UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
CHEMICAL SAFETY
AND POLLUTION

January 10, 2013

MEMORANDUM

SUBJECT: Efficacy Review for Peroxide Multi Surface Cleaner and Disinfectant;
EPA Reg. No. 1677-EGI;
DP Barcode: D405936

FROM: Karen M. Hill, Ph.D.
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K. Hill 1/10/13

THRU: Emily Mitchell
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TO: Marshall Swindell RM33/ Martha Terry
Regulatory Management Branch I
Antimicrobials Division (7510P)

APPLICANT: ECOLAB
655 Lone Oak Drive
Eagan, MN 55121-1560

Formulation from the Label:

Active Ingredient(s):	% by wt.
Hydrogen Peroxide	8.00%
Inert Ingredients	92.00%
Total	100.00%

I. BACKGROUND:

The product, Peroxide Multi Surface Cleaner and Disinfectant (EPA Reg. No. 1677-EGI), is seeking new pesticide registration as a disinfectant (bactericidal, virucidal, and fungicidal) and sanitizer on hard non-porous surfaces in residential, commercial, and institutional environments. Studies were conducted at ECOLAB, located at 655 Lone Oak Drive Eagan, MN 55121-1560 and ATS LABS located at 1285 Corporate Center Drive, suite 110 Eagan, MN 55121.

This data package contained a letter from the applicant to EPA dated September 17, 2012 (MRID 489474-00), EPA Form 8570-4 (CSF), EPA Form 8570-35 (Data Matrix), thirty five studies (MRID 489474-04 and 489474-38), and Statement of No Data Confidentiality Claims for all studies.

II. USE DIRECTIONS:

The product is intended to be used as a disinfectant and sanitizer on hard non-porous surfaces including shower doors, shower curtains, tables, cabinets, walls, desks, coolers, freezers, appliances, shelves, racks and carts, highchairs, fiberglass sinks, tubs, windows, mirrors, showers, shower stalls, shower fixtures, garbage cans, bed frames, doorknobs, computer keyboards, telephones, exterior surfaces of refrigerators, sinks, non-food contact equipment, urinals, exterior surfaces of toilets, restroom fixtures, shower fixtures, restroom floors, floors (tile, finished), tubs, tiles, vinyl, glazed tile, linoleum, glazed porcelain, stainless steel, painted surfaces, plastic surfaces, plastic (such as polyethylene, polypropylene, polyvinylchloride), and glass.

The products proposed label includes the following directions of use:

<u>For use as a (Daily) (Multi-Surface) Cleaner/Disinfectant or (Daily) Restroom Cleaner/Disinfectant:</u>
Dilute at 1:32 (4 oz.) – 1:21.3 (6 oz.) per gallon of water. Pre-Clean heavily soiled areas. Apply Use Solution by coarse trigger sprayer to hard non-porous surfaces. Spray 6 – 8 inches from the surface; making sure to wet surfaces thoroughly. All surfaces must remain wet for (5 minutes) (3 minutes) (90 seconds). Wipe surfaces (allow to air dry). Rinsing is not necessary on non-food contact surfaces. Do not use this product to clean or disinfectant glassware, dishes, or silverware.

Instructions for directions of use for sanitization of non-food contact surfaces were not given in the proposed label.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS:

<u>Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments:</u>
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 Test (for spray products), or the AOAC Hard Surface Carrier Test. The tests require that sixty carriers must be tested with each of 3 samples, representing 3 different batches, one of which is at least 60 days old, against a mean log density of at least 6 for

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. To pass performance requirements when using AOAC Hard Surface Carrier Test, tests must result in killing in 58 out of each set of 60 carriers for *Staphylococcus aureus* ATCC 6538; 57 out of each set of 60 carriers for *Pseudomonas aeruginosa* ATCC 15442 within ten minutes.

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. The product should kill all the test microorganisms on all carriers in ≤ten minutes. The minimum carrier count to make the test valid should be 1×10^4.

Virucidal:

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. If the product is intended to be represented as a one-step (ready to use) virucidal, an appropriate organic soil (i.e. - 5 percent blood serum) should be included with the viral inoculum.

Disinfectants for Use as Fungicides (Against Pathogenic Fungi):

Effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data derived from each of 2 samples representing 2 different batches using the AOAC Fungicidal Test. The test should be conducted at 5, 10, and 15 minute exposure times. Alternatively, the AOAC Use Dilution Method, modified to conform with appropriate elements in the AOAC Fungicidal Test, may be employed. If the product is intended for use as a spray, the AOAC Germicidal Spray Products Test must be employed. Performance requirements for this test: the highest dilution that kills all fungal spores is the minimum effective concentration. Ten carriers for each of two samples representing two different batches of the product should be evaluated against *Trichophyton mentagrophytes* (ATCC 9533). The inoculum employed should provide a concentration of $1 \times 10^4 - 1 \times 10^5$ conidia per carrier. For the AOAC International Fungicidal Activity of Disinfectants test, all fungal spores at 10 and 15 minutes should be

killed to support a 10 minute exposure time. For the AOAC International Use-Dilution Methods, all fungal spores on all 10 carriers should be killed in ≤ten minutes.

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). 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 STUDY:

The below studies were all conducted using the appropriate lower certified levels of the product tested. The ECOLAB studies included certificate of analysis for all batches tested and the below formula was used to determine the dilution needed to achieve the lower certified limit.

$$\text{Resulting ppm of active ingredient} = \left[\frac{\%\ \text{Active}}{100\%}\right]\left[\frac{\%\text{Dilution}}{100\%}\right] \text{X (Specific Gravity) } (10^6)$$

Active Ingredient	CSF Lower Certified Limit	Specific Gravity	Percent Dilution*	Resulting ppm of Active Ingredient
Hydrogen Peroxide	7.1%	1.04	3.125%	2308
Hydrogen Peroxide	7.1%	1.04	4.688%	3462

*Study proposed dilution: (4 oz/gallon) X (1 gallon/128 oz) X (100%) = 3.125%
*Study proposed dilution: (6 oz/gallon) X (1 gallon/128 oz) X (100%) = 4.688%

1. MRID 489474-04 "3-in-1 Disinfecting Cleaner Supplemental Non-Food Contact Surface Sanitizing Efficacy," Test Organism: *Pseudomonas aeruginosa* (ATCC 15442), *Escherichia coli* (ATCC 11229), *Salmonella enterica* (ATCC 10708), and *Enterococcus faecalis* (ATCC 29212) for product 3-in-1 Disinfecting Cleaner. Study director: Laurinda Holen. Study conducted at ECOLAB 655 Lone Oak Drive Eagan, MN 55121-1560. Study completion date – September 10, 2012. Study Number 1200001.

This study was conducted against *Pseudomonas aeruginosa* (ATCC 15442), *Escherichia coli* (ATCC 11229), *Salmonella enterica* (ATCC 10708), and *Enterococcus*

faecalis (ATCC 29212). Two lots, Batch P093012 and Batch P100611, of the product were tested using the provided ECOLAB Protocol No. MS016-23 prior to 07/02/12 and Protocol No. MS016-24 after 07/02/12. The test substance was diluted at 4 oz. per gallon in 400 ppm synthetic hard water resulting in the lower certified limit of 2308 ppm of active ingredient. A culture of each challenge microorganism was prepared by at least three but less than fifteen daily consecutive transfers in appropriate growth medium for each microorganism with no more than one day missed per seven-day period. Each culture was incubated at 35 ± 2°C for 24 ± 4 hours with the exception of *Salmonella enterica* on 08/09/12 which was incubated at 35 ± 2°C for 48 ± 4 hours. Each test system was mixed and after ≥15 minutes the upper portion was removed. Preparations and testing of the microorganisms were done on different dates. For *Pseudomonas aeruginosa* on 04/12/12, 15 mLs of the upper portion was centrifuged and the pellet was resuspended in 7 mLs of Nutrient broth. For *Escherichia coli* on 04/20/12, 16 mLs of the upper portion was centrifuged and the pellet was resuspended in 4.75 mLs of Nutrient broth. Fetal bovine serum was added to achieve a 5% organic soil load. Stainless steel test squares (1" X 1") were inoculated with 0.03 mL of the test system suspension. For *Salmonella enterica* on 08/09/12 stainless steel carriers (1" X 1") were inoculated with 0.02mL of the test system suspension and dried solely in desiccator at indicated below*. Drying for the other test system suspensions were initially performed in a desiccator containing 86.5% glycerin with the incubator following drying outside of the desiccator with Petri dish lids ajar to obtain adequate recovery of the test system. The chart below indicates the drying conditions for each organism*. The carriers were transferred into individual medicine jars containing the test substance and remained for an exposure period of 90 seconds at room temperature (15 – 30°C). Afterwards, 20 mLs of 2 X DE broth with Catalase was added to the jars containing the test system for *E. coli, Pseudomonas aeruginosa, and Salmonella enterica* for neutralization. For *Enterococcus faecalis*, 20 mLs of 2X DE broth was added to the jars for neutralization. Following, 1.0 mL and 0.1 mL of the solutions from all carrier neutralized suspensions were plated in duplicate using the pour plate technique. Brain Heart Infusion Agar was used for the subcultures for *Salmonella enterica* and *Enterococcus faecalis*. Tryptone Glucose Extract agar was used for the subcultures for *Escherichia coli* and *Pseudomonas aeruginosa*. All plates were incubated for 48 ± 4 hours at 35 ± 2°C. Controls include enumeration, purity, sterility, and neutralization confirmation.

*

Test Date	Organism	Desiccator Dry Time at 30 ± 2°C	Total Dry Time at 30 ± 2°C
02/16/12	*Enterococcus Faecalis*	13 minutes	31 minutes
04/12/12	*Pseudomonas aeruginosa*	30 minutes	35 minutes
04/20/12	*Escherichia coli*	32 minutes	36 minutes
08/09/12	*Salmonella enterica*	35 minutes	35 minutes

Note- Techniques, if any, used to remove the *Pseudomonas aeruginosa* pellicle from culture were not given in study.

2. MRID 489474-05 "3-in-1 Disinfecting Cleaner Germicidal Spray Disinfectant Efficacy," Test Organism: *Acinetobacter baumanni*- MDR (ATCC BAA-1605), *Staphylococcus aureus*- VISA (ATCC 700788), *Staphylococcus aureus* – CA-MRSA (ATCC BAA-1683), *Staphylococcus*

***aureus*- MRSA (ATCC 33592), *Klebsiella pneumoniae* carbapenemase producer (KPC) (ATCC BAA-1705), and *Enterococcus faecalis*- VRE (ATCC 51299) for product 3-in-1 Disinfecting Cleaner. Study director: Laurinda Holen. Study conducted at ECOLAB 655 Lone Oak Drive Eagan, MN 55121-1560. Study completion date – September 10, 2012. Study Number 1100062.**

This study was conducted against *Acinetobacter baumanni*- MDR (ATCC BAA-1605), *Staphylococcus aureus*- VISA (ATCC 700788), *Staphylococcus aureus* – CA-MRSA (ATCC BAA-1683), *Staphylococcus aureus*- MRSA (ATCC 33592), *Klebsiella pneumoniae* carbapenemase producer (KPC) (ATCC BAA-1705), and *Enterococcus faecalis*- VRE (ATCC 51299). Two lots, Batch P093012 and Batch P100611, of the product were tested using the provided ECOLAB Protocol No. MS010-20. The test substance was diluted at 4 oz. per gallon in 400 ppm synthetic hard water resulting in the lower certified limit of 2308 ppm of active ingredient. A culture of each challenge microorganism was prepared by at least three but less than fifteen daily consecutive transfers in appropriate growth medium for each microorganism with no more than one day missed per seven-day period. Each culture was incubated at 35 ± 2°C for 48 – 54 hours. Fetal bovine serum was added to achieve a 5% organic soil load. Non-corrosive microscope slides (1" X 1") were inoculated with 0.01 mL of the test system suspension. The inoculum was spread uniformly over the carrier to within approximately 1/8 inch of the edges. The carriers were dried for a total drying time of 30 minutes at 35 ± 2°C. The inoculated carriers were sprayed at a distance of 6-8 inches with 3 sprays. The test substance remained for an exposure period of 5 minutes at room temperature (15 – 30°C). Afterwards, the excess test substance was drained and the carrier was transferred to individual test tubes containing 20 mLs of appropriate subculture medium. *Acinetobacter baumanni*- MDR (ATCC BAA-1605), *Staphylococcus aureus*- VISA (ATCC 700788), *Staphylococcus aureus* – CA-MRSA (ATCC BAA-1683), and *Klebsiella pneumoniae* carbapenemase producer (KPC) (ATCC BAA-1705) were placed in Letheen Broth with 0.5% Sodium Thiosulfate subculture medium. *Staphylococcus aureus*- MRSA (ATCC 33592) and *Enterococcus faecalis*- VRE (ATCC 51299) were placed in Brain Heart Infusion Broth with Lecithin, Tween 80, and catalase subculture medium. All subcultures were incubated at 35 ± 2°C for 48 ± 4 hours. Controls include enumeration, sterility, purity, neutralization confirmation, and antibiotic susceptibility testing.

Note- The antibiotic susceptibility testing was done using disk diffusion method. Antibiotic resistance was confirmed for *Acinetobacter baumanni*- MDR (ATCC BAA-1605), *Staphylococcus aureus* – CA-MRSA (ATCC BAA-1683), *Staphylococcus aureus*-MRSA (ATCC 33592), and *Enterococcus faecalis*- VRE (ATCC 51299). Antibiotic susceptibility testing was done using MIC method for *Staphylococcus aureus* – VISA and the results confirmed resistance to vancomycin. The Modified Hodge test for carbapenamase detection was performed for *Klebsiella pneumoniae-KPC* (ATCC BAA-1705) and confirmed it not to be a carbapenamase producer.

3. MRID 489474-06 "3-in-1 Disinfecting Cleaner Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Rhinovirus type 37 (ATCC VR-1147) for product 3-in-1 Disinfecting Cleaner. Study director: Lisa Hellickson. Study conducted at ECOLAB 655 Lone Oak Drive Eagan, MN 55121. Study completion date – May 23, 2012. Study Number 1200016.

This study was conducted against Rhinovirus type 37, Strain 151-1 (ATCC VR-1147). HeLa cells (ATCC CCL-2) were used as the host cell line. Two lots, Batch P093012 and Batch P100611, of the product were tested using the provided ECOLAB Protocol No. MS505-06. The test substance was diluted at 4 oz. per gallon in 400 ppm synthetic hard water resulting in the lower certified limit of 2308 ppm of active ingredient. A culture of the challenge microorganism was prepared by thawing and pooling several vials of stored stock culture that contains 5% Fetal Bovine Serum organic soil load. Glass petri dishes (100 X 15 mm) were inoculated with 20 µL of the test system suspension. The inoculum was uniformly spread over the entire bottom surface of the Petri dishes. The carriers were dried for 5 - 13 minutes (until visibly dried). The inoculated carriers were sprayed at a distance of 6-8 inches with 3 sprays. The test substance remained for an exposure period of 5 minutes at 15 - 30°C. Afterwards, the dried film/test substance was scraped and 0.1 mL of the virus/test substance mixture was placed in a prepared Sephacryl column (five columns were used per batch). After centrifuge of the columns, 0.1 mL and 0.1 mL of ten-fold serial dilutions of the supernatant were inoculated into assay plates containing Minimum Essential Medium with Earle's balanced salt solution and non-essential amino acids supplemented with 5% heat inactivated fetal bovine serum and seeded with the test cell culture in quadruplicates. The plates were incubated for 7 to 10 days at 35 ± 2°C with 5 ± 2% CO_2. Controls include viral stock titer, dried virus film recovery, cytotoxicity, viability, and neutralization confirmation.

4. MRID 489474-07 "3-in-1 Disinfecting Cleaner Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Herpes Simplex Virus Type 1(ATCC VR-733) for product 3-in-1 Disinfecting Cleaner. Study director: Lisa Hellickson. Study conducted at ECOLAB 655 Lone Oak Drive Eagan, MN 55121. Study completion date – May 23, 2012. Study Number 1200013.

This study was conducted against Herpes Simplex Virus Type I, Strain F (ATCC VR-733). Vero cells (ATCC CCL-81) were used as the host cell line. Two lots, Batch P093012 and Batch P100611, of the product were tested using the provided ECOLAB Protocol No. MS505-06. The test substance was diluted at 4 oz. per gallon in 400 ppm synthetic hard water resulting in the lower certified limit of 2308 ppm of active ingredient. A culture of the challenge microorganism was prepared by thawing and pooling several vials of stored stock culture that contains 5% Fetal Bovine Serum organic soil load. Glass petri dishes (100 X 15 mm) were inoculated with 20 µL of the test system suspension. The inoculum was uniformly spread over the entire bottom surface of the petri dishes. The carriers were dried for 19 - 31 minutes (until visibly dried). The inoculated carriers were sprayed at a distance of 6-8 inches with 3 sprays. The test substance remained for an exposure period of 5 minutes at 15 - 30°C. Afterwards, the dried film/test substance was scraped and 0.1 mL of the virus/test substance mixture was placed in a prepared Sephacryl column (five columns were used per batch). After centrifuge of the columns, 0.1 mL and 0.1 mL of ten-fold serial dilutions of the supernatant were inoculated into assay plates containing Minimum Essential Medium with Earle's balanced salt solution and non-essential amino acids supplemented with 10% heat inactivated fetal bovine serum and seeded with the test cell culture in quadruplicates. The plates were incubated for 7 to 10 days at 35 ± 2°C with 5 ± 2% CO_2. Controls include viral stock titer, dried virus film recovery, cytotoxicity, viability, and neutralization confirmation.

5. MRID 489474-08 "3-in-1 Disinfecting Cleaner Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Adenovirus Type 5 (ATCC VR-5) for product 3-in-1 Disinfecting Cleaner. Study director: Lisa Hellickson. Study conducted at ECOLAB 655 Lone Oak Drive Eagan, MN 55121. Study completion date – June 5, 2012. Study Number 1200031.

This study was conducted against Adenovirus Type 5, Strain Adenoid 75 (ATCC VR-5). HeLa cells (ATCC CCL-2) were used as the host cell line. Two lots, Batch P093012 and Batch P100611, of the product were tested using the provided ECOLAB Protocol No. MS505-06. The test substance was diluted at 4 oz. per gallon in 400 ppm synthetic hard water resulting in the lower certified limit of 2308 ppm of active ingredient. A culture of the challenge microorganism was prepared by thawing and pooling several vials of stored stock culture that contains 5% Fetal Bovine Serum organic soil load. Glass petri dishes (100 X 15 mm) were inoculated with 20 µL of the test system suspension. The inoculum was uniformly spread over the entire bottom surface of the petri dishes. The carriers were dried for 15 - 23 minutes (until visibly dried). The inoculated carriers were sprayed at a distance of 6-8 inches with 3 sprays. The test substance remained for an exposure period of 5 minutes at 15 - 30°C. Afterwards, the dried film/test substance was scraped and 0.1 mL of the virus/test substance mixture was placed in a prepared Sephacryl column (five columns were used per replicate). After centrifuge of the columns, 0.1 mL and 0.1 mL of ten-fold serial dilutions of the supernatant were inoculated into assay plates containing Minimum Essential Medium with Earle's balanced salt solution and non-essential amino acids supplemented with 10% heat inactivated fetal bovine serum and seeded with the test cell culture in quadruplicates. The plates were incubated for 7 to 10 days at 35 ± 2°C with 5 ± 2% CO_2. Controls include viral stock titer, dried virus film recovery, cytotoxicity, viability, and neutralization confirmation.

6. MRID 489474-09 "3-in-1 Disinfecting Cleaner Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Rhinovirus Type 37 (ATCC VR-1147) for product 3-in-1 Disinfecting Cleaner. Study director: Lisa Hellickson. Study conducted at ECOLAB 655 Lone Oak Drive Eagan, MN 55121. Study completion date – June 29, 2012. Study Number 1200015.

This study was conducted against Rhinovirus Type 37, Strain 151-1 (ATCC VR-1157). HeLa cells (ATCC CCL-2) were used as the host cell line. Two lots, Batch P093012 and Batch P100611, of the product were tested using the provided ECOLAB Protocol No. MS505-06. The test substance was diluted at 6 oz. per gallon in 200 ppm synthetic hard water resulting in the lower certified limit of 3462 ppm of active ingredient. A culture of the challenge microorganism was prepared by thawing and pooling several vials of stored stock culture that contains 5% Fetal Bovine Serum organic soil load. Glass petri dishes (100 X 15 mm) were inoculated with 20 µL of the test system suspension. The inoculum was uniformly spread over the entire bottom surface of the petri dishes. The carriers were dried for 59 minutes (until visibly dried) at 19.82°C with 24.45% relative humidity. The inoculated carriers were sprayed at a distance of 6-8 inches with 3 sprays. The test substance remained for an exposure period of 3 minutes at 15 - 30°C. Afterwards, the dried film/test substance was scraped and 0.1 mL of the virus/test substance mixture was placed in a prepared Sephacryl column (0.1 mL per

column - five columns was used per batch). After centrifuge of the columns, 0.1 mL and 0.1 mL of ten-fold serial dilutions of the supernatant were inoculated into assay plates containing Minimum Essential Medium with Earle's balanced salt solution and non-essential amino acids supplemented with 10% heat inactivated fetal bovine serum and seeded with the test cell culture in quadruplicates. The plates were incubated for 7 to 10 days at 35 ± 2°C with 5 ± 2% CO_2. Controls include viral stock titer, dried virus film recovery, cytotoxicity, viability, and neutralization confirmation.

7. MRID 489474-10 "3-in-1 Disinfecting Cleaner Germicidal Spray Disinfectant Efficacy," Test Organism: *Enterobacter aerogenes* (ATCC 13048), and *Escherichia coli* O157:H7 (ATCC 43895) for product 3-in-1 Disinfecting Cleaner. Study director: Laurinda Holen. Study conducted at ECOLAB 655 Lone Oak Drive Eagan, MN 55121-1560. Study completion date – July 03, 2012. Study Number 1200048.

This study was conducted against *Enterobacter aerogenes* (ATCC 13048), and *Escherichia coli* O157:H7 (ATCC 43895). Two lots, Batch P093012 and Batch P100611, of the product were tested using the provided ECOLAB Protocol No. MS010-20. The test substance was diluted at 6 oz. per gallon in 200 ppm synthetic hard water resulting in the lower certified limit of 3462 ppm of active ingredient. A culture of each challenge microorganism was prepared by at least three but less than fifteen daily consecutive transfers in appropriate growth medium for each microorganism with no more than one day missed per seven-day period. *Enterobacter aerogenes* culture was incubated at 30 ± 2°C and *Escherichia coli* O157:H7 culture was incubated at 35 ± 2°C for 48 – 54 hours. Fetal bovine serum was added to achieve a 5% organic soil load. Non-corrosive microscope slides (1" X 1") were inoculated with 0.01 mL of the test system suspension. The inoculum was spread uniformly over the carrier to within approximately 1/8 inch of the edges. *Enterobacter aerogenes* carriers were dried for 32 minutes and *Escherichia coli* O157:H7 carriers were dried for 30 minutes at 35 ± 2°C. The inoculated carriers were sprayed at a distance of 6-8 inches with 3 sprays. The test substance remained for an exposure period of 5 minutes at room temperature (15 – 30°C). Afterwards, the excess test substance was drained and the carrier was transferred to individual test tubes containing 20 mLs of appropriate subculture medium. *Enterobacter aerogenes* carriers were placed in Letheen Broth with 0.5% Sodium Thiosulfate subculture medium. *Escherichia coli* O157:H7 carriers were placed in Brain Heart Infusion Broth with 0.5% Sodium Thiosulfate subculture medium. All subcultures were incubated at the indicated degrees for 48 ± 4 hours. Controls include enumeration, sterility, purity, and neutralization confirmation.

8. MRID 489474-11 "3-in-1 Disinfecting Cleaner Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Feline Calicivirus as a Surrogate for Norovirus (ATCC VR-782) for product 3-in-1 Disinfecting Cleaner. Study director: Lisa Hellickson. Study conducted at ECOLAB 655 Lone Oak Drive Eagan, MN 55121. Study completion date – May 23, 2012. Study Number 1200011.

This study was conducted against Feline Calicivirus, Strain F-9 (ATCC VR-782). CRFK cells (ATCC CCL-94) were used as the host cell line. Two lots, Batch P093012 and Batch P100611, of the product were tested using the provided ECOLAB Protocol No. MS505-06. The test substance was diluted at 6 oz. per gallon in 200 ppm synthetic hard water resulting in the lower certified limit of 3462 ppm of active ingredient. A

culture of the challenge microorganism was prepared by thawing and pooling several vials of stored stock culture that contains 5% horse serum organic soil load. Glass petri dishes (100 X 15 mm) were inoculated with 20 µL of the test system suspension. The inoculum was uniformly spread over the entire bottom surface of the petri dishes. The carriers were dried for 23 - 27 minutes (until visibly dried). The inoculated carriers were sprayed at a distance of 6-8 inches with 3 sprays. The test substance remained for an exposure period of 3 minutes at 15 - 30°C. Afterwards, the dried film/test substance was scraped and 0.1 mL of the virus/test substance mixture was placed in a prepared Sephacryl column (0.1 mL per column - five columns was used per replicate). After centrifuge of the columns, the 0.1 mL and 0.1 mL of ten-fold serial dilutions of the supernatant were inoculated into assay plates containing Minimum Essential Medium with Earle's balanced salt solution and non-essential amino acids supplemented with 10% heat inactivated horse serum and seeded with the test cell culture in quadruplicates. The plates were incubated for 7 to 10 days at 35 ± 2°C with 5 ± 2% CO_2. Controls include viral stock titer, dried virus film recovery, cytotoxicity, viability, and neutralization confirmation.

9. MRID 489474-12 "3-in-1 Disinfecting Cleaner Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Influenza A Virus H1N1 (ATCC VR-1736) for product 3-in-1 Disinfecting Cleaner. Study director: Lisa Hellickson. Study conducted at ECOLAB 655 Lone Oak Drive Eagan, MN 55121. Study completion date – June 01, 2012. Study Number 1200030.

This study was conducted against Influenza A Virus H1N1 strain A/Virginia/ATCC1/2009 (ATCC VR-1736). Rhesus Monkey Kidney (RMK) cells (ViroMed Laboratories, MN.) were used as the host cell line. Two lots, Batch P093012 and Batch P100611, of the product were tested using the provided ECOLAB Protocol No. MS505-06. The test substance was diluted at 4 oz. per gallon in 400 ppm synthetic hard water resulting in the lower certified limit of 2308 ppm of active ingredient. A culture of the challenge microorganism was prepared by thawing and pooling several vials of stored stock culture. Fetal Bovine Serum was added to obtain a 5% organic soil load. Glass petri dishes (100 X 15 mm) were inoculated with 20 µL of the test system suspension. The inoculum was uniformly spread over the entire bottom surface of the petri dishes. The carriers were dried for 15 - 19 minutes (until visibly dried). The inoculated carriers were sprayed at a distance of 6-8 inches with 3 sprays. The test substance remained for an exposure period of 5 minutes at 15 - 30°C. Afterwards, the dried film/test substance was scraped and 0.1 mL of the virus/test substance mixture was placed in a prepared Sephacryl column (0.1 mL per column - five columns was used per replicate). After centrifuge of the columns, the 0.1 mL and 0.1 mL of ten-fold serial dilutions of the supernatant were inoculated into assay plates containing Minimum Essential Medium supplemented with 2% heat inactivated fetal bovine serum and seeded with the test cell culture in quadruplicates. The plates were incubated for 7 to 10 days at 35 ± 2°C with 5 ± 2% CO_2. Controls include viral stock titer, dried virus film recovery, cytotoxicity, viability, and neutralization confirmation.

10. MRID 489474-13 "3-in-1 Disinfecting Cleaner Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Feline Calicivirus as a Surrogate for Norovirus (ATCC VR-782) for product 3-in-1 Disinfecting Cleaner. Study director: Lisa Hellickson.

Study conducted at ECOLAB 655 Lone Oak Drive Eagan, MN 55121. Study completion date – June 01, 2012. Study Number 1200012.

This study was conducted against Feline Calicivirus, Strain F-9 (ATCC VR-782). CRFK cells (ATCC CCL-94) were used as the host cell line. Two lots, Batch P093012 and Batch P100611, of the product were tested using the provided ECOLAB Protocol No. MS505-06. The test substance was diluted at 4 oz. per gallon in 400 ppm synthetic hard water resulting in the lower certified limit of 2308 ppm of active ingredient. A culture of the challenge microorganism was prepared by thawing and pooling several vials of stored stock culture that contains 5% Fetal Bovine Serum organic soil load. Glass petri dishes (100 X 15 mm) were inoculated with 20 µL of the test system suspension. The inoculum was uniformly spread over the entire bottom surface of the petri dishes. The carriers were dried for 19 - 40 minutes (until visibly dried). The inoculated carriers were sprayed at a distance of 6-8 inches with 3 sprays. The test substance remained for an exposure period of 5 minutes at 15 - 30°C. Afterwards, the dried film/test substance was scraped and 0.1 mL of the virus/test substance mixture was placed in a prepared Sephacryl column (0.1 mL per column - five columns were u sed per replicate). After centrifuge of the columns, the 0.1 mL and 0.1 mL of ten-fold serial dilutions of the supernatant were inoculated into assay plates containing Minimum Essential Medium with Earle's balanced salt solution and non-essential amino acids supplemented with 5% heat inactivated horse serum and seeded with the test cell culture in quadruplicates. The plates were incubated for 7 to 10 days at 35 ± 2°C with 5 ± 2% CO_2. Controls include viral stock titer, dried virus film recovery, cytotoxicity, viability, and neutralization confirmation.

11. MRID 489474-14 "3-in-1 Disinfecting Cleaner Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Influenza A Virus (ATCC VR-1736) for product 3-in-1 Disinfecting Cleaner. Study director: Lisa Hellickson. Study conducted at ECOLAB 655 Lone Oak Drive Eagan, MN 55121. Study completion date – March 15, 2012. Study Number 1200022.

This study was conducted against Influenza A Virus H1N1 strain A/Virginia/ATCC1/2009 (ATCC VR-1736). Rhesus Monkey Kidney (RMK) (ViroMed Laboratories, MN.) were used as the host cell line. Two lots, Batch P093012 and Batch P100611, of the product were tested using the provided ECOLAB Protocol No. MS505-06. The test substance was diluted at 6 oz. per gallon in 200 ppm synthetic hard water resulting in the lower certified limit of 3462 ppm of active ingredient. A culture of the challenge microorganism was prepared by thawing and pooling several vials of stored stock culture. Fetal Bovine Serum was added to obtain a 5% organic soil load. Glass petri dishes (100 X 15 mm) were inoculated with 20 µL of the test system suspension. The inoculum was uniformly spread over the entire bottom surface of the petri dishes. The carriers were dried for 17 - 25 minutes (until visibly dried). The inoculated carriers were sprayed at a distance of 6-8 inches with 3 sprays. The test substance remained for an exposure period of 1 minute at 15 - 30°C. Afterwards, the dried film/test substance was scraped and 0.1 mL of the virus/test substance mixture was placed in a prepared Sephacryl column (0.1 mL per column - five columns was used per batch). After centrifuge of the columns, the 0.1 mL and 0.1 mL of ten-fold serial dilutions of the supernatant were inoculated into assay plates containing Minimum Essential Medium supplemented with 2% heat inactivated fetal bovine serum and seeded with the test cell culture in quadruplicates. The plates were incubated for 7 to 10 days at 35 ± 2°C with 5

± 2% CO_2. Controls include viral stock titer, dried virus film recovery, cytotoxicity, viability, and neutralization confirmation.

12. MRID 489474-15 "3-in-1 Disinfecting Cleaner Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Herpes Simplex Virus Type 1 (ATCC VR-733) for product 3-in-1 Disinfecting Cleaner. Study director: Lisa Hellickson. Study conducted at ECOLAB 655 Lone Oak Drive Eagan, MN 55121. Study completion date – January 25, 2012. Study Number 1100070.

This study was conducted against Herpes Simplex Virus Type 1, strain F (ATCC VR-733). Vero cells (ATCC CCL-81) were used as the host cell line. Two lots, Batch P093012 and Batch P100611, of the product were tested using the provided ECOLAB Protocol No. MS505-06. The test substance was diluted at 4 oz. per gallon in 200 ppm synthetic hard water resulting in the lower certified limit of 2308 ppm of active ingredient. A culture of the challenge microorganism was prepared by thawing and pooling several vials of stored stock culture which contained 5% Fetal Bovine Serum organic soil load. Glass petri dishes (100 X 15 mm) were inoculated with 20 µL of the test system suspension. The inoculum was uniformly spread over the entire bottom surface of the petri dishes. The carriers were dried for 53 - 59 minutes (until visibly dried) at 20.90°C with 59.62% relative humidity. The inoculated carriers were sprayed at a distance of 6-8 inches with 3 sprays. The test substance remained for an exposure period of 3 minutes at 15 - 30°C. Afterwards, the dried film/test substance was scraped and 0.1 mL of the virus/test substance mixture was placed in a prepared Sephacryl column (0.1 mL per column - five columns was used per batch). After centrifuge of the columns, the 0.1 mL and 0.1 mL of ten-fold serial dilutions of the supernatant were inoculated into assay plates containing Minimum Essential Medium with Earle's balanced salt solution and non-essential amino acids supplemented with 10% heat inactivated fetal bovine serum and seeded with the test cell culture in quadruplicates. The plates were incubated for 7 to 10 days at 35 ± 2°C with 5 ± 2% CO_2. Controls include viral stock titer, dried virus film recovery, cytotoxicity, viability, and neutralization confirmation.

13. MRID 489474-16 "3-in-1 Disinfecting Cleaner Germicidal Spray Disinfectant Efficacy," Test Organism: *Staphylococcus aureus* (ATCC 6538), *Salmonella enterica* (ATCC 10708), and *Pseudomonas aeruginosa* (ATCC 15442) for product 3-in-1 Disinfecting Cleaner. Study director: Laurinda Holen. Study conducted at ECOLAB 655 Lone Oak Drive Eagan, MN 55121-1560. Study completion date – February 22, 2012. Study Number 1100061.

This study was conducted against *Staphylococcus aureus* (ATCC 6538), *Salmonella enterica* (ATCC 10708), and *Pseudomonas aeruginosa* (ATCC 15442). Three lots, Batch 081911, Batch P093012 and Batch P100611, of the product were tested using the provided ECOLAB Protocol No. MS010-20. The test substance was diluted at 6 oz. per gallon in 200 ppm synthetic hard water resulting in the lower certified limit of 3462 ppm of active ingredient. A culture of each challenge microorganism was prepared by at least three but less than fifteen daily consecutive transfers in appropriate growth medium for each microorganism with no more than one day missed per seven-day period. The cultures were incubated at 35 ± 2°C for 48 – 54 hours. Fetal bovine serum was added to achieve a 5% organic soil load. Non-corrosive microscope slides (1" X 1") were inoculated with 0.01 mL of the test system suspension. The inoculum was

spread uniformly over the carrier to within approximately 1/8 inch of the edges. The carriers were dried for 30 minutes at 35 ± 2°C. The inoculated carriers were sprayed at a distance of 6-8 inches with 3 sprays. The test substance remained for an exposure period of 3 minutes. Afterwards, the excess test substance was drained and the carrier was transferred to individual test tubes containing 20 mLs of Letheen Broth with 0.5% Sodium Thiosulfate subculture medium. All subcultures were incubated at 35 ± 2°C for 48 ± 4 hours. Controls include enumeration, sterility, purity, and neutralization confirmation.

14. MRID 489474-17 "3-in-1 Disinfecting Cleaner Germicidal Spray Disinfectant Efficacy," Test Organism: *Staphylococcus aureus* (ATCC 6538), *Salmonella enterica* (ATCC 10708), and *Pseudomonas aeruginosa* (ATCC 15442) for product 3-in-1 Disinfecting Cleaner. Study director: Laurinda Holen. Study conducted at ECOLAB 655 Lone Oak Drive Eagan, MN 55121-1560. Study completion date – January 09, 2012. Study Number 1100060.

This study was conducted against *Staphylococcus aureus* (ATCC 6538), *Salmonella enterica* (ATCC 10708), and *Pseudomonas aeruginosa* (ATCC 15442). Three lots, Batch 081911, Batch P093012 and Batch P100611, of the product were tested using the provided ECOLAB Protocol No. MS010-20. The test substance was diluted at 4 oz. per gallon in 400 ppm synthetic hard water resulting in the lower certified limit of 2308 ppm of active ingredient. A culture of each challenge microorganism was prepared by at least three but less than fifteen daily consecutive transfers in appropriate growth medium for each microorganism with no more than one day missed per seven-day period. The cultures were incubated at 35 ± 2°C for 48 – 54 hours. Fetal bovine serum was added to achieve a 5% organic soil load. Non-corrosive microscope slides (1" X 1") were inoculated with 0.01 mL of the test system suspension. The inoculum was spread uniformly over the carrier to within approximately 1/8 inch of the edges. The carriers were dried for 30-32 minutes at 35 ± 2°C. The inoculated carriers were sprayed at a distance of 6-8 inches with 3 sprays. The test substance remained for an exposure period of 5 minutes. Afterwards, the excess test substance was drained and the carrier was transferred to individual test tubes containing 20 mLs of Letheen Broth with 0.5% Sodium Thiosulfate subculture medium. All subcultures were incubated at 35 ± 2°C for 48 ± 4 hours. Controls include enumeration, sterility, purity, and neutralization confirmation.

15. MRID 489474-17 "3-in-1 Disinfecting Cleaner Germicidal Spray Disinfectant Efficacy," Test Organism: *Staphylococcus aureus* (ATCC 6538), *Salmonella enterica* (ATCC 10708), and *Pseudomonas aeruginosa* (ATCC 15442) for product 3-in-1 Disinfecting Cleaner. Study director: Laurinda Holen. Study conducted at ECOLAB 655 Lone Oak Drive Eagan, MN 55121-1560. Study completion date – January 09, 2012. Study Number 1100060.

This study was conducted against *Staphylococcus aureus* (ATCC 6538), *Salmonella enterica* (ATCC 10708), and *Pseudomonas aeruginosa* (ATCC 15442). Three lots, Batch 081911, Batch P093012 and Batch P100611, of the product were tested using the provided ECOLAB Protocol No. MS010-20. The test substance was diluted at 4 oz. per gallon in 400 ppm synthetic hard water resulting in the lower certified limit of 2308 ppm of active ingredient. A culture of each challenge microorganism was

prepared by at least three but less than fifteen daily consecutive transfers in appropriate growth medium for each microorganism with no more than one day missed per seven-day period. The cultures were incubated at 35 ± 2°C for 48 – 54 hours. Fetal bovine serum was added to achieve a 5% organic soil load. Non-corrosive microscope slides (1" X 1") were inoculated with 0.01 mL of the test system suspension. The inoculum was spread uniformly over the carrier to within approximately 1/8 inch of the edges. The carriers were dried for 30-32 minutes at 35 ± 2°C. The inoculated carriers were sprayed at a distance of 6-8 inches with 3 sprays. The test substance remained for an exposure period of 5 minutes. Afterwards, the excess test substance was drained and the carrier was transferred to individual test tubes containing 20 mLs of Letheen Broth with 0.5% Sodium Thiosulfate subculture medium. All subcultures were incubated at 35 ± 2°C for 48 ± 4 hours. Controls include enumeration, sterility, purity, and neutralization confirmation.

16. MRID 489474-18 "3-in-1 Disinfecting Cleaner Germicidal Spray Disinfectant Efficacy," Test Organism: *Serratia marcescens* (ATCC 14756), *Enterobacter aerogenes* (ATCC 13048), and *Bordetella bronchiseptica* (ATCC 31437) for product 3-in-1 Disinfecting Cleaner. Study director: Laurinda Holen. Study conducted at ECOLAB 655 Lone Oak Drive Eagan, MN 55121-1560. Study completion date – March 01, 2012. Study Number 1200029.

This study was conducted against *Serratia marcescens* (ATCC 14756), *Enterobacter aerogenes* (ATCC 13048), and *Bordetella bronchiseptica* (ATCC 31437). Three lots, Batch 081911, Batch P093012 and Batch P100611, of the product were tested using the provided ECOLAB Protocol No. MS010-20. The test substance was diluted at 6 oz. per gallon in 200 ppm synthetic hard water resulting in the lower certified limit of 3462 ppm of active ingredient. A culture of each challenge microorganism was prepared by at least three but less than fifteen daily consecutive transfers in appropriate growth medium for each microorganism with no more than one day missed per seven-day period. All cultures were incubated at the appropriate temperatures listed below for subculture incubation for 48 ± 4 hours. Fetal bovine serum was added to achieve a 5% organic soil load. Non-corrosive microscope slides (1" X 1") were inoculated with 0.01 mL of the test system suspension. The inoculum was spread uniformly over the carrier to within approximately 1/8 inch of the edges. The carriers were dried for 30-33 minutes at 35 ± 2°C. The inoculated carriers were sprayed at a distance of 6-8 inches with 3 sprays. The test substance remained for an exposure period of 3 minutes. Afterwards, the excess test substance was drained and the carrier was transferred to individual test tubes containing 20 mLs of Letheen Broth with 0.5% Sodium Thiosulfate subculture medium. *Serratia marcescens* culture was incubated at 26 ± 2°C for 48 ± 4 hours. *Enterobacter aerogenes* culture was incubated at 30 ± 2°C for 48 ± 4 hours. *Bordetella bronchiseptica* culture was incubated at 35 ± 2°C for 3 – 4 days. Controls include enumeration, sterility, purity, and neutralization confirmation.

17. MRID 489474-19 "3-in-1 Disinfecting Cleaner Germicidal Spray Disinfectant Efficacy," Test Organism: *Proteus mirabilis* (ATCC 7002) for product 3-in-1 Disinfecting Cleaner. Study director: Laurinda Holen. Study conducted at ECOLAB 655 Lone Oak Drive Eagan, MN 55121-1560. Study completion date – July 03, 2012. Study Number 1200036.

This study was conducted against *Proteus mirabilis* (ATCC 7002). Two lots, Batch P093012 and Batch P100611, of the product were tested using the provided ECOLAB Protocol No. MS010-20. The test substance was diluted at 4 oz. per gallon in 400 ppm synthetic hard water resulting in the lower certified limit of 2308 ppm of active ingredient. A culture of each challenge microorganism was prepared by at least three but less than fifteen daily consecutive transfers in appropriate growth medium for each microorganism with no more than one day missed per seven-day period. *Proteus mirabilis* culture was incubated at 35 ± 2°C for 48 – 54 hours. Fetal bovine serum was added to achieve a 5% organic soil load. Non-corrosive microscope slides (1" X 1") were inoculated with 0.01 mL of the test system suspension. The inoculum was spread uniformly over the carrier to within approximately 1/8 inch of the edges. The carriers were dried for 30 minutes at 35 ± 2°C. The inoculated carriers were sprayed at a distance of 6-8 inches with 3 sprays. The test substance remained for an exposure period of 3 minutes at room temperature (15 – 30°C). Afterwards, the excess test substance was drained and the carrier was transferred to individual test tubes containing 20 mLs of Letheen Broth with 0.5% Sodium Thiosulfate subculture medium. The subcultures were incubated at the 35 ± 2°C for 48 ± 4 hours. Controls include enumeration, sterility, purity, and neutralization confirmation.

18. MRID 489474-20 "3-in-1 Disinfecting Cleaner Non-Food Contact Surface Sanitizing Efficacy," Test Organism: *Staphylococcus aureus* (ATCC 6538), and *Enterococcus aerogenes* (ATCC 13048) for product 3-in-1 Disinfecting Cleaner. Study director: Laurinda Holen. Study conducted at ECOLAB 655 Lone Oak Drive Eagan, MN 55121-1560. Study completion date – July 03, 2012. Study Number 1100066.

This study was conducted against *Staphylococcus aureus* (ATCC 6538), and *Enterococcus aerogenes* (ATCC 13048). Three lots, Batch P081911, Batch P093012 and Batch P100611, of the product were tested using the provided ECOLAB Protocol No. MS016-22. One lot was ≥60 days aged, Batch P081911. The test substance was diluted at 4 oz. per gallon in 400 ppm synthetic hard water resulting in the lower certified limit of 2308 ppm of active ingredient. A culture of each challenge microorganism was prepared by at least three but less than fifteen daily consecutive transfers in appropriate growth medium for each microorganism with no more than one day missed per seven-day period. Each culture was incubated at 35 ± 2°C for 24 ± 4 hours. Each test system was mixed and after ≥15 minutes the upper portion was removed. Fetal bovine serum was added to achieve a 5% organic soil load. Stainless steel test squares (1" X 1") were inoculated with 0.03 mL of the test system suspension. For *Staphylococcus aureus*, the carriers were dried for a total of 30 minutes at 35 ± 2°C. For *Enterobacter aerogenes*, drying for the initial 20 minutes was performed in a desiccator containing 86.5% glycerin with the incubator and followed by 20 minutes drying outside of the desiccator with petri dish lids ajar for a total drying time of 40 minutes at 30 ± 2°C to obtain adequate recovery of the test system. The carriers were transferred into individual medicine jars containing the test substance and remained for an exposure period of 90 seconds at room temperature (15 – 30°C). Afterwards, 20 mLs of 2 X DE broth was added to the jars containing the test system for neutralization. Following, 1.0 mL and 0.1 mL of the solutions from all carrier neutralized suspensions were plated in duplicate using the pour plate technique. All plates were incubated for 48 ± 4 hours at 35 ± 2°C. Controls include enumeration, purity, sterility, and neutralization confirmation.

19. MRID 489474-21 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Human Immunodeficiency virus type 1 (Advanced Biotechnologies, MD.) for product 3-in-1 Disinfecting Cleaner. Study author: Shanen Conway. Study conducted at ATS Labs 1285 Corporate Center Drive, Suite 110 Eagan, MN. 55121. Study completion date – May 23, 2012. Study Number 12973.

This study was conducted against Human Immunodeficiency Virus type 1, Strain HTLV-III_B (Advanced Biotechnologies, MD.). MT-2 (human T-cell leukemia cells) (obtained from AIDS Research and Reference Program, Division of AIDS, NIAID, NIH from Dr. Douglas Richman) were used as the host cell line. Two lots, Batch P093012-CL and Batch P100611-CL, of the product were tested using the provided ATS Labs Protocol No. ECO01031412.HIV. The test substance was diluted at 4 oz. per gallon in 400 ppm synthetic hard water. The stock virus was prepared by collecting the supernatant culture fluid from infected culture cells that were disrupted and the cell debris removed by centrifugation. The high titer stock virus supernatant was aliquoted and stored at ≤-70°C until the day of use then three aliquots of stock virus (ATS Lot HIV-11) were removed, thawed, combined, and maintained at a refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain a final organic soil load of 5% fetal bovine serum. A volume of 200 µL of virus inoculum was spread uniformly over the bottoms of fifteen (15) separate 100 X 15 mm glass Petri dishes. The virus films were dried for 20 minutes until visibly dry at 24°C in 33.1% relative humidity. For each batch of test substance, five dried inoculated carriers were sprayed at a distance of 6-8 inches with 3 sprays and were exposed for 1 minute at 22°C. Following exposure, the plates were scraped and the virus-test substance mixtures were immediately passed through individual Sephadex columns. The filtrates were serially diluted 10-fold and assayed for infectivity and/or cytotoxicity of the MT-2 cell cultures. Cells in multi well dishes containing RPMI-1640 supplemented with 15% heat inactivated fetal bovine serum and 2.0 mM L-glutamine and 50 µg/mL gentamicin were inoculated in quadruplicate with 200 µL of the virus-test substance dilutions. The cultures were incubated at 36 – 38°C with 5 – 7% CO_2 and scored periodically for fourteen (14) days for the absence or presence of cytopathy, cytotoxicity, and viability. Titers were calculated using the Spearman Karber method. Controls included neutralization, cytotoxicity, and input virus control.

20. MRID 489474-22 "3-in-1 Disinfecting Cleaner Non-Food Contact Surface Sanitizing Efficacy," Test Organism: *Listeria monocytogenes* (ATCC 7644) for product 3-in-1 Disinfecting Cleaner. Study director: Laurinda Holen. Study conducted at ECOLAB 655 Lone Oak Drive Eagan, MN 55121-1560. Study completion date – July 03, 2012. Study Number 1200002.

This study was conducted against *Listeria monocytogenes* (ATCC 7644). Three lots, Batch P081911, Batch P093012, and Batch P100611, of the product were tested using the provided ECOLAB Protocol No. MS016-23. One lot was ≥60 days aged, Batch P081911. The test substance was diluted at 6 oz. per gallon in 200 ppm synthetic hard water resulting in the lower certified limit of 3462 ppm of active ingredient. A culture of the challenge microorganism was prepared by at least three but less than fifteen daily consecutive transfers in appropriate growth medium for each microorganism with no more than one day missed per seven-day period. Each culture was incubated at 35 ± 2°C for 24 ± 4 hours. Each test system was mixed and after ≥15 minutes the upper portion was removed. Fetal bovine serum was added to achieve a 5% organic soil load.

Stainless steel test squares (1" X 1") were inoculated with 0.03 mL of the test system suspension. The carriers were dried for a total drying time of 35 minutes at 30 ± 2°C. The carriers were transferred into individual medicine jars containing the test substance and remained for an exposure period of 90 seconds at room temperature (15 – 30°C). Afterwards, 20 mLs of 2 X DE broth was added to the jars containing the test system for neutralization. Following, 1.0 mL and 0.1 mL of the solutions from all carrier neutralized suspensions were plated in duplicate with Brain Heart Infusion Agar using the pour plate technique. All plates were incubated for 48 ± 4 hours at 35 ± 2°C. Controls include enumeration, purity, sterility, and neutralization confirmation.

21. MRID 489474-23 "3-in-1 Disinfecting Cleaner Germicidal Spray Disinfectant Efficacy," Test Organism: *Staphylococcus aureus* –CA-MRSA (ATCC BAA-1683), *Staphylococcus aureus*- MRSA (ATCC 33592), *Klebsiella pneumoniae* (ATCC 4352) and *Escherichia coli* O157:H7 (ATCC 43895) for product 3-in-1 Disinfecting Cleaner. Study director: Laurinda Holen. Study conducted at ECOLAB 655 Lone Oak Drive Eagan, MN 55121-1560. Study completion date – August 14, 2012. Study Number 1100065.

This study was conducted against *Staphylococcus aureus* –CA-MRSA (ATCC BAA-1683), *Staphylococcus aureus*- MRSA (ATCC 33592), *Klebsiella pneumoniae* (ATCC 4352) and *Escherichia coli* O157:H7 (ATCC 43895). Two lots, Batch P093012 and Batch P100611, of the product were tested using the provided ECOLAB Protocol No. MS010-20. The test substance was diluted at 6 oz. per gallon in 200 ppm synthetic hard water resulting in the lower certified limit of 3462 ppm of active ingredient. A culture of each challenge microorganism was prepared by at least three but less than fifteen daily consecutive transfers in appropriate growth medium for each microorganism with no more than one day missed per seven-day period. The cultures were incubated at 35 ± 2°C for 48 – 54 hours. Fetal bovine serum was added to achieve a 5% organic soil load. Non-corrosive microscope slides (1" X 1") were inoculated with 0.01 mL of the test system suspension. The inoculum was spread uniformly over the carrier to within approximately 1/8 inch of the edges. The carriers were dried for 30-32 minutes at 35 ± 2°C. The inoculated carriers were sprayed at a distance of 6-8 inches with 3 sprays. The test substance remained for an exposure period of 3 minutes. Afterwards, the excess test substance was drained and the carrier was transferred to individual test tubes containing 20 mLs of Letheen Broth with 0.5% Sodium Thiosulfate subculture medium for *S. aureus* –CA-MRSA and *K. pneumoniae* cultures, Brain Heart Infusion Broth with Lecithin and Tween 80 with 1 mL of 0.2% Catalase subculture medium for *S. aureus*-MRSA cultures, and Brain Heart Infusion Broth with 0.5% Sodium Thiosulfate subculture medium for *E. coli* O157:H7 cultures. All subcultures were incubated at 35 ± 2°C for 48 ± 4 hours. Controls include enumeration, sterility, purity, and neutralization confirmation.

Note- Antibiotic susceptibility testing was performed using disk diffusion method. The test confirmed Methicillin resistance for *Staphylococcus aureus* –CA-MRSA (ATCC BAA-1683) and *Staphylococcus aureus*- MRSA (ATCC 33592) using 1mcg oxacillin disks.

22. MRID 489474-24 "3-in-1 Disinfecting Cleaner Supplemental Germicidal Spray Disinfectant Efficacy," Test Organism: *Acinetobacter baumanni* (ATCC 19606), *Klebsiella pneumoniae* (ATCC 4352), *Serratia marcescens* (ATCC 14756), *Shigella flexneri* (ATCC 9380), *Enterobacter aerogenes* (ATCC 13048), *Escherichia coli* O157:H7 (ATCC 43895), *Streptococcus*

***pyogenes* (ATCC 19615), *Shigella dysenteriae* (ATCC 29026), *Bordetella bronchiseptica* (ATCC 31437), and *Listeria monocytogenes* (ATCC 7644) for product 3-in-1 Disinfecting Cleaner. Study director: Laurinda Holen. Study conducted at ECOLAB 655 Lone Oak Drive Eagan, MN 55121-1560. Study completion date – August 14, 2012. Study Number 1100064.**

This study was conducted against *Acinetobacter baumanni* (ATCC 19606), *Klebsiella pneumoniae* (ATCC 4352), *Serratia marcescens* (ATCC 14756), *Shigella flexneri* (ATCC 9380), *Enterobacter aerogenes* (ATCC 13048), *Escherichia coli* O157:H7 (ATCC 43895), *Streptococcus pyogenes* (ATCC 19615), *Shigella dysenteriae* (ATCC 29026), *Bordetella bronchiseptica* (ATCC 31437), and *Listeria monocytogenes* (ATCC 7644). Two lots, Batch P093012 and Batch P100611, of the product were tested using the provided ECOLAB Protocol No. MS010-20. The test substance was diluted at 4 oz. per gallon in 400 ppm synthetic hard water resulting in the lower certified limit of 2308 ppm of active ingredient. Each challenge microorganism was prepared by at least three but less than fifteen daily consecutive transfers in appropriate growth medium for each microorganism with no more than one day missed per seven-day period with the exception of *Listeria monocytogenes* which used to inoculate the test system on the 15th consecutive transfer. Another exception included the transfer of *Bordetella bronchiseptica* organism every other day. The cultures were incubated at 35 ± 2°C for 48 – 54 hours prior to testing except *Serratia marcescens* which was incubated at 26 ± 2°C for 48 – 54 hours. Fetal bovine serum was added to achieve a 5% organic soil load. Non-corrosive microscope slides (1" X 1") were inoculated with 0.01 mL of the test system suspension. The inoculum was spread uniformly over the carrier to within approximately 1/8 inch of the edges. The carriers were dried for 30 minutes at 35 ± 2°C. The inoculated carriers were sprayed at a distance of 6-8 inches with 3 sprays. The test substance remained for an exposure period of 5 minutes at room temperature (15 – 30°C). Afterwards, the excess test substance was drained and the carriers for *Acinetobacter baumanni*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Shigella flexneri*, *Enterobacter aerogenes, and Shigella dysenteriae* were transferred to individual test tubes containing Letheen Broth with 0.5% Sodium Thiosulfate subculture medium and Tryptone Glucose Extract agar for plating medium. The carriers for and *Bordetella bronchiseptica* were transferred to individual test tubes containing Tryptose Phosphate Broth with 0.5% Sodium Thiosulfate medium and Tryptose Phosphate agar for plating medium. The carriers for *Streptococcus pyogenes, Escherichia coli* O1557:H7, and *Listeria monocytogenes* were transferred to individual test tubes containing Brain Heart Infusion Broth with Lecithin and Tween 80 with Catalase medium and Brain Heart Infusion agar for plating medium. The subcultures were incubated at the 35 ± 2°C for 48 ± 4 hours with the exception of *Bordetella bronchiseptica* which was incubated at 30 ± 2°C for 3-4 days and *Serratia marcescens* which was incubated at 26 ± 2°C for 48 ± 4 hours. Controls include enumeration, sterility, purity, and neutralization confirmation.

23. MRID 489474-25 "3-in-1 Disinfecting Cleaner Germicidal Spray Disinfectant Efficacy," Test Organism: *Candida albicans* (ATCC 10231) for product 3-in-1 Disinfecting Cleaner. Study director: Lisa Hellickson. Study conducted at ECOLAB 655 Lone Oak Drive Eagan, MN 55121-1560. Study completion date – August 16, 2012. Study Number 1200039.

This study was conducted against *Candida albicans* (ATCC 10231). Two lots, Batch P093012 and Batch P100611, of the product were tested using the provided ECOLAB Protocol No. MS010-20. The test substance was diluted at 4 oz. per gallon in

400 ppm synthetic hard water resulting in the lower certified limit of 2308 ppm of active ingredient. A culture of the challenge microorganism was prepared by at least three but less than fifteen daily consecutive transfers in appropriate growth medium for each microorganism with no more than one day missed per seven-day period. The culture was incubated at 35 ± 2°C for 48 – 54 hours. Fetal bovine serum was added to achieve a 5% organic soil load. Non-corrosive microscope slides (1" X 1") were inoculated with 0.01 mL of the test system suspension. The carriers were dried for 30 minutes at 35 ± 2°C. The inoculated carriers were sprayed at a distance of 6-8 inches with 3 sprays at 15 second intervals. The test substance remained for an exposure period of 5 minutes at room temperature (15 – 30°C). Afterwards, the excess test substance was drained and the carrier was transferred to individual test tubes containing Sabouraud Dextrose Broth with 0.025% catalase subculture medium. The subcultures were incubated at the 26 ± 2°C for 72 ± 4 hours. Controls include enumeration, sterility, purity, and neutralization confirmation.

24. MRID 489474-26 "3-in-1 Disinfecting Cleaner Germicidal Spray Disinfectant Efficacy," Test Organism: *Bordetella pertussis* (ATCC 12743) for product 3-in-1 Disinfecting Cleaner. Study director: Nicole Albert. Study conducted at ATS LABS 1285 Corporate Center Drive, suite 110 Eagan, MN 55121. Study completion date – January 16, 2012. Study Number 12363.

This study was conducted against *Bordetella pertussis* (ATCC 12743). Two lots, Batch P093012-CL and Batch P100611-CL, of the product were tested using the provided ATS Labs Protocol No. ECO01112211.GS. The test substance was diluted at 4 oz. per gallon in 400 ppm synthetic hard water resulting in the lower certified limit of 2308 ppm of active ingredient. A culture of the challenge microorganism was prepared by inoculating Bordet Gengou agar plates from stock and incubating them at 35 – 37°C for 6 days. Following the organism was suspended in Butterfield's buffer to approximately match a 4.0 McFarland turbidity standard. Fetal bovine serum was added to achieve a 5% organic soil load. Glass slides (1" X 1") were inoculated with 10 µL of the test system suspension. The inoculum was uniformly spread over the entire surface of the slide and the carrier slides were dried at 25 -30°C for 30 minutes at 63% relative humidity. The carriers were dried for 30 minutes at 35 ± 2°C. The inoculated carriers were sprayed at a distance of 6-8 inches with 3 sprays. The test substance remained for an exposure period of 5 minutes at 21°C with 32% relative humidity. Afterwards, the excess test substance was drained and the carrier was transferred to individual test tubes containing 20 mLs of Letheen broth + 0.5% Sodium Thiosulfate subculture medium. The subcultures were filtered through a pre-wet filter membrane of ≥10.0 mL of sterile saline using a vacuum pump. Each filter membrane was washed with ≥50 mL of sterile saline and placed on the surface of a Bordet Gengou Agar plate. All subculture were incubated at the 35 - 37°C for 4 days. Controls include carrier population, sterility, purity, viability, and neutralization confirmation.

25. MRID 489474-27 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Human Coronavirus (ATCC VR-740) for product 3-in-1 Disinfecting Cleaner. Study author: Mary J. Miller. Study conducted at ATS Labs 1285 Corporate Center Drive, Suite 110 Eagan, MN. 55121. Study completion date – January 20, 2012. Study Number 12391.

This study was conducted against Human Coronavirus, Strain 229E (ATCC VR-740). WI-38 (human embryonic lung fibroblasts (ATCC CCL-75) were used as the host cell line. Two lots, Batch P093012-CL and Batch P100611-CL, of the product were tested using the provided ATS Labs Protocol No. ECO01112211.COR. The test substance was diluted at 4 oz. per gallon in 400 ppm synthetic hard water. The stock virus was prepared by collecting the supernatant culture fluid from 75 – 100% infected culture cells that were disrupted and the cell debris removed by centrifugation. The high titer stock virus supernatant was aliquoted and stored at ≤-70°C until the day of use then an aliquot of stock virus (ATS Lot HCV- 63) was removed, thawed, and maintained at a refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain a final organic soil load of 5% fetal bovine serum. A volume of 200 µL of virus inoculum was spread uniformly over the bottoms of 3 separate 100 X 15 mm glass Petri dishes. The virus films were dried for 20 minutes until visibly dry at 20°C in 50% relative humidity. The inoculated carriers were sprayed at a distance of 6-8 inches with 3 sprays and were exposed for 5 minutes at 20°C. Following exposure, the plates were scraped and the virus-test substance mixtures were immediately passed through individual Sephadex columns. The filtrates were serially diluted 10-fold and assayed for infectivity and/or cytotoxicity of the WI-38 cell cultures. Cells in multi well dishes containing Minimum Essential Medium supplemented with 2% heat-inactivated fetal bovine serum were inoculated in quadruplicate with 100 µL of the virus-test substance dilutions. The cultures were incubated at 31 – 35°C with 5 – 7% CO_2 and scored periodically for eleven (11) days for the absence or presence of cytopathy, cytotoxicity, and viability. Titers were calculated using the Spearman Karber method. Controls included neutralization, cytotoxicity, and input virus control.

26. MRID 489474-28 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Vaccinia Virus (ATCC VR-119) for product 3-in-1 Disinfecting Cleaner. Study author: Shanen Conway. Study conducted at ATS Labs 1285 Corporate Center Drive, Suite 110 Eagan, MN. 55121. Study completion date – January 17, 2012. Study Number 12392.

This study was conducted against Vaccinia Virus, Strain WR (ATCC VR-119). Vero cells (ATCC CCL-81) were used as the host cell line. Two lots, Batch P093012-CL and Batch P100611-CL, of the product were tested using the provided ATS Labs Protocol No. ECO01112211.VAC. The test substance was diluted at 4 oz. per gallon in 400 ppm synthetic hard water. The stock virus was prepared by collecting the supernatant culture fluid from 75 – 100% infected culture cells that were disrupted and the cell debris removed by centrifugation. The high titer stock virus supernatant was aliquoted and stored at ≤-70°C until the day of use an aliquot of stock virus (ATS Lot V19) was removed, thawed, and maintained at a refrigerated temperature until used in the assay. The stock virus culture contained an organic soil load of 5% fetal bovine serum. A volume of 200 µL of virus inoculum was spread uniformly over the bottoms of three (3) separate 100 X 15 mm glass Petri dishes. The virus films were dried for 20 minutes until visibly dry at 20°C in 50% relative humidity. For each batch of test substance, one inoculated carrier was sprayed at a distance of 6-8 inches with 3 sprays and was exposed for 5 minutes at 20°C. Following exposure, the plates were scraped and the virus-test substance mixtures were immediately passed through individual Sephadex columns. The filtrates were serially diluted 10-fold and assayed for infectivity and/or cytotoxicity of the Vero cell cultures. Cells in multi well dishes containing Minimum Essential Medium supplemented with 5% heat-inactivated fetal bovine serum

were inoculated in quadruplicate with 100 µL of the virus-test substance dilutions. The cultures were incubated at 36 – 38°C with 5 – 7% CO_2 and scored periodically for seven (7) days for the absence or presence of cytopathy, cytotoxicity, and viability. Titers were calculated using the Spearman Karber method. Controls included neutralization, cytotoxicity, and input virus control.

27. MRID 489474-29 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Canine Parvovirus (ATCC VR-2017) for product 3-in-1 Disinfecting Cleaner. Study author: Shanen Conway. Study conducted at ATS Labs 1285 Corporate Center Drive, Suite 110 Eagan, MN. 55121. Study completion date – January 18, 2012. Study Number 12373.

This study was conducted against Canine Parvovirus, Strain Cornell-780916-80 (ATCC VR-2017). Canine tumor (A-72) cells (ATCC CRL-1542) were used as the host cell line. Two lots, Batch P093012-CL and Batch P100611-CL, of the product were tested using the provided ATS Labs Protocol No. ECO01112211.CPV. The test substance was diluted at 4 oz. per gallon in 400 ppm synthetic hard water. The stock virus was prepared by collecting the supernatant culture fluid from 75 – 100% infected culture cells that were disrupted and the cell debris removed by centrifugation. The high titer stock virus supernatant was aliquoted and stored at ≤-70°C until the day of use an aliquot of stock virus (ATS Lot CPV1-19) was removed, thawed, and maintained at a refrigerated temperature until used in the assay. The stock virus culture contained an organic soil load of 5% fetal bovine serum. A volume of 200 µL of virus inoculum was spread uniformly over the bottoms of three (3) separate 100 X 15 mm glass Petri dishes. The virus films were dried for 20 minutes until visibly dry at 20°C in 40% relative humidity. For each batch of test substance, one inoculated carrier was sprayed at a distance of 6-8 inches with 3 sprays and was exposed for 5 minutes at 20°C. Following exposure, the plates were scraped and the virus-test substance mixtures were immediately passed through individual Sephadex columns. The filtrates were serially diluted 10-fold and assayed for infectivity and/or cytotoxicity of the A-72 cell cultures. Cells in multi well dishes containing Eagle's Minimum Essential Medium supplemented with 5% heat-inactivated fetal bovine serum were inoculated in quadruplicate with 100 µL of the virus-test substance dilutions. The cultures were incubated at 36 – 38°C with 5 – 7% CO_2 and scored periodically for seven (7) days for the absence or presence of cytopathy, cytotoxicity, and viability. Titers were calculated using the Spearman Karber method. Controls included neutralization, cytotoxicity, and input virus control.

28. MRID 489474-30 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Canine Distemper Virus (ATCC VR-128) for product 3-in-1 Disinfecting Cleaner. Study author: Kelleen Gutzmann. Study conducted at ATS Labs 1285 Corporate Center Drive, Suite 110 Eagan, MN. 55121. Study completion date – February 15, 2012. Study Number 12544.

This study was conducted against Canine Distemper Virus, Strain Lederle (ATCC VR-128). Vero cells (ATCC CCL-81) were used as the host cell line. Two lots, Batch P093012-CL and Batch P100611-CL, of the product were tested using the provided ATS Labs Protocol No. ECO01121611.CDIS. The test substance was diluted at 4 oz. per gallon in 400 ppm synthetic hard water. The stock virus was prepared by collecting the supernatant culture fluid from 75 – 100% infected culture cells that were disrupted and

the cell debris removed by centrifugation. The high titer stock virus supernatant was aliquoted and stored at ≤-70°C until the day of use an aliquot of stock virus (ATS Lot CDV-11) was removed, thawed, and maintained at a refrigerated temperature until used in the assay. The stock virus culture contained an organic soil load of 5% fetal bovine serum. A volume of 200 µL of virus inoculum was spread uniformly over the bottoms of three (3) separate 100 X 15 mm glass Petri dishes. The virus films were dried for 20 minutes until visibly dry at 20°C in 40% relative humidity. For each batch of test substance, one inoculated carrier was sprayed at a distance of 6-8 inches with 3 sprays and was exposed for 5 minutes at 20°C. Following exposure, the plates were scraped and the virus-test substance mixtures were immediately passed through individual Sephadex columns. The filtrates were serially diluted 10-fold and assayed for infectivity and/or cytotoxicity of the Vero cell cultures. Cells in multi well dishes containing Minimum Essential Medium supplemented with 5% heat-inactivated fetal bovine serum were inoculated in quadruplicate with 100 µL of the virus-test substance dilutions. The cultures were incubated at 36 – 38°C with 5 – 7% CO_2 and scored periodically for seven (7) days for the absence or presence of cytopathy, cytotoxicity, and viability. Titers were calculated using the Spearman Karber method. Controls included neutralization, cytotoxicity, and input virus control.

29. MRID 489474-31 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Hepatitis B Virus as a Surrogate Virus for Human Hepatitis B Virus (Hepadnavirus Testing, CA.) for product 3-in-1 Disinfecting Cleaner. Study author: Shanen Conway. Study conducted at ATS Labs 1285 Corporate Center Drive, Suite 110 Eagan, MN. 55121. Study completion date – February 24, 2012. Study Number 12469.

This study was conducted against Hepatitis B Virus (HBV) Strain 7/31/07 (obtained from Hepadnavirus Testing Inc., Palo Alto, CA.). Two day old Pekin breed hatchlings ducks blood Hepatocyte cells (obtained from Metzer Farms by Valley Research Institute and isolated by ATS Labs) were used as the host cell line. Two lots, Batch P093012-CL and Batch P100611-CL, of the product were tested using the provided ATS Labs Protocol No. ECO01112211.DHBV.1. The test substance was diluted at 4 oz. per gallon in 400 ppm synthetic hard water. The stock virus was prepared by collecting the supernatant culture fluid from infected culture cells that were disrupted and the cell debris removed by centrifugation. The high titer stock virus supernatant was aliquoted and stored at ≤-70°C until the day of use then two aliquots of stock virus (ATS Lot 7/31/07 pool) were removed, thawed, combined, and maintained at a refrigerated temperature until used in the assay. The stock virus culture contained an organic soil load of 100% duck serum. A volume of 200 µL of virus inoculum was spread uniformly over the bottoms of six (6) separate 100 X 15 mm glass Petri dishes. The virus films were dried for 20 minutes until visibly dry at 20°C in 50% relative humidity. For each batch of test substance, two dried inoculated carriers were sprayed at a distance of 6-8 inches with 3 sprays and were exposed for 5 minutes at 20°C. Following exposure, the plates were scraped and the virus-test substance mixtures were immediately passed through individual Sephadex columns. The filtrates were serially diluted 10-fold and assayed for infectivity and/or cytotoxicity of the primary duck hepatocytes cell cultures. Cells in multi well dishes containing Leibovitz L-15 medium supplemented with 0.1% glucose, 10 µM dexamethasone, 10 µg/mL insulin, 20 mM HEPES, 10 µg/mL gentamicin, and 100 units/mL penicillin were inoculated in quadruplicate with 250 µL of the virus-test substance dilutions. The cultures were

incubated at 36 – 38°C with 5 – 7% CO_2 and scored periodically for nine (9) days prior to observation microscopically for test substance cytotoxicity and then the cells were fixed with ethanol. Using a monoclonal antibody specific for the envelope protein of the DHBV, an indirect immunoflorescence assay was performed. Titers were calculated using the Spearman Karber method. Controls included neutralization, cytotoxicity, and input virus control.

30. MRID 489474-32 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Respiratory Syncytial Virus (ViroMed Laboratories) for product 3-in-1 Disinfecting Cleaner. Study author: Shanen Conway. Study conducted at ATS Labs 1285 Corporate Center Drive, Suite 110 Eagan, MN. 55121. Study completion date – January 6, 2012. Study Number 12381.

This study was conducted against Respiratory syncytial (RSV) virus, Strain Long (ATCC VR-26). Human larnyx carcinoma (Hep-2) cells (ViroMed Laboratories) were used as the host cell line. Two lots, Batch P093012-CL and Batch P100611-CL, of the product were tested using the provided ATS Labs Protocol No. ECO01112211.RSV. The test substance was diluted at 4 oz. per gallon in 400 ppm synthetic hard water. The stock virus was prepared by collecting the supernatant culture fluid from 75 – 100% infected culture cells that were disrupted and the cell debris removed by centrifugation. The high titer stock virus supernatant was aliquoted and stored at ≤-70°C until the day of use an aliquot of stock virus (ATS Lot NRSV-23) was removed, thawed, and maintained at a refrigerated temperature until used in the assay. The stock virus culture contained an organic soil load of 5% fetal bovine serum. A volume of 200 µL of virus inoculum was spread uniformly over the bottoms of three (3) separate 100 X 15 mm glass Petri dishes. The virus films were dried for 20 minutes until visibly dry at 20°C in 50% relative humidity. For each batch of test substance, one inoculated carrier was sprayed at a distance of 6-8 inches with 3 sprays and was exposed for 5 minutes at 20°C. Following exposure, the plates were scraped and the virus-test substance mixtures were immediately passed through individual Sephadex columns. The filtrates were serially diluted 10-fold and assayed for infectivity and/or cytotoxicity of the Hep-2 cell cultures. Cells in multi well dishes containing Minimum Essential Medium supplemented with 2% heat-inactivated fetal bovine serum were inoculated in quadruplicate with 100 µL of the virus-test substance dilutions. The cultures were incubated at 36 – 38°C with 5 – 7% CO_2 and scored periodically for ten (10) days for the absence or presence of cytopathy, cytotoxicity, and viability. Titers were calculated using the Spearman Karber method. Controls included neutralization, cytotoxicity, and input virus control.

31. MRID 489474-33 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Confirmatory Assay," Test Organism: Duck Hepatitis B Virus as a Surrogate Virus for Human Hepatitis B Virus (Hepadnavirus Testing, Inc., CA.) for product 3-in-1 Disinfecting Cleaner. Study author: Mary J. Miller. Study conducted at ATS Labs 1285 Corporate Center Drive, Suite 110 Eagan, MN. 55121. Study completion date – February 22, 2012. Study Number 12428.

This study was conducted against Duck Hepatitis B virus (DHBV), Strain 7/31/07 (obtained from Hepadnavirus Testing Inc., Palo Alto, CA.). Two day old Pekin breed hatchlings ducks blood Hepatocyte cells (obtained from Metzer Farms by Valley Research Institute and isolated by ATS Labs) were used as the host cell line. One lot,

Batch P100611-CL, of the product was tested using the provided ATS Labs Protocol No. ECO01112211.DHBV.2. The test substance was diluted at 4 oz. per gallon in 400 ppm synthetic hard water. The stock virus was prepared by collecting the supernatant culture fluid from infected culture cells that were disrupted and the cell debris removed by centrifugation. The high titer stock virus supernatant was aliquoted and stored at ≤-70°C until the day of use then an aliquot of stock virus (ATS Lot 7/31/07 pool) was removed, thawed, and maintained at a refrigerated temperature until used in the assay. The stock virus culture contained an organic soil load of 100% duck serum. A volume of 200 µL of virus inoculum was spread uniformly over the bottoms of four (4) separate 100 X 15 mm glass Petri dishes. The virus films were dried for 30 minutes until visibly dry at 20°C in 50% relative humidity. For each batch of test substance, two inoculated carriers were sprayed at a distance of 6-8 inches with 3 sprays and were exposed for 5 minutes at 20°C. Following exposure, the plates were scraped and the virus-test substance mixtures were immediately passed through individual Sephadex columns. The filtrates were serially diluted 10-fold and assayed for infectivity and/or cytotoxicity of the primary duck hepatocyte cell cultures. Cells in multi well dishes Leibovitz L-15 medium supplemented with 0.1% glucose, 10 µM dexamethasone, 10 µg/mL insulin, 20 mM HEPES, 10 µg/mL gentamicin, and 100 units/mL penicillin were inoculated in quadruplicate with 250 µL of the virus-test substance dilutions. The cultures were incubated for 60 minutes to allow for absorption. The cultures were incubated at 36 – 38°C with 5 – 7% CO_2 and scored periodically for nine (9) days for the absence or presence of cytopathy, cytotoxicity, and viability. Titers were calculated using the Spearman Karber method. Controls included neutralization, cytotoxicity, and input virus control.

32. MRID 489474-34 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Herpes Simplex Virus Type 2 (ATCC VR-734) for product 3-in-1 Disinfecting Cleaner. Study author: Dawn Pierson. Study conducted at ATS Labs 1285 Corporate Center Drive, Suite 110 Eagan, MN. 55121. Study completion date – August 2, 2012. Study Number 13533.

This study was conducted against Herpes simplex virus type 2, Strain G (ATCC VR-734). Rabbit Kidney (RK) cells (ViroMed Laboratories) were used as the host cell line. Two lots, Batch P093012-CL and Batch P100611-CL, of the product were tested using the provided ATS Labs Protocol No. ECO01061112.HSV. The test substance was diluted at 4 oz. per gallon in 400 ppm synthetic hard water. The stock virus was prepared by collecting the supernatant culture fluid from 75 – 100% infected culture cells that were disrupted and the cell debris removed by centrifugation. The high titer stock virus supernatant was aliquoted and stored at ≤-70°C until the day of use an aliquot of stock virus (ATS Lot H2-64) was removed, thawed, and maintained at a refrigerated temperature until used in the assay. The stock virus culture contained an organic soil load of 5% fetal bovine serum. A volume of 200 µL of virus inoculum was spread uniformly over the bottoms of three (3) separate 100 X 15 mm glass Petri dishes. The virus films were dried for 20 minutes until visibly dry at 20°C in 50% relative humidity. For each batch of test substance, one inoculated carrier was sprayed at a distance of 6-8 inches with 3 sprays and was exposed for 5 minutes at 21°C. Following exposure, the plates were scraped and the virus-test substance mixtures were immediately passed through individual Sephadex columns. The filtrates were serially diluted 10-fold and assayed for infectivity and/or cytotoxicity of the RK cell cultures. Cells in multi well

dishes containing Minimum Essential Medium supplemented with 5% heat-inactivated fetal bovine serum were inoculated in quadruplicate with 100 µL of the virus-test substance dilutions. The cultures were incubated at 36 – 38°C with 5 – 7% CO_2 and scored periodically for seven (7) days for the absence or presence of cytopathy, cytotoxicity, and viability. Titers were calculated using the Spearman Karber method. Controls included neutralization, cytotoxicity, and input virus control.

33. MRID 489474-35 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Rotavirus (University of Ottawa, Ontario, Canada) for product 3-in-1 Disinfecting Cleaner. Study author: Shanen Conway. Study conducted at ATS Labs 1285 Corporate Center Drive, Suite 110 Eagan, MN. 55121. Study completion date – May 24, 2012. Study Number 12972.

This study was conducted against Rotavirus, Strain WA (obtained from University of Ottawa, Ontario, Canada). Rhesus monkey kidney (MA-104) cells (obtained from Diagnostic Hybrids, Athens, OH.) were used as the host cell line. Two lots, Batch P093012-CL and Batch P100611-CL, of the product were tested using the provided ATS Labs Protocol No. ECO01031412.ROT. The test substance was diluted at 4 oz. per gallon in 400 ppm synthetic hard water. The stock virus was prepared by collecting the supernatant culture fluid from 75 – 100% infected culture cells that were disrupted and the cell debris removed by centrifugation. The high titer stock virus supernatant was aliquoted and stored at ≤-70°C until the day of use four aliquots of stock virus (ATS Lot XR-139) were removed, thawed, combined, and maintained at a refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain an organic soil load of 5% fetal bovine serum. A volume of 200 µL of virus inoculum was spread uniformly over the bottoms of fifteen (15) separate 100 X 15 mm glass Petri dishes. The virus films were dried for 20 minutes until visibly dry at 20°C in 50% relative humidity. For each batch of test substance, five inoculated carriers were sprayed at a distance of 6-8 inches with 3 sprays and were exposed for 5 minutes at 20°C. Following exposure, the plates were scraped and the virus-test substance mixtures were immediately passed through individual Sephadex columns. The filtrates were serially diluted 10-fold and assayed for infectivity and/or cytotoxicity of the MA-104 cell cultures. Cells in multi well dishes containing Minimum Essential Medium were inoculated in quadruplicate with 100 µL of the virus-test substance dilutions. The cultures were incubated at 36 – 38°C with 5 – 7% CO_2 and scored periodically for seven (7) days for the absence or presence of cytopathy, cytotoxicity, and viability. Titers were calculated using the Spearman Karber method. Controls included neutralization, cytotoxicity, and input virus control.

34. MRID 489474-36 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Polio Type 1 (ATCC VR-1562) for product 3-in-1 Disinfecting Cleaner. Study author: Mary J. Miller. Study conducted at ATS Labs 1285 Corporate Center Drive, Suite 110 Eagan, MN. 55121. Study completion date – March 12, 2012. Study Number 12671.

This study was conducted against Poliovirus type 1, Strain Chat (ATCC VR-1562). Vero cells (ATCC CCL-81) were used as the host cell line. Three lots, Batch P081911-CL, Batch P093012-CL and Batch P100611-CL, of the product were tested using the provided ATS Labs Protocol No. ECO01011912.POL. One batch was ≥60

days aged, Batch P081911-CL. The test substance was diluted at 4 oz. per gallon in 400 ppm synthetic hard water. The stock virus was prepared by collecting the supernatant culture fluid from 75 – 100% infected culture cells that were disrupted and the cell debris removed by centrifugation. The high titer stock virus supernatant was aliquoted and stored at ≤-70°C until the day of use five aliquots of stock virus (ATS Lot PC2-14) were removed, thawed, combined, and maintained at a refrigerated temperature until used in the assay. The stock virus culture contained an organic soil load of 5% fetal bovine serum. A volume of 200 µL of virus inoculum was spread uniformly over the bottoms of twenty (20) separate 100 X 15 mm glass Petri dishes. The virus films were dried for 20 minutes until visibly dry at 15.5°C in 55% relative humidity. For each batch of test substance, five inoculated carriers were sprayed at a distance of 6-8 inches with 3 sprays and were exposed for 5 minutes at 21°C. Following exposure, the plates were scraped and the virus-test substance mixtures were immediately passed through individual Sephadex columns. The filtrates were serially diluted 10-fold and assayed for infectivity and/or cytotoxicity of the A-72 cell cultures. Cells in multi well dishes containing Minimum Essential Medium supplemented with 5% heat-inactivated fetal bovine serum were inoculated in quadruplicate with 100 µL of the virus-test substance dilutions. The cultures were incubated at 36 – 38°C with 5 – 7% CO_2 and scored periodically for seven (7) days for the absence or presence of cytopathy, cytotoxicity, and viability. Titers were calculated using the Spearman Karber method. Controls included neutralization, cytotoxicity, and input virus control.

35. MRID 489474-37 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Newcastle Disease Virus (ATCC VR-108) for product 3-in-1 Disinfecting Cleaner. Study author: Kelleen Gutzmann. Study conducted at ATS Labs 1285 Corporate Center Drive, Suite 110 Eagan, MN. 55121. Study completion date – March 19, 2012. Study Number 12543.

This study was conducted against Newcastle disease virus, Strain B1, Hitcher or Blacksburg (ATCC VR-108). Chicken embryo fibroblast (CEF) cells (obtained from Charles River Laboratories) were used as the host cell line. Two lots, Batch P093012-CL and Batch P100611-CL, of the product were tested using the provided ATS Labs Protocol No. ECO01121611.NEW. The test substance was diluted at 4 oz. per gallon in 400 ppm synthetic hard water. The stock virus was prepared by collecting the allantoic fluid from inoculated ten day old fertilized, embryonated chicken eggs. The fluid was centrifuged and the high titer stock virus supernatant was aliquoted and stored at ≤-70°C until the day of use an aliquot of stock virus (ATS Lot NDV-39A) was removed, thawed, and maintained at a refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain an organic soil load of 5% fetal bovine serum. A volume of 200 µL of virus inoculum was spread uniformly over the bottoms of three (3) separate 100 X 15 mm glass Petri dishes. The virus films were dried for 20 minutes until visibly dry at 20°C in 50% relative humidity. For each batch of test substance, one inoculated carrier was sprayed at a distance of 6-8 inches with 3 sprays and was exposed for 5 minutes at 21°C. Following exposure, the plates were scraped and the virus-test substance mixtures were immediately passed through individual Sephadex columns. The filtrates were serially diluted 10-fold and assayed for infectivity and/or cytotoxicity of the CEF cell cultures. Cells in multi well dishes containing Minimum Essential Medium supplemented with 2% heat-inactivated fetal bovine serum were inoculated in quadruplicate with 100 µL of the virus-test substance dilutions. The cultures were incubated at 36 – 38°C with 5 – 7% CO_2 and scored periodically for seven (7) days for

the absence or presence of cytopathy, cytotoxicity, and viability. Titers were calculated using the Spearman Karber method. Controls included neutralization, cytotoxicity, and input virus control.

36. MRID 489474-38 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Test Organism: Murine Norovirus (Washington University, St. Louis, MO.) for product 3-in-1 Disinfecting Cleaner. Study author: Shanen Conway. Study conducted at ATS Labs 1285 Corporate Center Drive, Suite 110 Eagan, MN. 55121. Study completion date – May 23, 2012. Study Number 12974.

This study was conducted against Murine Norovirus, Strain MNV-1.CW1 (obtained from Washington University, St. Louis, MO.). A continuous mouse macrophage cell line (RAW 264.7) (obtained from Washington University, St. Louis, MO.) were used as the host cell line. Two lots, Batch P093012-CL and Batch P100611-CL, of the product were tested using the provided ATS Labs Protocol No. ECO01031412.MNV. The test substance was diluted at 4 oz. per gallon in 400 ppm synthetic hard water. The stock virus was prepared by collecting the supernatant culture fluid from infected culture cells that were disrupted and the cell debris removed by centrifugation. The high titer stock virus supernatant was aliquoted and stored at ≤-70°C until the day of use an aliquot of stock virus (ATS Lot MNV-3) was removed, thawed, combined, and maintained at a refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain an organic soil load of 5% fetal bovine serum. A volume of 200 µL of virus inoculum was spread uniformly over the bottoms of three (3) separate 100 X 15 mm glass Petri dishes. The virus films were dried for 20 minutes until visibly dry at 20°C in 50% relative humidity. For each batch of test substance, one inoculated carrier was sprayed at a distance of 6-8 inches with 3 sprays and was exposed for 5 minutes at 22°C. Following exposure, the plates were scraped and the virus-test substance mixtures were immediately passed through individual Sephadex columns. The filtrates were serially diluted 10-fold and assayed for infectivity and/or cytotoxicity of the RAW 264.7 cell cultures. Cells in multi well dishes containing complete 2X Minimum Essential Medium were inoculated in quadruplicate with 250 µL of the virus-test substance dilutions. The cultures were incubated for 60 minutes to allow for absorption. Afterwards, an aliquot of MNV Overlay Agarose I was inoculated into each well of the cell culture plates and allowed to incubate for two (2) days at 36 – 38°C with 5 – 7% CO_2. Following an aliquot of MNV Overlay Agarose II containing neutral red stain was added and the cultures were incubated at 36 – 38°C with 5 – 7% CO_2 for four hours prior to visually and microscopically observing for viral specific plaques and test substance cytotoxicity. Titers were calculated using the Spearman Karber method. Controls included neutralization, cytotoxicity, and input virus control.

Non-Food Contact Surface Sanitize Efficacy Results:						
MRID #	Organism	Percent Reduction Results/ Geometric Mean			Contact Time	Enumeration Geometric Mean CFU/Square
		Batch P081911	Batch P093012	Batch P100611		
489474 -04	*Enterococcus faecalis* Test date: 02/16/12	---	>99.9 <3.0 X 10^1	>99.9 <9.6 X 10^1	90 seconds	2.1 X 10^7
489474 -04	*Pseudomonas aeruginosa* Test date: 04/12/12	---	>99.9 <2.5 X 10^1	>99.9 <2.5 X 10^1	90 seconds	1.0 X 10^6
489474 -04	*Escherichia coli* Test date: 04/20/12	---	>99.9 <6.3 X 10^2	>99.9 <1.1 X 10^2	90 seconds	1.9 X 10^6
489474 -04	Salmonella enterica Test date: 08/13/12	---	>99.9 <5.4 X 10^1	>99.9 <6.4 X 10^1	90 seconds	1.6 X 10^6
489474 -20	*Staphylococcus aureus*	>99.9 <6.6 X 10^1	>99.9 <2.4 X 10^2	>99.9 <4.9 X 10^2	90 seconds	8.8 X 10^6
489474 -20	*Enterobacter aerogenes*	>99.9 <5.2 X 10^1	>99.9 <5.9 X 10^2	>99.9 <3.9 X 10^1	90 seconds	7.8 X 10^6
489474 -22	*Listeria monocytogenes*	---	>99.9 <2.5 X 10^1	>99.9 <1.0 X 10^2	90 seconds	2.5 X 10^5

Disinfection Efficacy Results:							
MRID #	Organism	Number showing no growth/ Total number tested			ppm tested	Average CFU/carrier	Contact Time (minutes)
		Batch P081911	Batch P093012	Batch P100611			
89474-16	*Staphylococcus aureus*	58/60	60/60	59/60	2308	3.7 X 10^6	3
89474-16	*Staphylococcus aureus*	60/60	---	---	3462	3.3 X 10^6	3
89474-16	*Salmonella enterica*	60/60	60/60	60/60	3462	1.5 X 10^4	3
89474-16	*Pseudomonas aeruginosa*	60/60	59/60	60/60	3462	7.9 X 10^5	3
89474-17	*Pseudomonas aeruginosa*	60/60	60/60	60/60	2308	6.8 X 10^5	5
89474-17	*Staphylococcus aureus*	60/60	59/60	60/60	2308	5.5 X 10^6	5
89474-17	*Salmonella enterica*	60/60	60/60	60/60	2308	1.3 X 10^4	5
89474-18	*Serratia marcescens*	---	10/10	10/10	3462	1.9 X 10^6	3
89474-18	*Enterobacter aerogenes*	---	9/10	10/10	3462	2.7 X 10^6	3
89474-18	*Bordetella bronchiseptica*	---	10/10	10/10	3462	1.9 X 10^6	3
89474-23	*S. aureus* – CA-MRSA	---	10/10	10/10	3462	6.2 X 10^5	3
89474-23	*E. coli* O157:H7	---	7/10	9/10	3462	2.2 X 10^6	3
89474-23	*K. pneumoniae*	---	10/10	10/10	3462	8.1 X 10^4	3
89474-23	*S. aureus* – MRSA test date 3/15/12	---	10/10		3462	2.2 X 10^6	3
89474-23	*S. aureus* - MRSA test date 4/05/12			10/10	3462	2.3 X 10^6	3

Disinfection Efficacy Results:						
MRID #	**Organism**	**Number showing no growth/ Total number tested**			**Contact Time (minutes)**	**Enumeration Geometric Mean CFU/carrier**
		Batch P081911	**Batch P093012**	**Batch P100611**		
489474-05	*Acinetobacter baumanni*-MDR	---	9/10	10/10	5	1.1×10^5
489474-05	*Staphylococcus aureus*-VISA	---	10/10	10/10	5	2.4×10^6
489474-05	*Staphylococcus aureus-* *CA-MRSA* *Test date: 1/24/12* *Test date: 3/15/12*	---	10/10	10/10	5	7.4×10^5 6.9×10^5
489474-05	*Staphylococcus aureus-* MRSA	---	10/10	10/10	5	2.1×10^6
489474-05	*Klebsiella pneumoniae- KPC*	---	10/10	10/10	5	9.0×10^5
489474-05	*Enterococcus faecalis- VRE* *Test date: 3/15/12* *Test date: 6/07/12*	---	10/10	10/10	5	1.3×10^6 2.5×10^6
489474-10	*Enterobacter aerogenes*	---	10/10	10/10	5	7.1×10^5
489474-10	*Escherichia coli O157:H7*	---	10/10	10/10	5	1.5×10^6
489474-19	*Proteus mirabilis*	---	10/10	10/10	3	3.1×10^5
489474-24	*Acinetobacter baumannii*	---	10/10	10/10	5	3.2×10^5
489474-24	*Klebsiella pneumoniae*	---	10/10	10/10	5	3.8×10^5
489474-24	*Serratia marcescens*	---	9/10	7/10	5	2.6×10^6
489474-24	*Shigella flexneri*	---	10/10	10/10	5	2.2×10^4
489474-24	*Enterobacter aerogenes*	---	8/10	8/10	5	4.0×10^6
489474-24	*Escherichia coli O157:H7*	---	10/10	7/10	5	5.8×10^6
489474-24	*Streptococcus pyogenes*	---	10/10	10/10	5	1.0×10^5
489474-24	*Shigella dysenteriae*	---	10/10	10/10	5	1.2×10^4
489474-24	*Bordetella bronchiseptica*	---	9/10	9/10	5	6.4×10^4
489474-24	*Listeria monocytogenes*	---	10/10	10/10	5	5.7×10^4
489474-25	*Candida albicans*	---	10/10	10/10	5	9.0×10^4
489474-26	*Bordetella pertussis* ** A Passing result is accomplished by demonstrating no growth of the test organism on all test filters from 10 inoculated carriers.*	---	Pass*	Pass*	5	8.7×10^4

Virucidal Efficacy of Disinfection Results:							
MRID #	Organism	RESULTS (Log_{10})			Dilution/ Cytotoxicity (CT)	Dried Virus Control Average (Log_{10}) $TCID_{50}$/0.1 mL	Contact Time (minutes)
			Batch P093012	Batch P100611			
489474 -06	Rhinovirus type 37		$\leq 10^{1.50}$	$\leq 10^{1.50}$	10^{-1} to 10^{-7} CT- 10^{-1}	$10^{5.40}$	5
		Log_{10} Reduction:	≥3.90	≥3.90			
489474 -07	Herpes Simplex virus type 1		$\leq 10^{1.50}$	$\leq 10^{1.50}$	10^{-1} to 10^{-7} CT- 10^{-1}	$10^{4.90}$	5*
		Log_{10} Reduction:	≥3.40	≥3.40			
489474 -08	Adenovirus Type 5		$\leq 10^{1.50}$	$\leq 10^{1.50}$	10^{-1} to 10^{-7} CT- 10^{-1}	$10^{6.20}$	5
		Log_{10} Reduction:	≥4.70	≥4.70			
489474 -09	Rhinovirus Type 37		$\leq 10^{1.50}$	$\leq 10^{1.50}$	10^{-1} to 10^{-7} CT- 10^{-1}	$10^{5.62}$	3
		Log_{10} Reduction:	≥4.12	≥4.12			
489474 -11	Norovirus (Feline Calicivirus)		$\leq 10^{1.50}$	$\leq 10^{1.50}$	10^{-1} to 10^{-7} CT- 10^{-1}	$10^{5.50}$	3¥
		Log_{10} Reduction:	≥3.00	≥3.00			
489474 -12	Influenza A Virus		$\leq 10^{0.50}$	$\leq 10^{0.50}$	10^{-1} to 10^{-7} CT- None	$10^{4.25}$	5§
		Log_{10} Reduction:	≥3.75	≥3.75			
489474 -13	Norovirus (Feline Calicivirus)		$\leq 10^{1.50}$	$\leq 10^{1.50}$	10^{-1} to 10^{-7} CT- 10^{-1}	$10^{5.55}$	5¥
		Log_{10} Reduction:	≥4.05	≥4.05			
489474 -14	Influenza A Virus		$\leq 10^{1.50}$	$\leq 10^{1.50}$	10^{-1} to 10^{-7} CT- 10^{-1}	$10^{5.00}$	1§
		Log_{10} Reduction:	≥3.50	≥3.50			
489474 -15	Herpes Simplex virus type 1		$\leq 10^{1.50}$	$\leq 10^{1.50}$	10^{-1} to 10^{-7} CT- 10^{-1}	$10^{4.50}$	3*
		Log_{10} Reduction:	≥3.00	≥3.00			
489474 -21	HIV Type 1 **$TCID_{50}$/0.2 mL**		$\leq 10^{1.50}$	$\leq 10^{1.50}$	10^{-1} to 10^{-7} CT- 10^{-1}	$10^{5.85}$	1
		Log_{10} Reduction:	≥4.35	≥4.35			
489474 -27	Human Coronavirus		$\leq 10^{0.50}$	$\leq 10^{0.50}$	10^{-1} to 10^{-7} CT- None	$10^{5.50}$	5
		Log_{10} Reduction:	≥5.00	≥5.00			
489474 -28	Vaccinia Virus		$\leq 10^{0.50}$	$\leq 10^{0.50}$	10^{-1} to 10^{-7} CT- None	$10^{7.50}$	5
		Log_{10} Reduction:	≥7.00	≥7.00			
489474 -29	Canine Parvovirus		$\leq 10^{0.50}$	$\leq 10^{0.50}$	10^{-1} to 10^{-7} CT- None	$10^{6.50}$	5
		Log_{10} Reduction:	≥5.00	≥5.00			
489474 -30	Canine Distemper virus		$\leq 10^{0.50}$	$\leq 10^{0.50}$	10^{-1} to 10^{-7} CT- None	$10^{6.00}$	5
		Log_{10} Reduction:	≥5.50	≥5.50			
489474 -31	Hepatitis B **$TCID_{50}$/0.25 mL**		$\leq 10^{0.50}$	$\leq 10^{0.50}$	10^{-1} to 10^{-7} CT- None	$10^{5.00}$	5
		Log_{10} Reduction:	≥4.50	≥4.50			
489474 -32	Respiratory Syncytial virus		$\leq 10^{1.50}$	$\leq 10^{1.50}$	10^{-1} to 10^{-7} CT- 10^{-1}	$10^{4.75}$	5
		Log_{10} Reduction:	≥3.25	≥3.25			
489474 -33	Hepatitis B **$TCID_{50}$/0.25 mL**		$\leq 10^{0.50}$	$\leq 10^{0.50}$	10^{-1} to 10^{-7} CT- None	$10^{5.12}$	5
		Log_{10} Reduction:	≥4.63	≥4.63			
489474 -34	Herpes simplex virus type 2		$\leq 10^{0.50}$	$\leq 10^{0.50}$	10^{-1} to 10^{-7} CT- None	$10^{4.50}$	5
		Log_{10} Reduction:	≥4.00	≥4.00			
489474 -35	Rotavirus		$\leq 10^{0.50}$	$\leq 10^{0.50}$	10^{-1} to 10^{-7} CT- None	$10^{5.29}$	5
		Log_{10} Reduction:	≥4.79	≥4.79			
489474 -36	Poliovirus type 1		$\leq 10^{0.50}$	$\leq 10^{0.50}$	10^{-1} to 10^{-7} CT- None	$10^{5.03}$	5
		Log_{10} Reduction:	≥4.53	≥4.53			
489474 -37	Newcastle disease virus		$\leq 10^{0.50}$	$\leq 10^{0.50}$	10^{-1} to 10^{-7} CT- None	$10^{6.00}$	5
		Log_{10} Reduction:	≥5.50	≥5.50			
489474 -38	Murine Norovirus		$\leq 10^{0.50}$	$\leq 10^{0.50}$	10^{-1} to 10^{-7} CT- None	$10^{5.50}$	5
		Log_{10} Reduction:	≥5.00	≥5.00			

VI. CONCLUSIONS:

1. The submitted efficacy data does support the use of the product, 3-in-1 Disinfecting Cleaner, as a non-food contact sanitizer with bactericidal activity against the following microorganism on hard, non-porous surfaces at 2308 ppm of test substance with 400 ppm synthetic hard water and 5% organic soil load for a 90 seconds contact time:

Enterococcus faecalis	MRID # 489474-04
Pseudomonas aeruginosa	MRID # 489474-04
Escherichia coli	MRID # 489474-04
Salmonella enterica	MRID # 489474-04
Staphylococcus aureus	MRID # 489474-20
Enterobacter aerogenes	MRID # 489474-20

The data results show a bacterial reduction of at least 99.9 percent over the parallel control within 5 minutes. Neutralization confirmation testing showed positive growth of the microorganisms. Sterility controls were reported with no growth.

2. The submitted efficacy data does support the use of the product, 3-in-1 Disinfecting Cleaner, as a non-food contact sanitizer with bactericidal activity against the following microorganism on hard, non-porous surfaces at 3462 ppm of test substance with 200 ppm synthetic hard water and 5% organic soil load for a 90 seconds contact time:

Listeria monocytogenes	MRID # 489474-22

The data results show a bacterial reduction of at least 99.9 percent over the parallel control within 5 minutes. Neutralization confirmation testing showed positive growth of the microorganisms. Sterility controls were reported with no growth.

3. The submitted efficacy data does support the use of the product, 3-in-1 Disinfecting Cleaner, as a disinfectant with bactericidal activity against the following microorganism on hard, non-porous surfaces at 3462 ppm of test product with 200 ppm synthetic hard water and 5% organic soil load for a 3 minutes contact time:

Staphylococcus aureus	MRID # 489474-16
Salmonella enterica	MRID # 489474-16
Pseudomonas aeruginosa	MRID # 489474-16
Serratia marcescens	MRID # 489474-18
Bordetella bronchiseptica	MRID # 489474-18
S. aureus – CA-MRSA	MRID # 489474-23
K. pneumoniae	MRID # 489474-23
S. aureus – MRSA	MRID # 489474-23

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Antibiotic resistance was confirmed for *S. aureus* – CA-MRSA and *S. aureus* – MRSA. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Sterility controls did not show growth.

4. The submitted efficacy data does support the use of the product, 3-in-1 Disinfecting Cleaner, as a disinfectant with bactericidal activity against the following microorganism on hard, non-porous surfaces at 2308 ppm of test product with 400 ppm synthetic hard water and 5% organic soil load for a 5 minutes contact time:

Acinetobacter baumanni	MRID # 489474-24
Klebsiella pneumoniae	MRID # 489474-24
Shigella flexneri	MRID # 489474-24
Streptococcus pyogenes	MRID # 489474-24
Shigella dysenteriae	MRID # 489474-24
Listeria monocytogenes	MRID # 489474-24
Staphylococcus aureus	MRID # 489474-17
Salmonella enterica	MRID # 489474-17
Pseudomonas aeruginosa	MRID # 489474-17
Staphylococcus aureus-VISA	MRID # 489474-05
Staphylococcus aureus-CA-MRSA	MRID # 489474-05
Staphylococcus aureus- MRSA	MRID # 489474-05
Klebsiella pneumoniae- KPC	MRID # 489474-05
Enterococcus faecalis- VRE	MRID # 489474-05
Candida albicans	MRID # 489474-25
Bordetella pertussis	MRID # 489474-26

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Antibiotic resistance was confirmed for *Staphylococcus aureus*-VISA, *Staphylococcus aureus*-CA-MRSA, *Staphylococcus aureus*- MRSA, *Klebsiella pneumoniae*- KPC and *Enterococcus faecalis*- VRE. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Sterility controls did not show growth.

5. The submitted efficacy data does support the use of the product, 3-in-1 Disinfecting Cleaner, as a disinfectant with bactericidal activity against the following microorganism on hard, non-porous surfaces at 3462 ppm of test product with 200 ppm synthetic hard water and 5% organic soil load for a 5 minutes contact time:

Enterobacter aerogenes	MRID # 489474-10
Escherichia coli O157:H7	MRID # 489474-10

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Sterility controls did not show growth.

6. The submitted efficacy data does support the use of the product, 3-in-1 Disinfecting Cleaner, as a disinfectant with bactericidal activity against the following microorganism on hard, non-porous surfaces at 2308 ppm of test product with 400 ppm synthetic hard water and 5% organic soil load for a 3 minutes contact time:

Proteus mirabilis	MRID # 489474-19

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Sterility controls did not show growth.

7. The submitted efficacy data does support the use of the product, 3-in-1 Disinfecting Cleaner, as a disinfectant with virucidal activity against the following microorganism on hard, non-porous surfaces at 2308 ppm of test product with 400 ppm synthetic hard water and 5% organic soil load for a 5 minutes contact time:

Rhinovirus type 37	MRID # 489474-06
Herpes Simplex virus type 1	MRID # 489474-07
Adenovirus Type 5	MRID # 489474-08
Norovirus (Feline Calicivirus)	MRID # 489474-13
Influenza A Virus	MRID # 489474-12
Human Coronavirus	MRID # 489474-27
Vaccinia Virus	MRID # 489474-28
Canine Parvovirus	MRID # 489474-29
Canine Distemper virus	MRID # 489474-30
Hepatitis B	MRID # 489474-31
Respiratory Syncytial virus	MRID # 489474-32
Hepatitis B	MRID # 489474-33
Herpes simplex virus type 2	MRID # 489474-34
Rotavirus	MRID # 489474-35
Poliovirus type 1	MRID # 489474-36
Newcastle disease virus	MRID # 489474-37
Murine Norovirus	MRID # 489474-38

At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level. Input virus controls demonstrated positive growth. Cytotoxicity controls and neutralization control showed growth beyond the cytotoxic level.

8. The submitted efficacy data does support the use of the product, 3-in-1 Disinfecting Cleaner, as a disinfectant with virucidal activity against the following microorganism on hard, non-porous surfaces at 2308 ppm of test product with 400 ppm synthetic hard water and 5% organic soil load for a 3 minutes contact time:

Rhinovirus Type 37	MRID # 489474-09
Norovirus (Feline Calicivirus)	MRID # 489474-11
Herpes Simplex virus type 1	MRID # 489474-15

At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level. Input virus controls demonstrated positive growth. Cytotoxicity controls and neutralization control showed growth beyond the cytotoxic level.

9. The submitted efficacy data does support the use of the product, 3-in-1 Disinfecting Cleaner, as a disinfectant with virucidal activity against the following microorganism on

hard, non-porous surfaces at 3462 ppm of test product with 200 ppm synthetic hard water and 5% organic soil load for a 1 minutes contact time:

Influenza A Virus	MRID # 489474-14

At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level. Input virus controls demonstrated positive growth. Cytotoxicity controls and neutralization control showed growth beyond the cytotoxic level.

10. The submitted efficacy data does support the use of the product, 3-in-1 Disinfecting Cleaner, as a disinfectant with virucidal activity against the following microorganism on hard, non-porous surfaces at 2308 ppm of test product with 200 ppm synthetic hard water and 5% organic soil load for a 1 minute contact time:

HIV Type 1	MRID # 489474-21

At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level. Input virus controls demonstrated positive growth. Cytotoxicity controls and neutralization control showed growth beyond the cytotoxic level.

11. The submitted efficacy data does **not** support the use of the product, 3-in-1 Disinfecting Cleaner, as a disinfectant with bactericidal activity against the following microorganism on hard, non-porous surfaces at 3462 ppm of test product with 200 ppm synthetic hard water and 5% organic soil load for a 3 minutes contact time:

Enterobacter aerogenes	MRID # 489474-18
Escherichia coli O157:H7	MRID # 489474-23

There was not acceptable killing observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Sterility controls did not show growth.

12. The submitted efficacy data does **not** support the use of the product, 3-in-1 Disinfecting Cleaner, as a disinfectant with bactericidal activity against the following microorganism on hard, non-porous surfaces at 2308 ppm of test product with 400 ppm synthetic hard water and 5% organic soil load for a 5 minutes contact time:

Acinetobacter baumanni	MRID # 489474-05
Enterobacter aerogenes	MRID # 489474-24
Escherichia coli O157:H7	MRID # 489474-24
Serratia marcescens	MRID # 489474-24
Bordetella bronchiseptica	MRID # 489474-24

There was not acceptable killing observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Sterility controls did not show growth.

VII. RECOMMENDATIONS:

1. **Note to PM-** Confirm that the tested product, 3-in-1 Disinfecting Cleaner, is the same product seeking registration, Peroxide Multi Surface Cleaner and Disinfectant.

2. The label claims the product, Peroxide Multi Surface Cleaner and Disinfectant, is an effective non-food contact sanitizer on hard non-porous surfaces for a contact period of 90 seconds with 400 ppm hard water and in the presence of a 5% organic soil load in for the following organisms:

Enterococcus faecalis	ATCC 29212
Pseudomonas aeruginosa	ATCC 15442
Escherichia coli	ATCC 11229
Salmonella enterica	ATCC 10708
Staphylococcus aureus	ATCC 6538
Enterobacter aerogenes	ATCC 13048

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

3. The label claims the product, Peroxide Multi Surface Cleaner and Disinfectant, is an effective non-food contact sanitizer on hard non-porous surfaces for a contact period of 90 seconds with 200 ppm hard water and in the presence of a 5% organic soil load for the following organism:

Listeria monocytogenes	ATCC 7644

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

4. The label claims the product, Peroxide Multi Surface Cleaner and Disinfectant, is an effective disinfectant on hard non-porous surfaces for a contact period of 5 minutes with 400 ppm hard water and in the presence of a 5% organic soil load in for the following organisms:

Staphylococcus aureus	ATCC 6538
Salmonella enterica	ATCC 10708
Pseudomonas aeruginosa	ATCC 15442
Staphylococcus aureus-VISA	ATCC 700788
Staphylococcus aureus-CA-MRSA	ATCC BAA-1683
Staphylococcus aureus- MRSA	ATCC33592
Klebsiella pneumoniae- KPC	ATCC BAA-1705
Enterococcus faecalis- VRE	ATCC 51299
Candida albicans	ATCC 10231
Bordetella pertussis	ATCC 9797
Acinetobacter baumanni	ATCC 19606
Klebsiella pneumoniae	ATCC 4352
Shigella flexneri	ATCC 9380

Streptococcus pyogenes	ATCC 19615
Shigella dysenteriae	ATCC 29026
Listeria monocytogenes	ATCC 7644
Rhinovirus type 37	ATCC VR-1147
Herpes Simplex virus type 1	ATCC VR-733
Adenovirus Type 5	ATCC VR-5
Norovirus (Feline Calicivirus)	ATCC VR-782
Influenza A Virus	ATCC VR-1736
Human Coronavirus	ATCC VR-740
Vaccinia Virus	ATCC VR-119
Canine Parvovirus	ATCC VR-2017
Canine Distemper virus	ATCC VR-128
Hepatitis B	(Hepadnavirus Testing, CA.)
Respiratory Syncytial virus	ATCC VR-26
Herpes simplex virus type 2	ATCC VR-734
Rotavirus (Strain WA)	(University of Ottawa, Canada)
Poliovirus type 1 (Strain Chat)	ATCC VR-1562
Newcastle disease virus	ATCC VR-108
Murine Norovirus (Strain MNV-1.CW1)	(Washington University, St. Louis, MO.)

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

5. The label claims the product, Peroxide Multi Surface Cleaner and Disinfectant, is an effective disinfectant on hard non-porous surfaces for a contact period of 3 minutes with 200 ppm hard water and in the presence of a 5% organic soil load in for the following organisms:

Staphylococcus aureus	ATCC 15442
Salmonella enterica	ATCC 6538
Pseudomonas aeruginosa	ATCC 10708
Serratia marcescens	ATCC 14756
Bordetella bronchiseptica	ATCC 31437
S. aureus – CA-MRSA	ATCC BAA-1683
K. pneumoniae	ATCC 4352
S. aureus – MRSA	ATCC 33592
Rhinovirus Type 37	ATCC VR-1147
Norovirus (Feline Calicivirus)	ATCC VR-782
Influenza A Virus	ATCC VR-1736
Herpes Simplex virus type 1	ATCC VR-733

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

6. The label claims the product, Peroxide Multi Surface Cleaner and Disinfectant, is an effective disinfectant on hard non-porous surfaces for a contact period of 5 minutes with 200 ppm hard water and in the presence of a 5% organic soil load in for the following organisms:

Enterobacter aerogenes	ATCC 13048
Escherichia coli O157:H7	ATCC 43895

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

7. The label claims the product, Peroxide Multi Surface Cleaner and Disinfectant, is an effective disinfectant on hard non-porous surfaces for a contact period of 3 minutes with 400 ppm hard water and in the presence of a 5% organic soil load in for the following organisms:

Proteus mirabilis	ATCC 7002

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

8. The label claims the product, Peroxide Multi Surface Cleaner and Disinfectant, is an effective disinfectant on hard non-porous surfaces for a contact period of 1 minute with 400 ppm hard water and in the presence of a 5% organic soil load in for the following organisms:

HIV Type 1	(Advanced Biotechnologies, MD.)

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

9. Label claims for the product, Peroxide Multi Surface Cleaner and Disinfectant, as an effective disinfectant on hard non-porous surfaces for a contact period of 3 minutes with 200 ppm hard water or for a contact period of 5 minutes with 400 ppm hard water for the following organisms are **unacceptable** and are not supported by the submitted data:

Enterobacter aerogenes	ATCC 13048
Escherichia coli O157:H7	ATCC 43895

10. Label claims for the product, Peroxide Multi Surface Cleaner and Disinfectant, as an effective disinfectant on hard non-porous surfaces for a contact period of 5 minutes with 400 ppm hard water for the following organisms are **unacceptable** and are not supported by the submitted data:

Acinetobacter baumannii- MDR	ATCC BAA-1605
Serratia marcescens	ATCC 14756
Bordetella bronchiseptica	ATCC 31437

11. **Label:**

- The label must have clear instructions on the directions of use for sanitization.
- **Note to PM-** The label is confusing and is potentially misleading. The registrant grouped dilutions, ppm(s), and contact times together on the label. One should have one contact time for one dilution claim for each group of bacteria, viruses, and fungi.

- On page 3 and 4 on the label, optional contact times cannot be listed in parenthesis.
- On page 13 on the label, Sporicidal claims must be removed. Efficacy data was not submitted to support these claims.