 2009 (copy provided). Use solutions (1:10 dilution) were prepared using a sprayer system comprised of a product cartridge and a water bottle cartridge (filled with 400±2.9% ppm AOAC synthetic hard water) installed on applicant-provided custom spray bottles. The stock virus culture contained 100% duck serum. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked bottoms of separate sterile glass Petri dishes. The virus films were dried for 40 minutes at ambient temperature. Two replicates per product lot were tested. For each lot of product, separate dried virus films were sprayed until thoroughly wet with the use solution at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 20-21°C. Following exposure, each plate was neutralized with 2.0 mL of fetal bovine serum with 1% Polysorbate 80 and 0.5% sodium thiosulfate. Each plate was scraped with a cell scraper to re-suspend the contents. Ten-fold serial dilutions were prepared, using Leibovitz 15 Complete. Primary duck hepatocytes in culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 20-30 hours at 36±2°C in 5±1% CO_2 for viral adsorption. Post-adsorption, the cultures were re-fed. The cultures were returned to incubation for 9-13 days at 36±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the infectious virus was assayed by an immunofluorescence assay. Controls included those for cell viability/sterility, virus stock titer, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% fluorescent focus forming unit dose per mL ($FFFUD_{50}$/mL) was determined using the method of Spearman Karber.

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

11. MRID 481310-11 "Confirmatory Virucidal Effectiveness Test, Duck Hepatitis B Virus (Surrogate for Human Hepatitis B virus)" for Activate 5.25% Institutional Bleach, by Salimatu Jibril. Study conducted at MICROBIOTEST. Study completion date – November 30, 2009. Laboratory Project Identification Number 713-102.

This confirmatory study, under the direction of Study Director Salimatu Jibril, was conducted against Duck hepatitis B virus (strain not specified; obtained from

HepadnaVirus Testing, Inc.), using primary duck hepatocytes (ducklings obtained from Metzer Farms) as the host system. One lot (Lot No. 9281-1) of the product, Activate 5.25% Institutional Bleach, was tested according to a MicroBioTest Protocol titled "Confirmatory Virucidal Effectiveness Test, Duck Hepatitis B virus (Surrogate for Human Hepatitis B virus)," dated October 23, 2009 (copy provided). A use solution (1:10 dilution) was prepared using a sprayer system comprised of a product cartridge and a water bottle cartridge (filled with 400±2.9% ppm AOAC synthetic hard water) installed on an applicant-provided custom spray bottle. The stock virus culture contained 100% duck serum. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked bottoms of separate sterile glass Petri dishes. The virus films were dried for 26 minutes at ambient temperature. Two replicates were tested. For the single product lot, separate dried virus films were sprayed until thoroughly wet with the use solution at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 30 seconds at 20-21°C. Following exposure, the plates were neutralized with 2.0 mL of fetal bovine serum with 1% Polysorbate 80 and 0.5% sodium thiosulfate. The plates were scraped with a cell scraper to re-suspend the contents. Ten-fold serial dilutions were prepared, using Leibovitz 15 Complete. Primary duck hepatocytes in culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 20-30 hours at 36±2°C in 5±1% CO_2 for viral adsorption. Post-adsorption, the cultures were re-fed. The cultures were returned to incubation for 9-13 days at 36±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the infectious virus was assayed by an immunofluorescence assay. Controls included those for cell viability/sterility, virus stock titer, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% fluorescent focus forming unit dose per mL ($FFFUD_{50}$/mL) was determined using the method of Spearman Karber.

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

12. MRID 481310-12 "A GLP Hard Surface Disinfection Evaluation of One Formulation Versus Human Immunodeficiency Virus" for Activate 5.25% Institutional Bleach, by Volha Dzyakanava. Study conducted at BioScience Laboratories, Inc. Study completion date – December 31, 2009. Laboratory Study Number 090922-404.

This study was conducted against Human immunodeficiency virus type 1 (HIV-1; obtained from ZeptoMetrix Corporation), using C8166 cells (Human T-cell leukemia cells; ECACC #88051601) as the host system. Two lots (Lot Nos. 9281-1 and 9287-1) of the product, Activate 5.25% Institutional Bleach, were tested according to a BioScience Laboratories, Inc. protocol titled "A GLP Hard Surface Disinfection Evaluation of One Formulation Versus Human Immunodeficiency Virus," dated November 11, 2009 (copy provided). Use solutions were prepared using a sprayer system comprised of a product cartridge and a water bottle cartridge (filled with 432 ppm AOAC synthetic hard water) installed on applicant-provided custom spray bottles. Fetal bovine serum was added to the viral stock to achieve a 5% organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for ~10 minutes at 26-28°C. For each lot of product, separate dried virus films were sprayed (3 pumps) with the use solution at a distance of 6-8 inches from the carrier surface (according to the protocol). The carriers were allowed to remain wet for 30 seconds at ambient temperature. Following exposure, the plates were neutralized with 20 mL of D/E Broth with 0.5% sodium thiosulfate. The plates were scraped with a cell scraper to re-suspend

the contents. Ten-fold serial dilutions were prepared, using Advanced RPMI 1640 with 2% fetal bovine serum, 100 units/mL penicillin, 100 µg/mL Streptomycin, and 2 mM L-Glutamine. C8166 cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 13 days at 37±2°C. Following incubation, the cultures were examined microscopically for cytopathic effects (i.e., presence or absence of cell disintegration, presence of virus syncytia). Controls included a negative control (i.e., cell viability) and those for virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

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

V RESULTS

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested			Carrier Population (CFU/ carrier)
1-Minute Exposure Time					
		Lot No. 9281-1	**Lot No. 9287-1**	**Lot No. 9229-1**	
481310-01	*Staphylococcus aureus*	1/60 --- ---	--- 1/60 ---	--- --- 3/60	6.58×10^5 5.86×10^5 7.02×10^5
2-Minute Exposure Time					
		Lot No. 9299-1	**Lot No. 9358-1**	**Lot No. 9364-1**	
481310-02	*Staphylococcus aureus*	0/60	---	---	3.16×10^6
481310-03	*Salmonella enterica*	0/60 --- ---	--- 0/60 ---	--- --- 0/60	4.5×10^4 4.2×10^4 5.1×10^4
481310-05	*Pseudomonas aeruginosa*	0/60 --- ---	--- 0/60 ---	--- --- 0/60	3.6×10^6 2.76×10^6 2.84×10^6
481310-06	*Acinetobacter baumannii*	---	0/10 ---	--- 0/10	1.74×10^5 1.0×10^5
481310-07	Methicillin-Resistant *Staphylococcus aureus*	---	0/10 ---	--- 0/10	3.04×10^7 3.37×10^7
481310-08	*Streptococcus pyogenes*	---	0/10 ---	--- 0/10	3.32×10^5 3.54×10^5
481310-09	Vancomycin-Resistant *Enterococcus faecalis*	---	0/10 ---	--- 0/10	9.0×10^5 6.14×10^5

MRID Number	Organism	Results			Plate Recovery Control
			Lot No. 9281-1	Lot No. 9287-1	
30-Second Exposure Time					
481310-10	Duck hepatitis B virus	10^{-2} dilution	Cytotoxicity	Cytotoxicity	$t0^{5.18}$ $FFFUD_{50}$/ 0.2 mL
		10^{-3} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$FFFUD_{50}$/0.2 mL	$\leq 10^{1.80}$	$\leq 10^{1.80}$	
		Log reduction	≥ 3.38 $\log_{10}$	≥ 3.38 $\log_{10}$	
481310-11	Duck hepatitis B virus	10^{-2} dilution	Cytotoxicity	---	$10^{5.30}$ $FFFUD_{50}$/ 0.2 mL
		10^{-3} to 10^{-7} dilutions	Complete inactivation	---	
		$FFFUD_{50}$/0.2 mL	$\leq 10^{1.80}$	---	
		Log reduction	≥ 3.50 $\log_{10}$	---	
481310-12	Human immunodeficiency virus type 1	10^{-2} dilution	Cytotoxicity	Cytotoxicity	$t0^{6.75}$ $TCID_{50}$/ t.0 mL
		10^{-3} to 10^{-6} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/1.0 mL	$\leq 10^{3.00}$	$\leq 10^{3.00}$	
		Log reduction	≥ 3.75 $\log_{10}$	≥ 3.75 $\log_{10}$	

VI CONCLUSIONS

1. The submitted efficacy data support the use of a 1:10 dilution of the product, Activate 5.25% Institutional Bleach, as a disinfectant with bactericidal activity against the following microorganisms on hard, non-porous surfaces in the presence of 400 ppm hard water and a 7.5% organic soil load (a 5.0% organic soil load for Vancomycin-Resistant *Enterococcus faecalis*) for a 2-minute contact time:

Staphylococcus aureus	MRID 481310-01 and -02
Salmonella enterica	MRID 481310-03
Pseudomonas aeruginosa	MRID 481310-05
Acinetobacter baumannii	MRID 481310-06
Methicillin-Resistant *Staphylococcus aureus*	MRID 481310-07
Streptococcus pyogenes	MRID 481310-08
Vancomycin-Resistant *Enterococcus faecalis*	MRID 481310-09

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. [Note that a fourth product lot was tested against *Staphylococcus aureus* to evaluate for false positives.] In testing against *Staphylococcus aureus*, *Salmonella enterica*, and *Pseudomonas aeruginosa*, at least one of the product lots tested was at least 60 days old at the time of testing. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Sterility controls did not show growth. The registrant must provide a rationale for the extended incubation time (48-54 hours, instead of the method specified 18-24 hours) for *Pseudomonas aeruginosa*.

2. The submitted efficacy data support the use of a 1:10 dilution of the product, Activate 5.25% Institutional Bleach, as a disinfectant with virucidal activity against the following microorganisms on hard, non-porous surfaces in the presence of 400 ppm hard water and at least a 5% organic soil load for a 30-second contact time:

Duck hepatitis B virus	MRID 481310-10 and -11
Human immunodeficiency virus type 1	MRID 481310-12

Recoverable virus titers of at least 10^4 were achieved. Cytotoxicity was observed in the 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 Duck Hepatitis B Virus, the initial and confirmatory studies were performed at the same laboratory but under the direction of different study directors. Both the initial and confirmatory studies tested two replicates per product lot. The confirmatory study tested one product lot.

VII RECOMMENDATIONS

1. The proposed label claims that a 1:10 dilution of the product, Activate 5.25% Institutional Bleach, is an effective "one-step" disinfectant against the following microorganisms on hard, non-porous surfaces for a 2-minute contact time:

Staphylococcus aureus
Salmonella enterica
Pseudomonas aeruginosa
Acinetobacter baumannii
Methicillin-Resistant *Staphylococcus aureus*
Streptococcus pyogenes
Vancomycin-Resistant *Enterococcus faecalis*

These claims are acceptable as they are supported by the submitted data. The rationale for extended incubation time for Pseudomonas aeruginosa, as stated in the Conclusion section, is required.

2. The proposed label claims that a 1:10 dilution of the product, Activate 5.25% Institutional Bleach, is an effective "one-step" disinfectant against the following microorganisms on hard, non-porous surfaces for a 30-second contact time:

Human hepatitis B virus
Human immunodeficiency virus type 1

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

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

- On page 2 under the "To Clean and Disinfect in One Step" section, add the following (or similar) instructions: "Remove gross dirt and debris."

4. The following revisions to the proposed label are recommended:

- On page 1 of the proposed label, change "*Salmonella enteri*" to read

"*Salmonella enterica.*"

- On page 2, change "ceramic tile" to read "glazed ceramic tile." Ceramic is a porous surface.

- On page 2, change "porcelain" to read "glazed porcelain." Porcelain is a porous surface.

- On page 2 under the "Precautionary Statements," change "wash after handling" to read "wash after handing and before eating, drinking, chewing gum, using tobacco, or using the toilet."

- On page 3 under the "Storage & Disposal" section, add a "Pesticide Storage" subheading.

- Add ATCC numbers for all listed microorganisms.

- Add page numbers to each page of the proposed label.