UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

January 7, 2011

MEMORANDUM

Subject: Efficacy Review for Puma; EPA File Symbol 5813-RNN; DP Barcode: D384233

From: Ibrahim Laniyan, Ph.D.
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Applicant: The Clorox Company
1221 Broadway
Oakland, CA 94612

Formulation from the Label:

Active Ingredient	% by wt.
Sodium Hypochlorite	8.25 %
Other Ingredients:	91.75 %
Total	100.00 %

(Yields 7.85% available chlorine)

I. BACKGROUND

The product, Puma (EPA File Symbol 5813-RNN), is a new product. This data package contains efficacy data provided by the applicant in response to Agency concerns described in the Data Evaluation Record (DER), dated June 8, 2010. This data package contains efficacy studies for product lots with sodium hypochlorite concentrations at the lower limit of the certified limit range, which were at least 60 days old at the time of testing. The applicant requested to register the product for use as a disinfectant (bactericide, fungicide, virucide), sanitizing rinse, sanitizer, and deodorizer on hard, non-porous surfaces in household, commercial, institutional, food preparation, animal care, and hospital or medical environments. The applicant also requested to register the product for use as a laundry sanitizer in household and high efficiency (HE) washing machines. Studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121.

This data package contained a letter from the applicant to EPA (dated November 12, 2010), EPA Form 8570-34 (Certification with Respect to Citation of Data), EPA Form 8570-35 (Data Matrix), nine studies (MRID 482955-01 through 482955-09), Statements of No Data Confidentiality Claims for all nine studies, and the proposed label.

II. USE DIRECTIONS

The product is designed for disinfecting and sanitizing hard, non-porous surfaces. The product may be used to treat hard, non-porous surfaces such as appliances, baby bottles, bathtubs, bicycles, brushes, changing tables, combs, counter tops, cribs, cups and mugs, cutting boards, diaper pails, dishes, door handles, faucets, floors, flower pots, garbage cans, garbage disposals, glassware, golf balls and clubs, grills, handles, high chairs, litter boxes, lunchboxes, outdoor siding, plastic mattress covers, playground sets, play pens, pots and pans, shower curtains, shower doors, showers, sinks, sports equipment, steering wheels, thermometers, toilets, toys, trash cans, trash compactors, urinals, utensils, wading pools, and walls. The label indicates that the product may be used on finished woodwork, glass, glazed ceramic tile, glazed porcelain, laminated surfaces, linoleum, painted woodwork, plastic (e.g., vinyl), sealed brick, and sealed granite. The label indicates that the product is not for use on aluminum, chipped enamel, non-stainless steel, and silver. Directions on the proposed label provide the following information regarding preparation and use of the product:

> As a disinfectant: Prepare a use solution by adding 4 ounces of the product and 1 gallon of water (a 1:32 dilution). Wash surfaces/ items. Apply use solution. Let stand for 5 minutes (for 10 minutes against *Pseudomonas aeruginosa*). Rinse. Air dry.
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> As a sanitizing rinse on food contact surfaces: Prepare a use solution by adding 0.33 ounce of the product and 1 gallon of water (a 1:388 dilution; 200 ppm available chlorine). Wash, wipe, or rinse surfaces/ items with detergent and water. Apply use solution. Let stand for 2 minutes. Air dry.
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> As a sanitizer on non-food contact surfaces: Prepare a use solution by adding 4 ounces of the product and 1 gallon of water (a 1:32 dilution). Wash, wipe, or rinse surfaces/ items with detergent and water. Apply use solution. Let stand for 30 seconds. Air dry.

As a laundry sanitizer in standard washing machines (i.e., 69 L): Sort laundry by color. Add detergent. Add ¾ cup of product to wash water (i.e., a 1:388.6 dilution). Add clothes. Start wash.

As a laundry sanitizer in high efficiency washing machines (i.e., 96 L): Sort laundry by color. Add detergent. Add ¾ cup of product to wash water (i.e., a 1:541 dilution). Add clothes. Start wash.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

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

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

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

Sanitizing Rinses (For Previously Cleaned, Food Contact Surfaces): Sanitizing rinses may be formulated with iodophors, mixed halides, or chlorine-bearing chemicals. The effectiveness

of such sanitizing rinses for previously cleaned, food contact surfaces must be substantiated by data derived from the AOAC Available Chlorine Germicidal Equivalent Concentration Method. Data from one test on each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old against *Salmonella enterica* (formerly *Salmonella choleraesuis*) are required. Test results must show product concentrations equivalent in activity to 50, 100, and 200 ppm of available chlorine. The reference standard is sodium hypochlorite.

Laundry Sanitizer: The effectiveness of laundry sanitizers must be supported by data that show that the product will substantially reduce the numbers of test bacteria on fabric and in laundry water. Laundry additives may either be used as soaking treatments prior to laundering or as treatments added during laundry operations. The label must specify the type of use. Laundry additives may be recommended for household/coin-operated machine use or commercial-industrial-institutional use. The label must specify the type of use. There is a significant difference in the water to fabric ratio between these two uses, which may affect the efficacy of the product. Tests should be conducted using a simulated-use procedure such as Petrocci and Clarke's "Proposed Test Method for Antimicrobial Laundry Additives" or a simulated use study involving washing machines. Tests should be performed with each of 3 product samples, representing 3 different lots, one of which is at least 60 days old. Tests should be conducted against *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352). Products labeled as being suitable for hospital use must also be tested against *Pseudomonas aeruginosa* (ATCC 15442). Each product lot must be tested with 3 fabrics swatches against each of the test organisms. The method employed must include subculturing of both the fabric and the laundry water. The laundry water to media volume ratio must not exceed 1:40. Testing of a 0.5 mL sample of laundry water from the simulated washing device (or a 5 mL sample from the automatic washer) is recommended. Results from a quantitative bacteriological assay must be reported. Results must show a bacterial reduction of 99.9% over the control count for both fabric and laundry water for each organism tested. The label directions for use of laundry additives should specify the machine cycle in which the product is to be added, as well as water level, temperature, and treatment time. Compatibility of the treatment with other laundry additives should be determined in testing and addressed in labeling, when applicable. These Agency standards are presented in DIS/TSS-13, and do not apply to sodium-calcium hypochlorites, sodium-potassium dichloro-s-triazinetriones, or trichloro-s-triazinetrione.

IV. BRIEF DESCRIPTION OF THE DATA

1. MRID 482955-01 "AOAC Use-Dilution Method," Test Organism: *Staphylococcus aureus* (ATCC 6538), for Puma F2009.0092, by Lynsey Wieland. Study conducted at ATS Labs. Study completion date – October 26, 2010. Project Number A10254.

This study was conducted against *Staphylococcus aureus* (ATCC 6538). One lot (Lot No. 10PUMA12) of the product, Puma F2009.0092, was tested using ATS Laboratory Protocol No. CX18090810.UD.1 (copy provided). The product lot tested (i.e., Lot No. 10PUMA12) was at least 60 days old at the time of testing. Testing was conducted on October 1, 2010 and October 12, 2010. Use solutions were prepared by adding 60.0 mL of the product and 1920 mL of 100 ppm AOAC synthetic hard water, or the equivalent (titrated at 99 ppm; a 1:33 dilution). A culture of the challenge microorganism was prepared in accordance with the published AOAC methods. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sixty (60) stainless steel penicylinder carriers were immersed for 15 minutes in a 48-54 hour old suspension of test organism, at a ratio of 1 carrier per 1.0 mL broth. The carriers were dried for

40 minutes at 35-37°C at 40-55% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 4.5 minutes at 20.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth to neutralize. All subcultures from testing on October 1, 2010 were incubated for 47.5 hours at 35-37°C. Subcultures from testing on October 1, 2010 were stored for 1 day at 2-8°C prior to examination. Subcultures from testing on October 12, 2010 were incubated for 45 hours at 35-37°C. Following incubation, or incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation. Sodium hypochlorite titration results for the use solution were provided.

Note: The laboratory reported a failed study set up on October 1, 2010. In the study, expected efficacy results were not obtained. Testing was repeated on October 12, 2010 to check for false positives. See pages 8 and 16 of the laboratory report.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

2. MRID 482955-02 "AOAC Use-Dilution Method," Test Organism: *Salmonella enterica* (ATCC 10708), for Puma F2009.0092, by Lynsey Wieland. Study conducted at ATS Labs. Study completion date – October 26, 2010. Project Number A10255.

This study was conducted against *Salmonella enterica* (ATCC 10708). One lot (Lot No. 10PUMA12) of the product, Puma F2009.0092, was tested using ATS Laboratory Protocol No. CX18090810.UD.2 (copy provided). The product lot tested (i.e., Lot No. 10PUMA12) was at least 60 days old at the time of testing. A use solution was prepared by adding 60.0 mL of the product and 1920 mL of 100 ppm AOAC synthetic hard water (titrated at 99 ppm; a 1:33 dilution). A culture of the challenge microorganism was prepared in accordance with the published AOAC methods. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sixty (60) stainless steel penicylinder carriers were immersed for 15 minutes in a 48-54 hour old suspension of test organism, at a ratio of 1 carrier per 1.0 mL broth. The carriers were dried for 40 minutes at 35-37°C at 40% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 4.5 minutes at 20.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth to neutralize. All subcultures were incubated for 47.5 hours at 35-37°C. Subcultures were stored for 1 day at 2-8°C prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation. Sodium hypochlorite titration results for the use solution were provided.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

3. MRID 482955-03 "AOAC Use-Dilution Method," Test Organism: *Pseudomonas aeruginosa* (ATCC 15442), for Puma F2009.0092, by Lynsey Wieland. Study conducted at ATS Labs. Study completion date – November 2, 2010. Project Number A10256.

This study was conducted against *Pseudomonas aeruginosa* (ATCC 15442). One lot (Lot No. 10PUMA12) of the product, Puma F2009.0092, was tested using ATS Laboratory

Protocol No. CX18090810.UD.3 (copy provided). The product lot tested (i.e., Lot No. 10PUMA12) was at least 60 days old at the time of testing. A use solution was prepared by adding 60.0 mL of the product and 1920 mL of 100 ppm AOAC synthetic hard water (titrated at 99 ppm; a 1:33 dilution). A culture of *Pseudomonas aeruginosa* culture was incubated for 48-54 hours at 35-37°C and the pellicle was aspirated from the culture on the day of use. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sixty (60) stainless steel penicylinder carriers were immersed for 15 minutes in a 48-54 hour old suspension of test organism, at a ratio of 1 carrier per 1.0 mL broth. The carriers were dried for 40 minutes at 35-37°C at 45% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 9 minutes at 19.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth to neutralize. All subcultures were incubated for 47.25 hours at 35-37°C. Subcultures were stored for 1 day at 2-8°C prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation. Sodium hypochlorite titration results for the use solution were provided.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

4. MRID 482955-04 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Virus: Influenza A virus, Strain Hong Kong (ATCC VR-544), for Puma F2009.0092, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – September 8, 2010. Project Number A09866.

This study was conducted against Influenza A virus (Strain Hong Kong; ATCC VR-544), using RMK cells (Rhesus monkey kidney cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. One lot (Lot No. 09PUMA3) of the product, Puma F2009.0092, was tested according to ATS Labs Protocol No. CX18070710.FLUA (copy provided). A use solution was prepared by adding 4.0 mL of the product and 128.0 mL of 100 ppm AOAC synthetic hard water (titrated at 100 ppm; a 1:33 dilution). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 50% relative humidity. For the single product lot, a dried virus film was exposed to 2.00 mL of the use solution for 4.5 minutes at 20.0°C. Following exposure, the plate was scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixture was passed immediately through a Sephadex column, and diluted serially in Minimum Essential Medium with 1% (v/v) heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. Sodium hypochlorite titration results for the use solution were provided.

5. MRID 482955-05 "AOAC Available Chlorine in Disinfectants," Test Organism: *Salmonella typhi* (ATCC 6539), for Puma F2009.0092, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – November 8, 2010. Project Number A10253.

This study was conducted against *Salmonella typhi* (ATCC 6539). One lot (Lot No. 10PUMA12) of the product, Puma F2009.0092, was tested using ATS Laboratory Protocol No. CX18090810.AVC (copy provided). The product lot tested (i.e., Lot No. 10PUMA12) was at least 60 days old at the time of testing. A use solution was prepared by adding 1.00 mL of the product and 294 mL of 100 ppm synthetic hard water (titrated at 99 ppm; a 1:295 dilution). NaOCl control solutions of 200, 100, and 50 ppm available chlorine were prepared. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. A 50 µL aliquot of the test suspension was added to 10 mL of the use solution and NaOCl control solutions at 20.0ºC. After one minute, a loopful (i.e., 10 µL) of each culture-solution mixture was transferred into 10 mL of Letheen Broth to neutralize (Letheen Broth with 0.1% sodium thiosulfate for the NaOCl control solutions). Each tube was then challenged with an additional 50 µL aliquot of the test suspension 30 seconds after subculturing. This procedure was repeated for a total of 10 subcultures for the use solution and NaOCl control solutions. All tubes were incubated for 48 hours at 35-37ºC. Subcultures were stored for 1 day at 2-8°C prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for initial suspension count, purity, sterility, and viability. Sodium hypochlorite titration results for the use solution were provided.

6. MRID 482955-06 "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces," Test Organism: *Staphylococcus aureus* (ATCC 6538), for Puma F2009.0092, by Becky Lien. Study conducted at ATS Labs. Study completion date – November 3, 2010. Project Number A10270.

This study was conducted against *Staphylococcus aureus* (ATCC 6538). One lot (Lot No. 10PUMA12) of the product, Puma F2009.0092, was tested using ATS Laboratory Protocol No. CX18090810.NFS.1 (copy provided). The product lot tested (i.e., Lot No. 10PUMA12) was at least 60 days old at the time of testing. A use solution was prepared by adding 5.0 mL of the product and 160.0 mL of 100 ppm AOAC synthetic hard water (titrated at 99 ppm; a 1:33 dilution). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Five sterile glass carriers (1 inch x 1 inch) were inoculated with 20.0 µL of a 48±4 hour old suspension of test organism. The inoculum was spread to within 1/8 inch of the edges of each carrier. The carriers were dried for 20 minutes at 36.1°C at 40% relative. Each carrier was transferred to a sterile vessel and treated with 5.0 mL of the use solution for 20 seconds at 20°C at 28% relative humidity. Following exposure, 20 mL of Letheen Broth was transferred to each vessel. The vessels were rotated vigorously on an even plane for ~50 rotations to suspend the surviving organisms. Within 30 minutes of the addition of the neutralizer, 1.00 mL aliquots of the 10^0 and 10^{-1} dilutions were plated in duplicate on tryptic soy agar with 5% sheep's blood. All subcultures were incubated for 50.75 hours at 35-37°C prior to examination. Following incubation, the subcultures were visually enumerated. Controls included those for inoculum count, carrier quantitation, purity, sterility, and neutralization confirmation. Sodium hypochlorite titration results for the use solution were provided.

7. MRID 482955-07 "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces," Test Organism: *Klebsiella pneumoniae* (ATCC 4352), for Puma F2009.0092, by Becky Lien. Study conducted at ATS Labs. Study completion date – October 28, 2010. Project Number A10271.

This study was conducted against *Klebsiella pneumoniae* (ATCC 4352). One lot (Lot No. 10PUMA12) of the product, Puma F2009.0092, was tested using ATS Laboratory Protocol No. CX18090810.NFS.2 (copy provided). The product lot tested (i.e., Lot No. 10PUMA12) was at least 60 days old at the time of testing. A use solution was prepared by adding 5.0 mL of the product and 160.0 mL of 100 ppm AOAC synthetic hard water (titrated at 99 ppm; a 1:33 dilution). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Five sterile glass carriers (1 inch x 1 inch) were inoculated with 20.0 µL of a 48±4 hour old suspension of test organism. The inoculum was spread to within 1/8 inch of the edges of each carrier. The carriers were dried for 30 minutes at 36.0°C at 40% relative humidity. Each carrier was transferred to a sterile vessel and treated with 5.0 mL of the use solution for 20 seconds at 20°C at 28% relative humidity. Following exposure, 20 mL of Letheen Broth was transferred to each vessel. The vessels were rotated vigorously on an even plane for ~50 rotations to suspend the surviving organisms. Within 30 minutes of the addition of the neutralizer, 1.00 mL aliquots of the 10^0 and 10^{-1} dilutions were plated in duplicate on tryptic soy agar with 5% sheep's blood. All subcultures were incubated for 51.5 hours at 35-37°C prior to examination. Following incubation, the subcultures were visually enumerated. Controls included those for inoculum count, carrier quantitation, purity, sterility, and neutralization confirmation. Sodium hypochlorite titration results for the use solution were provided.

8. MRID 482955-08 "Standard Test Method for the Evaluation of Laundry Sanitizers," Test Organisms: *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352), for Puma F2009.0092, by Becky Lien. Study conducted at ATS Labs. Study completion date – November 8, 2010. Project Number A10411.

This study was conducted against *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352). One lot (Lot No. 10PUMA12) of the product, Puma F2009.0092, was tested using ATS Protocol No. CX18101210.LSAN.1 (copy provided). The laboratory report referenced Petrocci and Clarke's "Proposed Test Method for Antimicrobial Laundry Additives." The product lot (i.e., Lot No. 10PUMA12) was at least 60 days old at the time of testing. A use solution of the product was prepared by diluting 2.00 mL of the product with 1166 mL of 100 ppm AOAC synthetic hard water (titrated at 99 ppm; a 1:584 dilution). Cultures of the challenge microorganisms were prepared and harvested. The culture suspension densities were determined using a spectrophotometer. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. A sterile bottle was filled with 150.0 g of the prepared use solution. The carriers prepared on August 18, 2010* were prepared by boiling 611.89 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 3.0067 grams of Na_2CO_3, 3.0454 grams of Triton X-100, and 6 L of deionized water for 60 minutes. The fabric then was rinsed in boiling water for 8 minutes and then rinsed in cold water for 10 minutes. During the rinsing procedure, the fabric was mixed in the water to help remove the wetting agent. The carriers prepared on September 18, 2010* were prepared by boiling 778 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 4.0 grams of Na_2CO_3, 4.0 grams of Triton X-100, and 8 L of deionized water for 60 minutes. The fabric then was rinsed in boiling water for 6 minutes and then rinsed in cold water for 5 minutes. During the rinsing procedure, the fabric was mixed in the water to help remove the wetting agent. After air-drying, the fabric was cut into 5 cm (~2 inch) wide strips weighing 15±1 grams. Each strip was wrapped around a stainless steel spindle between 12 and 13 times. Swatches (1 inch by 1.5 inch) were also cut from the remaining fabric. All carriers were autoclave sterilized, allowed to cool, and held at room temperature until use. Three swatches were inoculated with 30 µL of the prepared organism culture, and dried in an incubator for 10 minutes at 35-37°C. After drying, the swatches were each inserted between the 6^{th} and 7^{th} lap of a wrapped spindle. Each spindle

contained three dried, contaminated swatches. The spindles were placed in the bottles containing the use solution and subjected to a simulated tumble-wash at 45-60 RPM for 9.5 minutes at 21°C. A 1.00 mL aliquot of the wash water was transferred to a vessel containing 9.0 mL of Letheen Broth to neutralize. The fabric swatches were transferred to 10 mL of Letheen Broth to neutralize. The fabric swatches were then vortex mixed for a minimum of 10 seconds to extract fabric-bound microorganisms.A 1.00 mL aliquot of the neutralizing subculture medium was transferred to 9 mL of sterile diluent; representing the 10^{-1} dilution. Ten-fold serial dilutions were continued through the 10^{-4} dilution. Each dilution was plated using 1.00 mL of the 10^{0} to 10^{-4} dilutions in duplicate on tryptic soy agar with 5% sheep's blood. All subcultures were incubated for 44 hours at 35-37°C. The subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation and storage, standard plate count procedures were used to determine the average colony forming units per carrier (CFU/carrier) and per milliliter of wash water (CFU/mL). Controls included those for initial inoculum count, carrier population, purity, sterility, viability, and neutralization confirmation. Sodium hypochlorite titration results for the use solution were provided.

Note: Adding a ca. 15-gram cloth strip to 150.0 g of product use solution yields a 1:10 w/w ratio of simulated laundry to wash water, the appropriate ratio for household laundry operations.

*Note: The laboratory report describes carriers prepared on August 18, 2010 and September 18, 2010. This appears to be an error. The protocol is dated October 12, 2010. The test date in Tables 3, 4, and 8 is October 22, 2010.

9. MRID 482955-09 "Standard Test Method for the Evaluation of Laundry Sanitizers," Test Organisms: *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352), for Puma F2009.0092, by Becky Lien. Study conducted at ATS Labs. Study completion date – November 9, 2010. Project Number A10412.

This study was conducted against *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352). One lot (Lot No. 10PUMA12) of the product, Puma F2009.0092, was tested using ATS Protocol No. CX18101210.LSAN.2 (copy provided). The laboratory report referenced Petrocci and Clarke's "Proposed Test Method for Antimicrobial Laundry Additives." The product lot (i.e., Lot No. 10PUMA12) was at least 60 days old at the time of testing. A use solution of the product was prepared by diluting 2.00 mL of the product with 1166 mL of 100 ppm AOAC synthetic hard water (titrated at 99 ppm; a 1:584 dilution). Cultures of the challenge microorganisms were prepared and harvested. The culture suspension densities were determined using a spectrophotometer. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. A sterile bottle was filled with 60.00 g of the prepared use solution. The carriers prepared on August 12, 2010* were prepared by boiling 315.69 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 1.5161 grams of Na_2CO_3, 1.5310 grams of Triton X-100, and 3 L of deionized water for 61 minutes. The fabric then was rinsed in boiling water for 5 minutes and then rinsed in cold water for 7 minutes. During the rinsing procedure, the fabric was mixed in the water to help remove the wetting agent. The carriers prepared on September 18, 2009* were prepared by boiling 778 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 4.0 grams of Na_2CO_3, 4.0 grams of Triton X-100, and 8 L of deionized water for 60 minutes. The fabric then was rinsed in boiling water for 6 minutes and then rinsed in cold water for 5 minutes. During the rinsing procedure, the fabric was mixed in the water to help remove the wetting agent. After air-drying, the fabric was cut into 5 cm (~2 inch) wide strips weighing 15±1 grams. Each strip was wrapped around a stainless steel spindle between 12 and 13 times. Swatches (1 inch by 1.5 inch) were

also cut from the remaining fabric. All carriers were autoclave sterilized, allowed to cool, and held at room temperature until use. Three swatches were inoculated with 30 µL of the prepared organism culture, and dried in an incubator for 10 minutes at 35-37°C. After drying, the swatches were each inserted between the 6th and 7th lap of a wrapped spindle. Each spindle contained three dried, contaminated swatches. The spindles were placed in the bottles containing the use solution and subjected to a simulated tumble-wash at 45-60 RPM for 9.5 minutes at 21°C. A 1.00 mL aliquot of the wash water was transferred to a vessel containing 9.0 mL of Letheen Broth to neutralize. The fabric swatches were transferred to 10 mL of Letheen Broth to neutralize. The fabric swatches were then vortex mixed for a minimum of 10 seconds to extract fabric-bound microorganisms. A 1.00 mL aliquot of the neutralizing subculture medium was transferred to 9 mL of sterile diluent; representing the 10^{-1} dilution. Ten-fold serial dilutions were continued through the 10^{-4} dilution. Each dilution was plated using 1.00 mL of the 10^{0} to 10^{-4} dilutions in duplicate on tryptic soy agar with 5% sheep's blood. All subcultures were incubated for 44 hours at 35-37°C. The subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation and storage, standard plate count procedures were used to determine the average colony forming units per carrier (CFU/carrier) and per milliliter of wash water (CFU/mL). Controls included those for initial inoculum count, carrier population, purity, sterility, viability, and neutralization confirmation. Sodium hypochlorite titration results for the use solution were provided.

Note: Adding a ca. 15-gram cloth strip to 60.0 g of product use solution yields a 1:4 w/w ratio of simulated laundry to wash water, the appropriate ratio for high efficiency laundry operations.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

*Note: The laboratory report describes carriers prepared on August 12, 2010 and September 18, 2009. This appears to be an error. The protocol is dated October 12, 2010. The test date in Tables 3, 4, and 8 is October 22, 2010.

V. RESULTS

MRID Number	Lot or Concentration		Subculture Series									
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
482955-05	*Salmonella typhi*											
	10PUMA12		0	0	0	0	0	0	+	+	+	+
	NaOCl	200 ppm	0	0	0	0	0	0	+	+	+	+
	Control	100 ppm	0	0	0	+	+	+	+	+	+	+
		50 ppm	0	0	+	+	+	+	+	+	+	+

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested	Carrier Population (CFU/ carrier)
		Lot No. 10PUMA12	
482955-01	*Staphylococcus aureus* Test Date: 10/01/2010 Test Date: 10/12/2010	 2/60 0/60	 6.1×10^6 6.3×10^6
482955-02	*Salmonella enterica*	0/60	3.7×10^6
482955-03	*Pseudomonas aeruginosa*	0/60	1.08×10^7

MRID Number	Organism	Results		Dried Virus Count
			Lot No. 09PUMA3	
482955-04	Influenza A virus	10^{-1} to 10^{-7} dilutions	Complete inactivation	$\geq 10^{7.5}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	

MRID Number	Organism	Lot No.	Total No. Surviving	Control Carrier	Percent Reduction
			(CFU/carrier)		
482955-06	*Staphylococcus aureus*	10PUMA12	$<2.51 \times 10^1$	3.16×10^6	>99.9
482955-07	*Klebsiella pneumoniae*	10PUMA12	$<2.51 \times 10^1$	5.13×10^4†	>99.9

†Zero-time control counts did not demonstrate an average of at least 7.5×10^5 surviving organisms, which is the criterion set forth in ASTM 1153.

MRID/ Organism	Lot No.	Average No. Surviving (CFU/ swatch)	Microbes Initially Present (mean CFU/ swatch)	"Wash" Water Test Results (CFU/ mL)	"Wash" Water Control (CFU/ mL)	% Red.
Household Laundry Operations (1:10 ratio of laundry to wash water)						
482955-08	*Staphylococcus aureus*					
	10PUMA12	$<1 \times 10^1$	5.3×10^6	$<1 \times 10^1$	2.20×10^4	>99.9
482955-08	*Klebsiella pneumoniae*					
	10PUMA12	$<1 \times 10^1$	9.7×10^6	$<1 \times 10^1$	8.3×10^5	>99.9
High Efficiency Laundry Operations (1:4 ratio of laundry to wash water)						
482955-09	*Staphylococcus aureus*					
	10PUMA12	$<1 \times 10^1$	2.6×10^6	$<1 \times 10^1$	5.3×10^5	>99.9
	Klebsiella pneumoniae					
	10PUMA12	$<1 \times 10^1$	4.4×10^6	$<1 \times 10^1$	1.8×10^6	>99.9

VI. CONCLUSIONS

1. The submitted efficacy data in conjunction with **support** the use of a 1:33 dilution of the product, Puma, as a disinfectant with bactericidal activity against the following microorganisms on hard, non-porous surfaces in the presence of 100 ppm hard water and a 5% organic soil load for a 4.5-minute contact time:

Staphylococcus aureus	MRID 482955-01 & MRID 480175-04
Salmonella enterica	MRID 482955-02 & MRID 480175-05
Pseudomonas aeruginosa	MRID 482955-03 & MRID 480175-06

Complete killing was observed in the subcultures of the required number of carriers tested against a single product lot. The single product lot tested was at least 60 days old at the time of testing. [Note that repeat testing was conducted on one product lot against *Staphylococcus aureus* to evaluate for false positives.] Neutralization confirmation testing showed positive growth of the microorganisms. Purity controls were reported as pure. Viability controls were positive for growth. Sterility controls did not show growth.

2. The submitted efficacy data (MRID 482955-04) in conjunction with MRID 480175-11, **support** the use of a 1:33 dilution of the product, Puma, as a disinfectant with virucidal activity against Influenza A virus on hard, non-porous surfaces in the presence of 100 ppm hard water and a 5% organic soil load for a 4.5-minute contact time. One product lot was tested. A recoverable virus titer of at least 10^4 was achieved. Cytotoxicity was not observed. Complete inactivation (no growth) was indicated in all dilutions tested.

3. The submitted efficacy data (MRID 482955-05) in conjunction with MRID 480175-22, **support** the use of a 1:295 dilution of the product, Puma, as a sanitizing rinse against *Salmonella typhi* on hard, non-porous, food contact surfaces in the presence of 100 ppm hard water and a 5% organic soil load for a 1-minute contact time. The effectiveness of the use solution was observed to be equivalent to a 200 ppm NaOCl standard control. The single product lot tested was at least 60 days old at the time of testing. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

4. The submitted efficacy data (MRID 482955-06 and 07) in conjunction with MRID 480175-23 and 24, **support** the use of a 1:33 dilution of the product, Puma, as a sanitizer against *Staphylococcus aureus* and *Klebsiella pneumoniae* on hard, non-porous, non-food contact surfaces in the presence of 100 ppm hard water and a 5% organic soil load for a 20-second contact time. The single product lot tested was at least 60 days old at the time of testing. A bacterial reduction of at least 99.9 percent over the parallel control was observed within 5 minutes (i.e., 20 seconds specifically). Neutralization confirmation testing met the acceptance criterion of growth within 1 $\log_{10}$ of the numbers control. Purity controls were reported as pure. Sterility controls did not show growth.

5. The submitted efficacy data in conjunction with previously submitted efficacy data **support** the use of the product, Puma, as a laundry additive for sanitizing laundry during household laundry operations and high efficiency laundry operations against the following microorganisms in the presence of 100 ppm hard water and a 5% organic soil load for a 9.5-minute contact time:

Klebsiella pneumoniae	MRID 480175-26 and 29, MRID 482955-08 and -09
Staphylococcus aureus	MRID 480175-25 and 28, MRID 482955-08 and -09

A 99.9% reduction in population was observed for both the fabric swatches and the "wash" water. The single product lot tested was at least 60 days old at the time of testing. Three fabric swatches per product lot were tested. Neutralization confirmation testing met the acceptance criterion of growth within 1 $\log_{10}$ of the numbers control. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

VII. LABEL

1. The proposed label claims that a **1:32 dilution** (i.e., 4 ounces of the product and 1 gallon of water) of the product, Puma, is an effective disinfectant against the following microorganisms on **pre-cleaned**, hard, non-porous surfaces for a **5-minute** contact time (a **10-minute** contact time against ***Aspergillus niger*** *and* ***Pseudomonas aeruginosa***):

Pseudomonas aeruginosa
Staphylococcus aureus
Salmonella enterica
Influenza A virus
Shigella dysenteriae
Methicillin Resistant *Staphylococcus aureus*
Community Acquired Methicillin Resistant *Staphylococcus aureus* (NARSA NRS123) (Genotype USA400)
Escherichia coli with extended beta-lactamase resistance
Trichophyton mentagrophytes
Aspergillus niger
Candida albicans
Influenza A (H1N1)
Rhinovirus type 37
Rotavirus

Supplemental efficacy data provided in the data package in conjunction with previously submitted data support these claims.

2. The proposed label claims that a **1:21.3 dilution** (i.e., ¾ cup of the product and 1 gallon of water) of the product, Puma, is an effective disinfectant against ***Aspergillus niger*** on **pre-cleaned**, hard, non-porous surfaces for a **5-minute** contact time. **This claim is acceptable as it is supported by previously submitted data**.

3. The proposed label claims that a **200 ppm** chlorine equivalent dilution of the product, Puma, is an effective **sanitizing rinse** against *Salmonella typhi* on pre-cleaned, hard, non-porous, food contact surfaces for a **2-minute** contact time. Supplemental efficacy data provided in the data package support this claim. In conjunction with previously submitted data, we have the following microorganisms:

Salmonella typhi
Escherichia coli O157:H7
Listeria monocytogenes

4. The proposed label claims that a **1:32 dilution** of the product, Puma, is an effective sanitizer against *Staphylococcus aureus* and *Klebsiella pneumoniae* on pre-cleaned, hard, non-porous, non-food contact surfaces for a 30-second contact time. Supplemental efficacy data provided in the data package in conjunction with previously submitted data support these claims.

5. The proposed label claims that adding a **¾ cup** of the product, Puma, to a **standard washing machine will sanitize laundry** against *Staphylococcus aureus* and *Klebsiella pneumoniae*. Supplemental efficacy data provided in the data package support in conjunction with previously submitted data support these claims.

6. The proposed label claims that adding a **¾ cup** of the product, Puma, to a **high efficacy washing machine will sanitize laundry** against *Staphylococcus aureus* and *Klebsiella pneumoniae*. Supplemental efficacy data provided in the data package support in conjunction with previously submitted data support these claims.

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

- On page 8 of the proposed label under the "Sanitization" section, change "standard washer" to read "**standard washer or high efficacy washer**."

- On page 13 of the proposed label, change "driveways, walkways, and sidewalks" to read "sealed driveways, walkways, and sidewalks." These surfaces are porous.

- On page 13 of the proposed label, change "patio stone" to read "**sealed patio stone**." Stone is a porous surface.