UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

February 16, 2005

MEMORANDUM

Subject: Efficacy Review for Maquat 750-M; EPA Reg. No. 10324-115;
DP Barcode: D321242

From: Marcie Wawzysko Tidd, Microbiologist Marcie Tidd 2/16/06
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Applicant: Mason Chemical Company
721 W. Algonquin Road
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Formulation from the Label:

Active Ingredient(s)	% by wt.
Octyl Decyl Dimethyl Ammonium Chloride	15.0%
Didecyl Dimethyl Ammonium Chloride	7.5%
Dioctyl Dimethyl Ammonium Chloride	7.5%
Alkyl (C14 50%, C12 40%, C16 10%) dimethyl benzyl ammonium chloride	20.0%
Other Ingredients	50.0%
Total	100.0%

I. BACKGROUND

The product, Maquat 750-M (EPA Reg. No. 10324-115), is an EPA-approved disinfectant (bactericide, fungicide, virucide), sanitizing rinse, sanitizer, and mildewstat for use on hard, non-porous surfaces in household, commercial, institutional, industrial, food processing, agricultural, animal care, and hospital or medical environments. The applicant requested an amendment to the registration of this product to add claims for the product's effectiveness as a:

- Residual bacteriostat on fabric

- Laundry sanitizer against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* - MRSA, and the Human immunodeficiency virus type 1 (HIV-1). Effective in laundry rinse water up to 200 ppm hard water (400 ppm hard water against HIV-1).

The applicant also provided studies demonstrating the product's effectiveness as a sanitizing rinse on food contact surfaces against *Klebsiella pneumoniae* and *Shigella sonnei*. Studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 100, in Eagan MN 55121.

This data package contained a letter from the applicant to the Agency (dated June 13, 2005), EPA Form 8570-35 (Data Matrix), eight studies (MRID Nos. 465837-01 through 465837-08), Statements of No Data Confidentiality Claims for all eight studies, and a proposed label.

Note: The laboratory reports describe studies conducted using the product, Maquat 7.5M (EPA Reg. No. 10324-81). The product, Maquat 750-M, is the subject of this efficacy report. The applicant's letter to EPA (dated June 13, 2005), states that both products are similar in formulation. Information on the Data Matrix indicates that efficacy data developed for the product, Maquat 7.5M, has been used in the past to support label claims for the product, Maquat 750-M.

II. USE DIRECTIONS

The product is designed for use as a sanitizing rinse on hard, non-porous, food contact surfaces such as food processing equipment, dairy equipment, food utensils, dishes, silverware, eating utensils, glasses, sink tops, counter tops, and refrigerated storage and display equipment. Directions on the proposed label provided the following information regarding preparation and use of the product as a sanitizing rinse: Remove gross food particles and soil using a pre-flush, pre-scrape, or pre-soak treatment. Thoroughly wash objects with a good detergent. Rinse with potable water. Prepare a 150-400 ppm active use solution by adding 0.96-2.5 ounces of the product to 25 gallons of water. Apply the use solution by cloth, brush, or mechanical spray device, or by immersion. Treated surfaces must remain wet for at least 60 seconds. Do not rinse.

The product provides residual bacteriostatic activity against odor-causing bacteria. Directions on the proposed label provided the following information regarding use of the product

as a laundry bacteriostat: Use 2 ounces of this product per 100 pounds of dry laundry. Dilute the appropriate amount first in 1-2 gallons of water. Add this solution to the wash wheel at the beginning of the final rinse cycle. A minimum rinse cycle of 5 minutes is required. Repeat and re-treat fabric after each washing.

The product is also designed for use as a laundry sanitizer during commercial, institutional, and industrial laundry operations. Directions on the proposed label provided the following information regarding use of the product as a laundry sanitizer: Fill washer to low water level with a minimum water temperature of 95°F (i.e., 35°C). Inject product into the sanitizing rinse step at the rate of 0.2 ounces per gallon of water (780 ppm active quat; 2.4 ounces per 100 pounds of dry laundry). Treat the laundry for a minimum of 5 minutes.

The proposed label also lists directions for use of the product as a hospital/medical use disinfectant.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Sanitizing Rinses (For Previously Cleaned, Food Contact Surfaces; Additional Bacteria)

There are cases where an applicant requests to make claims of effectiveness against additional bacteria for a product that is already registered as a sanitizing rinse for previously cleaned, food contact surfaces. EPA staff indicated that the DIS/TSS-5 standards are silent on this matter and that confirmatory test standards would apply. EPA staff indicated that, for sanitizing rinses for previously cleaned, food contact surfaces, 2 product samples, representing 2 different product lots, must be tested against each additional microorganism. Results must show a bacterial reduction of at least 99.999% in the number of microorganisms within 30 seconds. The results must be reported according to the actual count and the percentage reduction over the control.

Furthermore, according to information in the AOAC test method itself, counts on number controls for the product should fall between 75 and 125 x 10^6/mL for percent reductions to be considered valid. Label directions for use, however, must state that a contact time of at least 1 minute is required for sanitization. These Agency standards are presented in DIS/TSS-4 and -17, as well as the AOAC Germicidal and Detergent Sanitizing Action of Disinfectants Method.

Residual Bacteriostatic Activity – Laundered Fabrics

The effectiveness of antimicrobial products that are intended to provide residual bacteriostatic treatment to laundered fabrics must be substantiated by data derived using the AOAC Bacteriostatic Activity of Laundry Additive Disinfectants Method. This method involves treating a fabric with a product during a simulated laundering operation and subsequently conducting bacteriostatic testing of the treated fabric. The method should be modified, depending on how the product is to be used (i.e., product recommended as final rinse additive in industrial laundering operations, product recommended as final rinse additive in home or coin-operated laundering operations, product recommended as final rinse additive in both industrial and home laundering

operations, product recommended as final rinse additive and described as compatible with other adjunct chemicals). Tests should be conducted against *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352). Five test samples are required for a single bacteriostatic test against each test organism. Results should be scored by determining the presence or absence of a clear zone of inhibition extending along the entire edge of each of 4 sides of 5 replicate fabric test samples. A total score of at least 18/20 sides demonstrating bacteriostatis is required. These Agency standards are presented in the AOAC method itself.

Laundry Sanitizer – For Use During Commercial-Industrial-Institutional Laundry Operations

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 product 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.

Note: The water to fabric ratio in industrial laundering operations is about 5:1. Dosages may be based on pounds of fabric for industrial machines.

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. These Agency standards are presented in DIS/TSS-7.

Supplemental Claims

On a product label, the hard water tolerance level may differ with the level of antimicrobial activity (e.g., sanitizer vs. disinfectant) claimed. To establish efficacy in hard water, all microorganisms (i.e., bacteria, fungi, viruses) claimed to be controlled must be tested by the appropriate Recommended Method at the same hard water tolerance level. These Agency standards are presented in DIS/TSS-2.

Supplemental Recommendations

Antimicrobial agents which claim to be "one-step" cleaner-disinfectants, or cleaner-sanitizers, or agents to be used in the presence of organic soil, must undergo appropriate efficacy testing modified to include a representative organic soil of 5% blood serum. A suggested method to simulate antimicrobial treatment of dry inanimate surfaces is to add the blood serum 5% v/v (19mL bacterial inoculum with 1mL blood serum) to bacterial inoculum prior to carrier contamination and drying. Control data should be produced as described in Supplemental Recommendation 6 of DIS/TSS-2 to confirm the validity of this test with this modification. The suggested organic soil level is appropriate for simulation of lightly to moderately soiled surfaces. For highly soiled surfaces, a prior cleaning step should be recommended on the product label. A suggested procedure for incorporating organic soil load where the antimicrobial agent is not tested against a dry inanimate surface, such as the AOAC Fungicidal Test involves adding 5% v/v blood serum directly to the test solution (e.g., 4.75 ml test solution + 0.25 ml blood serum) before adding 0.5 ml of the required level (5 X 10^6 /ml) of conidia. These agency standards can be found in DIS/TSS-2.

IV. SUMMARY OF SUBMITTED STUDIES

1. MRID 465837-01 "Germicidal and Detergent Sanitizing Action of Disinfectants, Test Organism: *Klebsiella pneumoniae* (ATCC 4352)" for Maquat 7.5M, by Sally Nada. Study conducted at ATS Labs. Study completion date – November 29, 2004. Project Number A02533.

This study was conducted against *Klebsiella pneumoniae* (ATCC 4352). Two lots (Lot Nos. MRII 45-1 and MRII 45-2) of the product, Maquat 7.5M, were tested in duplicate using the AOAC Germicidal and Detergent Sanitizing Action of Disinfectants Method

(modified) as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. A use solution was prepared by adding 1 mL of the product to 375.5 mL of 500 ppm AOAC synthetic hard water (titrated at 500 ppm; a 1.36 ounce/4 gallon use solution; 200 ppm active quat). A 99-mL aliquot of each use solution was transferred to individual sterile, 250-mL Erlenmeyer flasks, which were then placed in a water bath at 25°C. One-mL of 18-24 hour old bacterial suspension was added to each flask. One-mL aliquots of the bacterium-product mixture were transferred to 9 mL of Neutralizer Blanks exactly 30 seconds after the addition of the bacterial suspension. The neutralizer tubes were vortex mixed. Four 1.0 mL and four 0.1 mL aliquots of the neutralized use solution were transferred into individual sterile, Petri dishes. Approximately 15-20 mL of tryptone glucose extract agar was added to each plate. All plates were incubated for 48±4 hours at 35-37°C. Following incubation, the plates were visually examined for growth. Controls included those for purity, sterility, viability, numbers count, and neutralization confirmation.

2. MRID 465837-02 "Germicidal and Detergent Sanitizing Action of Disinfectants, Test Organism: *Shigella sonnei* (ATCC 25931)" for Maquat 7.5M, by Sally Nada. Study conducted at ATS Labs. Study completion date – November 24, 2004. Project Number A02534.

This study was conducted against *Shigella sonnei* (ATCC 25931). Two lots (Lot Nos. MRII 45-1 and MRII 45-2) of the product, Maquat 7.5M, were tested in duplicate using the AOAC Germicidal and Detergent Sanitizing Action of Disinfectants Method (modified) as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. A use solution was prepared by adding 1 mL of the product to 375.5 mL of 500 ppm AOAC synthetic hard water (titrated at 504 ppm; a 1.36 ounce/4 gallon use solution; 200 ppm active quat). A 99-mL aliquot of each use solution was transferred to individual sterile, 250-mL Erlenmeyer flasks, which were then placed in a water bath at 25°C. One-mL of bacterial suspension was added to each flask. One-mL aliquots of the bacterium-product mixture were transferred to 9 mL of neutralizer blanks exactly 30 seconds after the addition of the bacterial suspension. The neutralizer tubes were vortex mixed. Four 1.0 mL and four 0.1 mL aliquots of the neutralized test solution were transferred into individual sterile, Petri dishes. Approximately 15-20 mL of tryptone glucose extract agar was added to each plate. All plates were incubated for 48±4 hours at 35-37°C. Following incubation, the plates were visually examined for growth. Controls included those for purity, sterility, viability, numbers count, and neutralization confirmation.

3. MRID 465837-03 "Bacteriostatic Activity of Laundry Additive Disinfectants, Test Organisms: *Klebsiella pneumoniae* (ATCC 4352) and *Staphylococcus aureus* (ATCC 6538)" for Maquat 7.5M, by Sally Nada. Study conducted at ATS Labs. Study completion date – August 11, 2004. Project Number A02324.

This study was conducted against *Klebsiella pneumoniae* (ATCC 4352) and *Staphylococcus aureus* (ATCC 6538). Two lots (Lot Nos. MRII-45-1 and MRII- 45-2) of the product, Maquat 7.5M, were tested using the AOAC Bacteriostatic Activity of Laundry Additive Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. A use solution was prepared by adding 1 mL of the product to 115.4 mL of filter sterilized deionized water (a 1.1 ounce/gallon use solution; 650 ppm active quat). Four sets of sterile Nalgene jars were filled with 75 mL of the prepared use solution and equilibrated at 35±1°C. The carriers for this test were prepared by boiling 922 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 4.5 grams of Na_2CO_3, 4.5 grams of Triton X-100, and 9.0 L of deionized water for 60 minutes. The fabric then was rinsed in boiling water for 5 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 fabric strip was wrapped around a spindle between 12 and 13 times. All fabric wrapped spindles were autoclaved, allowed to cool, and held at room temperature until use. The fabric wrapped spindles were placed in the Nalgene jars containing the use solution and subjected to a simulated tumble-wash at 45-60 RPM at 5 minutes at 35±1°C. The fabric was removed from the spindle and placed into a sterile glass pan for drying in a bio-safety hood. After drying, 1-inch test squares were aseptically and randomly removed. Five test squares were removed per organism, and a minimum of 2 1-inch test squares were removed from the middle 20% of the dry fabric strip. An ~24-hour broth culture was inoculated into molten Nutrient Agar B, at a ratio of 1.0 mL culture to 100 mL agar. After mixing, 10 mL aliquots of the inoculated agar were transferred to individual sterile, Petri dishes and allowed to harden. Each 1-inch test square was transferred to the center of an individual inoculated agar plate, and pressed firmly onto the agar surface. The plates were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were examined for the presence of a clear zone of inhibition of the test organism adjacent to each side of the test square. Controls included those for purity, sterility, and viability.

4. MRID 465837-04 "Bacteriostatic Activity of Laundry Additive Disinfectants, Test Organism: *Corynebacterium ammoniagenes* (ATCC 6872)" for Maquat 7.5M, by Sally Nada. Study conducted at ATS Labs. Study completion date – August 11, 2004. Project Number A02325.

This study was conducted against *Corynebacterium ammoniagenes* (ATCC 6872). Two lots (Lot Nos. MRII-45-1 and MRII- 45-2) of the product, Maquat 7.5M, were tested using the AOAC Bacteriostatic Activity of Laundry Additive Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. A use solution was prepared by adding 1 mL of the product to 115.4 mL of filter sterilized deionized water (a 1.1 ounce/gallon use solution; 650 ppm active quat). Two sets of sterile Nalgene jars were filled with 75 mL of the prepared use solution and equilibrated at 35±1°C. The carriers for this test were prepared by boiling 922 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 4.5 grams of Na_2CO_3, 4.5 grams of

Triton X-100, and 9.0 L of deionized water for 60 minutes. The fabric then was rinsed in boiling water for 5 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 fabric strip was wrapped around a spindle between 12 and 13 times. All fabric wrapped spindles were autoclaved, allowed to cool, and held at room temperature until use. The fabric wrapped spindles were placed in the Nalgene jars containing the use solution and subjected to a simulated tumble-wash at 45-60 RPM at 5 minutes at 35±1°C. The fabric was removed from the spindle and placed into a sterile glass pan for drying in a bio-safety hood. After drying, 1-inch test squares were aseptically and randomly removed. Five 1-inch test squares were removed, and a minimum of 2 1-inch test squares were removed from the middle 20% of the dry fabric strip. An ~24-hour broth culture was inoculated into molten Nutrient Agar B, at a ratio of 1.0 mL culture to 100 mL agar. After mixing, 10 mL aliquots of the inoculated agar were transferred to sterile Petri dishes and allowed to harden. Each 1-inch test square was transferred to the center of an individual inoculated agar plate. The plates were incubated for 48±4 hours at 25-30°C. Following incubation, the subcultures were examined for the presence of a clear zone of inhibition of the test organism adjacent to each side of the test square. Controls included those for purity, sterility, and viability.

5. MRID 465837-05 "Standard Test Method for Evaluation of Laundry Sanitizers, Test Organisms: *Klebsiella pneumoniae* (ATCC 4352) and *Staphylococcus aureus* (ATCC 6538)" for Maquat 7.5M, by Amy Jeske. Study conducted at ATS Labs. Study completion date – April 6, 2005. Project Number A02756.

This study was conducted against *Klebsiella pneumoniae* (ATCC 4352) and *Staphylococcus aureus* (ATCC 6538). Three lots (Lot Nos. MRII 45-1, MRII 45-2, and MRII 45-3) of the product, Maquat 7.5M, were tested using ATS Labs Protocol No. MC03021705.LSAN (copy not provided). The laboratory report referenced Petrocci and Clarke's "Proposed Test Method for Antimicrobial Laundry Additives." Each of the three product lots tested was at least 60 days old at the time of testing. A use solution of the product was prepared by diluting 3.2 mL of the product with 304.77 mL of 200 ppm AOAC synthetic hard water (titrated at 202 ppm; a 1.33 ounce/gallon use solution; 780 ppm active quat). Numerous sets of sterile Nalgene jars were filled with 75 mL of the prepared use solution and equilibrated at 36.0°C. The carriers for this test were prepared by boiling 300 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 1.5 grams of Na_2CO_3, 1.5 grams of Triton X-100, and 3.0 L of deionized water for 60 minutes. The fabric then was rinsed in boiling water for 5 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 fabric strip was wrapped around a spindle between 12 and 13 times. Swatches (1 inch by 1.5 inch) were then cut from the remaining fabric. All carriers were autoclaved at 121°C, allowed to cool, and held at room temperature until use. Three swatches per organism per product lot were inoculated with 0.02 mL of the prepared organism culture, and dried in an incubator at 35-37°C for 30 minutes. 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 Nalgene jars containing the

use solution and subjected to a simulated tumble-wash at 45-60 RPM at 5 minutes at 35.0°C. A 1.0 mL aliquot of the "wash" water was transferred to a vessel containing 9 mL of Letheen Broth with 0.07% Lecithin and 0.5% Tween 80 to neutralize. The fabric swatches were transferred to 10 mL of Letheen Broth with 0.07% Lecithin and 0.5% Tween 80 to neutralize. The fabric swatches were then vortex mixed for a minimum of 10 seconds to extract fabric-bound microorganisms. The neutralizing subculture medium was vortex mixed and then serially diluted. A 1.0 mL aliquot of the neutralizing subculture medium was transferred to 9.0 mL of Butterfield's Buffer; representing the 10^{-1} dilution, and continuing in like manner through the 10^{-4} dilution. Each dilution was plated in duplicate in tryptic soy agar with 5% sheep blood in aliquots of 1.0 mL. In addition, 1.0 mL aliquots of the 10^{0} to 10^{-4} dilutions of the "wash" water were plated in duplicate. All subcultures were incubated for 48±4 hours at 35-37°C. Subcultures were stored at 2-8°C for 2 days prior to examination. Following incubation and storage, the subcultures were then examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, initial inoculum confirmation, carrier population, and neutralization confirmation.

6. MRID 465837-06 "Standard Test Method for Evaluation of Laundry Sanitizers, Test Organism: *Staphylococcus aureus* - MRSA (ATCC 33592)" for Maquat 7.5M, by Sally Nada. Study conducted at ATS Labs. Study completion date – November 24, 2004. Project Number A02521.

This study was conducted against *Staphylococcus aureus* - MRSA (ATCC 33592). Two lots (Lot Nos. MRII-45-1 and MRII-45-2) of the product, Maquat 7.5M, were tested using ATS Labs Protocol No. MC03102004.LSAN (copy not provided). The laboratory report referenced Petrocci and Clarke's "Proposed Test Method for Antimicrobial Laundry Additives." A use solution of the product was prepared by diluting 2.0 mL of the product with 190.4 mL of 200 ppm AOAC synthetic hard water (titrated at 200 ppm; a 1.33 ounce/gallon use solution; 780 ppm active quat). Two sets of sterile Nalgene jars were filled with 75 mL of the prepared use solution and equilibrated at 35±1°C. The carriers for this test were prepared by boiling 300 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 1.5 grams of Na_2CO_3, 1.5 grams of Triton X-100, and 3.0 L of deionized water for 60 minutes. The fabric then was rinsed in boiling water for 5 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 fabric strip was wrapped around a spindle between 12 and 13 times. Swatches (1 inch by 1.5 inch) were then cut from the remaining fabric. All carriers were autoclaved at 121°C for 20 minutes, allowed to cool, and held at room temperature until use. Three swatches per product lot were inoculated with 0.02 mL of the prepared organism culture, and dried in an incubator at 35-37°C for 30 minutes. 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 Nalgene jars containing the use solution and subjected to a simulated tumble-wash at 45-60 RPM at 4 minutes at 35.1°C. A 1.0 mL aliquot of the "wash" water was transferred to a vessel containing 9 mL of Letheen Broth with 0.07% Lecithin and 0.5% Tween 80 to neutralize. The fabric swatches were transferred to 10 mL of Letheen Broth with 0.07% Lecithin and 0.5% Tween 80 to neutralize. The fabric swatches were then vortex mixed for a

minimum of 10 seconds to extract fabric-bound microorganisms. The neutralizing subculture medium was vortex mixed and then serially diluted. A 1.0 mL aliquot of the neutralizing subculture medium was transferred to 9.0 mL of Butterfield's Buffer; representing the 10^{-1} dilution, and continuing in like manner through the 10^{-4} dilution. Each dilution was plated in duplicate in tryptic soy agar with 5% sheep blood in aliquots of 1.0 mL. In addition, 1.0 mL of the 10^{0} dilution of the "wash" water was plated in duplicate. All subcultures were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were then examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, initial inoculum confirmation, carrier population, and neutralization confirmation.

Note: Antibiotic resistance of *Staphylococcus aureus* - MRSA (ATCC 33592) was verified on a representative culture. The laboratory performed a Kirby Bauer Susceptibility assay. *Staphylococcus aureus* (ATCC 25923) was the control organism. The measured zone of inhibition confirmed antibiotic resistance of *Staphylococcus aureus* - MRSA to oxacillin. See Attachment I of the laboratory report.

7. MRID 465837-07 "Standard Test Method for Evaluation of Laundry Sanitizers, Test Organism: *Pseudomonas aeruginosa* (ATCC 15442)" for Maquat 7.5M, by Sally Nada. Study conducted at ATS Labs. Study completion date – February 22, 2005. Project Number A02694.

This study was conducted against *Pseudomonas aeruginosa* (ATCC 15442). Three lots (Lot Nos. MRII 45-1, MRII 45-2, and MRII 45-3) of the product, Maquat 7.5M, were tested using ATS Labs Protocol No. MC03011705.LSAN (copy not provided). The laboratory report referenced Petrocci and Clarke's "Proposed Test Method for Antimicrobial Laundry Additives." Each of the three product lots tested was at least 60 days old at the time of testing. A use solution of the product was prepared by diluting 2.0 mL of the product with 190.4 mL of 200 ppm AOAC synthetic hard water (titrated at 202 ppm; a 1.33 ounce/gallon use solution; 780 ppm active quat). Two sets of sterile Nalgene jars were filled with 75 mL of the prepared use solution and equilibrated at 35±1°C. The carriers for this test were prepared by boiling 300 grams of plain cotton weave fabric (approximately 80 x 80 threads/inch) in a solution of 1.5 grams of Na_2CO_3, 1.5 grams of Triton X-100, and 3.0 L of deionized water for 60 minutes. The fabric then was rinsed in boiling water for 5 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 fabric strip was wrapped around a spindle between 12 and 13 times. Swatches (1 inch by 1.5 inch) were then cut from the remaining fabric. All carriers were steam sterilized, allowed to cool, and held at room temperature until use. Three swatches per product lot were inoculated with 0.02 mL of the prepared organism culture, and dried in an incubator at 35-37°C for 30 minutes. 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 Nalgene jars containing the use solution and subjected to a simulated tumble-wash at 45-60 RPM at 5 minutes at 35.0-35.2°C. A 1.0 mL aliquot of the "wash" water was transferred to a vessel containing 9 mL of Letheen Broth with 0.07% Lecithin and 0.5% Tween 80 to neutralize. The fabric swatches were transferred to 10 mL of Letheen Broth with 0.07%

Lecithin and 0.5% Tween 80 to neutralize. The fabric swatches were then vortex mixed for a minimum of 10 seconds to extract fabric-bound microorganisms. A 1.0 mL aliquot of the neutralizing subculture medium was transferred to 9.0 mL of Butterfield's Buffer; representing the 10^{-1} dilution, and continuing in like manner through the 10^{-4} dilution. Each dilution was plated in duplicate in tryptic soy agar with 5% sheep blood in aliquots of 1.0 mL. Apparently, 1.0 mL of the 10^{0} to 10^{4} dilutions of the "wash" water were plated in duplicate. All subcultures were incubated for 48±4 hours at 35-37°C. Subcultures were stored at 2-8°C for 1 day prior to examination. Following incubation and storage, the subcultures were then examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, initial inoculum confirmation, carrier population, and neutralization confirmation.

8. MRID 465837-08 "Virucidal Efficacy of a Laundry Additive, Virus: Human Immunodeficiency Virus type 1" for Maquat 7.5M, by Karen M. Ramm. Study conducted at ATS Labs. Study completion date – February 7, 2005. Project Number A02659.

This study was conducted against the Human immunodeficiency virus type 1 (originally obtained from Advanced Biotechnologies, Inc., Columbia, MD; propagated in-house; Strain HTLV-III_B), using MT-2 cells (human CD4+ lymphocytes; originally obtained from the National Cancer Institute, Frederick, MD; propagated in-house) as the host system. Two lots (Lot Nos. MRII-45-1 and MRII-45-2) of the product, Maquat 7.5M, were tested according to ATS Labs Protocol No. MC03081004.HIV (copy not provided). The laboratory report referenced Petrocci and Clarke's "Proposed Test Method for Antimicrobial Laundry Additives." The stock virus culture was adjusted to contain a 5% organic soil load (fetal bovine serum). A use solution was prepared by adding 4.0 mL of the product to 381.0 mL of 400 ppm synthetic hard water (titrated at 408 ppm; a 1.33 ounces/gallon use solution; 780 ppm active quat). The carriers for this test were prepared from plain cotton weave fabric (approximately 80 x 80 threads/inch). The fabric was scoured in an unspecified solution. A 2-inch wide fabric strip weighing 15±1 grams was cut from the scoured fabric. The fabric strip was wrapped around a spindle between 12 and 13 times. Fabric swatches (1 inch by 1.5 inch) were cut. The fabric swatches were steam-sterilized and held at room temperature until use. Nine swatches per product lot were inoculated with 0.2 mL of the prepared virus culture, and air-dried at 26.0°C for 20 minutes in 10.3% relative humidity. After drying, the inoculated fabric 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 plastic bottles containing 75 mL of the use solution and subjected to a simulated tumble-wash at 60 RPM at 4 minutes at 34.0-34.5°C. A 2.0 mL aliquot of the "wash" water was passed through a Sephadex column. The fabric swatches were transferred to individual tubes containing 2.0 mL of the test medium. The tubes were vortex mixed and the contents were passed through a Sephadex column. Ten-fold serial dilutions of the "wash" water and fabric swatch filtrates were prepared using RPMI 1640 supplemented with 15% (v/v) heat- inactivated fetal bovine serum, 2.0 mM L-glutamine, and 50 µg/mL gentamicin. MT-2 cells in multi-well culture dishes were inoculated in quadruplicate with 0.2 mL of the dilutions. The cultures were incubated at 35-37°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 9 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for

cytotoxicity, input virus count, dried virus count, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

V. RESULTS

MRID Number	Organism	Lot No.	Average No. Surviving (CFU/carrier)	Microbes Initially Present (CFU/carrier)	Percent Reduction
465837-01	*Klebsiella pneumoniae*	MRII 45-1 MRII 45-2	$<1.04 \times 10^3$ <10	1.22×10^8 1.22×10^8	>99.999 >99.999
465837-02	*Shigella sonnei*	MRII 45-1 MRII 45-2	20 10	7.6×10^7 7.6×10^7	99.999 99.999

MRID Number	Organism	Lot No.	Score (Total Number of Sides Showing Zone of Inhibition)
465837-03	*Klebsiella pneumoniae*	MRII-45-1 MRII-45-2	18/20 20/20
	Staphylococcus aureus	MRII-45-1 MRII-45-2	20/20 20/20
465837-04	*Corynebacterium ammoniagenes*	MRII-45-1 MRII-45-2	20/20 20/20

MRID No. 465837-05

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.
Klebsiella pneumoniae	MRII 45-1 MRII 45-2 MRII 45-3	$<1 \times 10^1$ $<1 \times 10^1$ $<1 \times 10^1$	2.6×10^5	0, 0 0, 0 0, 0	8.5×10^4	>99.9 >99.9 >99.9
Staphylococcus aureus	MRII 45-1 MRII 45-2 MRII 45-3	$<1 \times 10^1$ $<1 \times 10^1$ $<1 \times 10^1$	1.89×10^5	0, 0 0, 0 0, 0	1.49×10^4	>99.9 >99.9 >99.9

MRID No. 465837-06

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.
Staphylococcus aureus - MRSA	MRII-45-1 MRII-45-2	$<1 \times 10^1$ $<1 \times 10^1$	2.34×10^6	0, 0 0, 0	1.04×10^4	>99.9 >99.9

MRID No. 465837-07

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.
Pseudomonas aeruginosa	MRII 45-1 MRII 45-2 MRII 45-3	2.4×10^2 6.0×10^1 $<1 \times 10^1$	9.1×10^5	0, 0 0, 0 0, 0	4.4×10^4	99.9 99.9 >99.9

MRID Number	Organism	Results			Dried Virus Control ($TCID_{50}$/0.2 mL)
			Lot No. MRII-45-1	Lot No. MRII-45-2	
465837-08	Human immuno-deficiency virus type 1 (fabric carriers)	10^{-1} dilution	Cytotoxicity	Cytotoxicity	$10^{5.35}$
		10^{-2} to 10^{-7} dilution	Complete inactivation	Complete inactivation	
		$TCID_{50}$/0.2 mL	$\leq 10^{1.5}$	$\leq 10^{1.5}$	
		Log reduction	≥ 3.85 $\log_{10}$	≥ 3.85 $\log_{10}$	
465837-08	Human immuno-deficiency virus type 1 ("wash" water)	10^{-1} dilution	Cytotoxicity	Cytotoxicity	$10^{5.0}$
		10^{-2} to 10^{-7} dilution	Complete inactivation	Complete inactivation	
		$TCID_{50}$/0.2 mL	$\leq 10^{1.5}$	$\leq 10^{1.5}$	
		Log reduction	3.5 $\log_{10}$	3.5 $\log_{10}$	

VI. CONCLUSIONS

1. The submitted efficacy data support the use of a 200 ppm active quat use solution of the product, Maquat 7.5M, as a sanitizing rinse against the following microorganisms on previously cleaned, hard, non-porous, food contact surfaces in the presence of 500 ppm hard water for a contact time of one minute (tested at 30 seconds):

Klebsiella pneumoniae	MRID No. 465837-01
Shigella sonnei	MRID No. 465837-02

A 99.999% reduction in population was observed. Numbers counts were between 75 and 125 x 10^6/mL. 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.

2. The submitted efficacy data support the use of a 650 ppm active quat use solution of the product, Maquat 7.5M, as a laundry additive with residual bacteriostatic activity against the following microorganisms at a temperature of 35°C for a contact time of 5 minutes:

Corynebacterium ammoniagenes	MRID No. 465837-04
Klebsiella pneumoniae	MRID No. 465837-03
Staphylococcus aureus	MRID No. 465837-03

Total "zone of inhibition" scores of at least 18/20 sides were demonstrated. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

3. The submitted efficacy data support the use of a 780 ppm active quat use solution of the product, Maquat 7.5M, as a laundry sanitizer against the following microorganisms in the presence of 200 ppm hard water for a contact time of 5 minutes (4 minutes for *Staphylococcus aureus* - MRSA):

Klebsiella pneumoniae	MRID No. 465837-05
Pseudomonas aeruginosa	MRID No. 465837-07
Staphylococcus aureus	MRID No. 465837-05
Staphylococcus aureus - MRSA	MRID No. 465837-06

A 99.9% reduction in population was observed for both the fabric carriers and the "wash" water. The carrier and "wash" water quantification controls were at least $1x10^4$ CFU/carrier and $1x10^4$ CFU/mL, respectively, and, therefore, were valid. Neutralizer confirmation testing met the acceptance criterion of growth within 1.0 $\log_{10}$ of the control. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

Each of the product lots tested against *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were at least 60 days old at the time of testing. Three fabric swatches per organism per product lot were tested.

4. The submitted efficacy data (MRID No. 465837-08) support the use of a 780 ppm active quat use solution of the product, Maquat 7.5M, as a laundry additive against Human immunodeficiency virus type 1 in the presence of a 5% organic soil load and 400ppm hard water for a contact time of 4 minutes. Recoverable virus titers of at least 10^4 were achieved. Cytotoxicity was observed in the 10^{-1} dilutions of the fabric carriers and "wash" water. Complete inactivation (no growth) was indicated in all higher dilutions tested. At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level.

VII RECOMMENDATIONS

A. Recommendations Regarding Submitted Efficacy Claims

1. The proposed label (page 10) claims that the product, Maquat 750-M, is effective as a one-step sanitizer at a 200ppm use solution on hard porous and non-porous, non-food contact surfaces with a 3 minute contact time against *Klebsiella pneumoniae*. This claim appeared on the previous label dated July 28, 2004, and was supported by previously submitted data.

The submitted data (MRID No. 465837-01) supports the use of the product as a food-contact sanitizer at a use dilution of 200 ppm in the presence of 500 ppm hard water at a contact time of 60 seconds. It is acceptable for the applicant to list *Klebsiella pneumoniae* under the list of organisms on page 13 of the proposed label (food-contact surface sanitization).

2. The proposed label (page 10) claims that the product, Maquat 750-M, is effective as a one-step sanitizer at a 200ppm use solution on hard porous and non-porous, non-food contact surfaces with a 3 minute contact time against *Shigella sonnei*. This claim is not acceptable. The test was not conducted for porous surfaces, and no soil load was included in the test design. The applicant must remove this organism from page 10 of the label, or remove the claims "one-step sanitizer" and "hard porous surfaces".

The submitted data (MRID No. 465837-02) supports the use of the product as a food-contact sanitizer at a use dilution of 200 ppm in the presence of 500 ppm hard water at a contact time of 60 seconds. It is acceptable for the applicant to list *Shigella sonnei* under the list of organisms on page 13 of the proposed label (food-contact surface sanitization).

3. The proposed label claims that the product, Maquat 750-M, provides residual bacteriostatic activity on fabric when 2 ounces of the product are used to treat 100 pounds of dry fabric (~650 ppm active quat) for a 5-minute contact time. Data provided by the applicant support this claim.

4. The proposed label claims that the product, Maquat 750-M, is an effective laundry sanitizer against the following microorganisms when 2.4 ounces of the product is used to treat 100 pounds of dry fabric (~780 ppm active quat) in rinse water up to 200 ppm hard water (400 ppm hard water against HIV-1) for a 5-minute contact time with a minimum temperature of 95°F (i.e., 35°C):

Staphylococcus aureus
Klebsiella pneumoniae

Pseudomonas aeruginosa
Staphylococcus aureus - MRSA
Human immunodeficiency virus type 1

Data provided by the applicant support these claims.

B. Other Label Recommendations

1. Page 2 of the proposed label (right column; first item) claims that the product is recommended for use as a sanitizer on food processing equipment and utensils at a 400 ppm active solution. This use level does not agree with the directions for food contact sanitization on page 13 of the label, where a 200 ppm use solution is recommended.

2. The proposed label claims that the product "works as a laundry sanitizer against harmful bacteria" (page 3; left column; 8th item). The applicant must delete the word "harmful" from this statement.

3. The proposed label claims [page 3 of the proposed label (left column; 10th item)] that the product "kills the bacteria that may cause allergy to skin on clothes." This is a public health claim. The applicant must remove this claim from the label.

4. Page 3 of the proposed label (left column; 11th item) claims that the "Fabric treated with this product which has become contaminated or soiled through use will inhibit the growth of many organisms..." The applicant must revise the statement to read "Fabric treated with this product which has become contaminated or soiled through use will inhibit the growth of odor-causing organisms..."

5. Page 3 of the proposed label (right column; 12th item) claims that the product is "Safe for most surfaces." The applicant must delete this claim, the use of this term (safe) is not acceptable.

6. The proposed label claims that the product "Kills common kitchen germs" [page 3 (right column; 17th item)]. An unqualified germ claim such as this must have public health data developed according to Agency guidelines for bacteria (a general/broad spectrum disinfectant), a pathogenic fungus, and both an enveloped and non-enveloped virus (See http://www.epa.gov/oppad001/germs.htm). Although the product meets the criteria for both bacteria and viruses, no data has been submitted on a pathogenic fungus. The applicant must remove the germ claim from the label or qualify the claim with an asterisk that indicates the organisms the term "germ" is referring to.

7. Page 5 of the proposed label (left column; 4th and 11th items) includes the statements "NO POTABLE WATER RINSE IS ALLOWED" and also "A potable water rinse is required after application on food contact surfaces." The applicant must indicate the use patterns these directions coincide with, i.e. food and non-food contact surface sanitization).

8. Page 5 of the proposed label (right column; 7th and 8th items) claims that the product is an effective disinfectant and sanitizer in the presence of 5% serum contamination (soils). Not all

organisms were tested in the presence of an organic soil load. The applicant must specify which claims and organisms these statements refer to, or remove them from the label.

9. Page 5 of the proposed label (right column; 12th item) claims that "work or dining surfaces could harbor hazardous microorganisms." Remove the word "hazardous" from the statement.

10. Page 7 of the proposed label (left column, 4th item) claims that the product is effective against HBV and HCV "in the presence of 400 ppm hard water (CaCo3)". Studies for HBV and HCV did not incorporate a hard water burden. The hard water claims must be deleted from the label unless data can be submitted to support them.

11. Page 7 of the proposed label (left column; 5th item) claims that the product is an effective fungicide in the presence of organic soil. Fungicidal claims are public health claims. Delete "fungicide" from this sentence unless data against *Trychophyton mentagrophytes*, in the presence of a 5% soil load, can be submitted.

12. The proposed label (page 7, right column; 5th item, and also page 11, right column; 5th and 6th items) claims that the product is both an effective disinfectant and sanitizer for whirlpool footbath units. Due to recent outbreaks of severe skin infections linked to rapidly growing Mycobacteria, the proper disinfection of these sites is critical. The directions provided for the sanitization and disinfection of these units are likely inadequate for the control of such infections. The applicant needs to delete these claims from the product label.

13. Page 10 of the proposed label (right column; under Sanitizer Directions for Non-Food Contact Surfaces) claims that the product is effective on "hard porous and non-porous environmental surfaces." Data has not been cited or submitted to support the use of the product on porous surfaces (MRID No. 454102-01, used in support of the non-food contact surface sanitizer claim did not evaluate efficacy of the product on porous surfaces). The applicant must delete the term "porous" or cite data to support this claim.

14. The proposed label [see page 10 under the "Laundry Sanitizer" section] states that "Other laundry additives, such as fabric softeners, laundry sours, starch and sizing can be used per manufacturer's instructions in subsequent rinse cycles after the sanitizing rinse cycle." The applicant has not provided data to support the compatibility of the sanitizer treatment with other laundry additives. Per DIS/TSS-13: "Compatibility of the treatment with other common laundry additives (e.g. soaps, detergents, bleach, starch, bluing, sours, fabric softeners) should be determined in testing and addressed in labeling, when applicable". The applicant must remove this sentence from the label, or submit data to support these claims.

15. Page 11 of the proposed label (left column; 5th item) lists instructions for a "Laundry Sanitizer (Against Odor Causing Bacteria)." Instructions call for a lessened use solution. The language "Against Odor Causing Bacteria" is not optional as it calls for different use directions than the general laundry sanitizer use, and must be taken out of the parentheses.

16. Page 11 of the proposed label (right column; first item) reads: "To prevent cross contamination of harmful organisms from area to area...." The applicant must delete the word "harmful" from the statement.

17. The general directions for use of the product as a sanitizer on non-food contact surfaces specify a 3-minute contact time. A number of applications [page 11 of the proposed label], however, allow a 60-second contact time (i.e., shoe bath sanitizer, shoe foam, salon/barber instruments and tools sanitizer). These are non-food contact surfaces and must be designated with a 3 minute contact time at a 1 ounce to 25 gallon use dilution.

18. The applicant needs to make the following changes to the proposed label, as appropriate:

- On page 2 (left column; 5th item), change "water dishes." to read "water dishes)." An end parenthesis is needed.

- On page 3 (left column; 8th item), change "...bacteria including the HIV Virus" to read "...bacteria and the HIV virus."

- On page 3 (left column; 8th item), change "For HIV, this product is effective in 400 ppm hard." to read "For HIV, this product is effective in 400 ppm hard water."

- On page 3 (left column; 11th item), change "...garments are laundered and treat with this product." to read "...garments are laundered and treated with this product."

- On page 15, under "Direction for Sanitizing Food Contact Surfaces . . ," delete the 4th step ("Rinse articles thoroughly with potable water.") as it is the same as the 3rd step. If this step is to be taken twice, label directions should indicate this.