UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION,
PESTICIDES
AND TOXIC
SUBSTANCES

January 26, 2006

MEMORANDUM

Subject: Efficacy Review for EPA Reg. No. 67619-8, CPPC Ultra Bleach 2;
DP Barcode: 323340

From: Tajah L. Blackburn, Ph.D., Microbiologist 1/26/06
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510C)

Thru: Nancy Whyte, Team Leader
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To: Emily Mitchell PM 32/ Delores Williams
Regulatory Management Branch II
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Applicant: Clorox Services Company
7200 Johnson Drive
PO Box 493
Pleasanton, CA 94566

Formulations from Label:

Active Ingredient(s)	% by wt.
Sodium Hypochlorite.....	6.15%
Inert Ingredients.....	93.85%
Total	100.00%

I BACKGROUND

The product, CPPC Ultra Bleach 2 (EPA Reg. No. 67619-8), is a registered disinfectant (bactericide, fungicide, tuberculocide, virucide), sanitizer, and mildewcide for use on hard, non-porous surfaces in household, commercial, institutional, food processing, animal care, and hospital or medical environments. **The applicant requested to amend the registration by adding claims for effectiveness against *Clostridium difficile* (vegetative), *Streptococcus pneumoniae*, Vancomycin resistant *Enterococcus faecalis*, Avian Influenza A virus, Human Hepatitis C virus, and Norwalk virus. Furthermore, the applicant has requested to add a new service bulletin covering treatment of Southern seaoats seeds.** In support of this new service bulletin, the current data package contains a manuscript entitled, "Influence of Selected Surface Disinfectants, Fungicides, and Temperature on Seed Germination and Initial Growth of Southern Seaoats (*Uniola paniculata*)," Department of Horticultural Science, North Carolina State University, Raleigh, NC. Studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121; and MicroBioTest, Inc., located at 105 Carpenter Drive in Sterling, VA 20164.

This data package contained a letter from the applicant to EPA (dated October 14, 2005), eight studies (MRID Nos. 466708-01 through 466708-08), Statements of No Data Confidentiality Claims for all eight studies, and the proposed label.

Note: The laboratory reports describe studies conducted for the products, F2001.0126 and Ultra Clorox Liquid Bleach." The applicant's letter to EPA (dated October 14, 2005) indicates that the tested products, F2001.0126 and Ultra Clorox Liquid Bleach, are the product, 67619-8 Basic CSF, which is the subject of this efficacy report.

II USE DIRECTIONS

The product is designed for use in disinfecting hard, non-porous surfaces such as appliances, bathtubs, bed frames, cabinets, carts, chairs, changing tables, combs and brushes, counters, desks, diaper pails, door knobs, faucets, floors, furniture, garbage cans, garbage disposals, hampers, kennels, lamps, light switch panels, litter boxes, patio furniture, playpens, recycling bins, showers, sinks, telephones, toilets, toys, urinals, and walls consisting of glass, ceramic, glazed tile, porcelain, laminated surfaces, linoleum, and vinyl. Directions on the proposed label provided the following information regarding preparation and use of the product as a disinfectant: Wash, wipe, or rinse items with water. Prepare a use solution by adding 2/3 cup of this product per gallon of water (2400 ppm available chlorine). Apply use solution. Let stand 2 minutes to treat against bacteria. Let stand 5 minutes to treat against viruses. Rinse thoroughly and air dry.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

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

Effectiveness of disinfectants against specific bacteria other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Method, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method, must be determined by either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants Method. Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different product lots. To support products labeled as "disinfectants" for specific bacteria (other than those bacteria named in the above test methods), killing of the specific microorganism on all carriers is required. In addition, plate count data must be submitted for each microorganism to demonstrate that a concentration of at least 10^4 microorganisms survived the carrier-drying step. These Agency standards are presented in DIS/TSS-1.

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.

Virucides – Novel Virus Protocol Standards

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

IV COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. MRID 466708-01 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Avian Influenza A virus (Influenza A Reassortant)" for F2001.0126, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – June 1, 2005. Project Number A02902.

This study was conducted against Avian Influenza A virus (Influenza A Reassortant) (ATCC VR-2072, Strain A/Washington/897/80 x A/Mallard/New York/6750/78), using Rhesus monkey kidney cells (RMK cells; obtained from ViroMed Laboratories, Inc.; maintained in-house) as the host system. Two lots (Lot Nos. A8500514 and A8501309) of the product, F2001.0126, were tested according to ATS Labs Protocol No. CLO17011405.AFLU (copy not provided). A use solution was prepared by adding 2 ml of the product to 48 ml of 100 ppm AOAC synthetic hard water (titrated at 101 ppm; a 2400 ppm solution). [The sodium hypochlorite concentration of each use solution was confirmed to be 2902-2942 ppm.] 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 at 20.0°C at 38% relative humidity for 20 minutes. For each lot of product, separate dried virus films were treated with 2.0 ml of the use solution for 5 minutes at 20.0°C. After exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixture was passed through a Sephadex column, and diluted serially in Minimum essential medium supplemented with 1% 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 and scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for cytotoxicity, dried virus counts, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

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

Note: Avian Influenza A virus (Avian Reassortant) (ATCC VR-2072) is no longer listed in ATCC's web-based catalog.

2. MRID 466708-02 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces – Confirmatory Assay, Virus: Bovine Viral Diarrhea virus as a surrogate for Human Hepatitis C virus" for F2001.0126, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – June 1, 2005. Project Number A02903.

This study was conducted against the Bovine viral diarrhea virus (NADL strain; ATCC VR-1422) using bovine turbinate cells (BT cells; ATCC CRL-1390; propagated in-house) as the host system. One lot (Lot No. A8500514) of the product, F2001.0126, was tested according to ATS Labs Protocol No. CLO17011405.BVD.2 (copy not provided). A use solution was prepared by adding 2 ml of the product to 48 ml of 100

ppm AOAC synthetic hard water (titrated at 101 ppm; a 2400 ppm solution). [The sodium hypochlorite concentration of the use solution was confirmed to be 2783 ppm.] The stock virus culture was adjusted to contain 5% horse serum as the organic soil load. Two glass carriers were tested for the single product lot against the target virus. Films of virus were prepared by spreading 0.2 ml of stock virus on the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 19.9-20.0°C and a relative humidity of 40-42%. A 2.0 ml aliquot of the use solution was added to the virus films for 5 minutes at 19.9°C. After the contact period, the virus-disinfectant mixture was scraped from the surface of the dish with a cell scraper to re-suspend the contents. The virus-disinfectant mixture was passed through a Sephadex column, using a syringe plunger. This initial dilution was considered the 10^{-1} dilution. A 0.2 ml aliquot of the test virus was resuspended in 2.0 ml of test substance, which equals a 1:10 dilution. Ten-fold serial dilutions were then prepared, using Minimum Essential Medium containing 2% non-heat inactivated horse serum, 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B. BT cells were inoculated in quadruplicate with a 0.1 ml aliquot of each dilution. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 7 days for the presence or absence of specified cytopathic effects (i.e., cell rounding; cells dark with a granular appearance), cytotoxicity, and viability. To verify the cytopathic effect readings, a direct immunofluorescence assay was performed on the final day of incubation. The $\log_{10}$ reduction in infectivity was calculated using EPA's Most Probable Number (MPN) method. Controls included those for input virus count, dried virus count, neutralization, cytotoxicity, and data consistency.

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

3. MRID 466708-03 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Bovine Viral Diarrhea virus as a surrogate for Human Hepatitis C virus" for F2001.0126, by Karen M. Ramm. Study conducted at ATS Labs. Study completion date – June 21, 2005. Project Number A02914.

This study was conducted against the Bovine viral diarrhea virus (NADL strain; ATCC VR-1422) using bovine turbinate cells (BT cells; ATCC CRL-1390; propagated in-house) as the host system. Two lots (Lot Nos. A8500514 and A8501309) of the product, F2001.0126, were tested according to ATS Labs Protocol No. CLO17011405.BVD.1 (copy not provided). Testing was conducted on May 18, 2005 and June 2, 2005. A use solution was prepared by adding 2 ml of the product to 48 ml of 100 ppm AOAC synthetic hard water (titrated at 99.4 ppm; a 2400 ppm solution). [The sodium hypochlorite concentration of each use solution was confirmed to be 2882-2945 ppm.] The stock virus culture was adjusted to contain 5% horse serum as the organic soil load. Two glass carriers were tested for each product lot against the target virus. Films of virus were prepared by spreading 0.2 ml of stock virus on the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 42% relative humidity. For each lot of product, 2.0 ml of the use solution was added to the virus films for 5 minutes at 20.0°C. After the contact period, the virus-disinfectant mixture was scraped from the surface of the dish with a cell scraper to re-suspend the contents. The virus-disinfectant mixture was passed through a Sephadex column, using a syringe plunger. This initial dilution was considered the 10^{-1} dilution. A 0.2 ml aliquot of the test virus was resuspended in 2.0 ml of test substance, which equals a 1:10 dilution. Ten-

fold serial dilutions were then prepared, using Minimum Essential Medium containing 2% non-heat inactivated horse serum, 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B. BT cells were inoculated in quadruplicate with 0.1 ml of each dilution. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 7 days for the presence or absence of specified cytopathic effects (i.e., cell rounding; cells dark with a granular appearance), cytotoxicity, and viability. To verify the cytopathic effect readings, a direct immunofluorescence assay was performed on the final day of incubation. The log_{10} reduction in infectivity was calculated using EPA's Most Probable Number (MPN) method. Controls included those for input virus count, dried virus count, neutralization, cytotoxicity, and data consistency.

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

Note: The applicant provided the data for a failed trial set up on May 18, 2005. In that trial, the dried virus control was invalid (i.e., test virus was not detected). Thus, the test was invalid. These data were not used to evaluate efficacy of the product. Testing was repeated on June 2, 2005. See Attachment I of the laboratory report.

4. MRID 466708-04 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Feline Calicivirus as a Surrogate for Norwalk and Norovirus – Confirmatory Assay" for F2001.0126, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – May 25, 2005. Project Number A02904.

This study was conducted against Feline calicivirus (Strain F-9; ATCC VR-782) using Crandel Reese feline kidney cells (CRFK cells; ATCC CCL-94; propagated in-house) as the host system. One lot (Lot No. A8500514) of the product, F2001.0126, was tested according to ATS Labs Protocol No. CLO17011405.FCAL.2 (copy not provided). A use solution was prepared by adding 2 ml of the product to 48 ml of 100 ppm AOAC synthetic hard water (titrated at 101 ppm; a 2400 ppm solution). [The sodium hypochlorite concentration of the use solution was confirmed to be 2894 ppm.] The stock virus culture contained 5% fetal bovine serum as the organic soil load. Two glass carriers were tested for the single product lot against the target virus. Films of virus were prepared by spreading 0.2 ml of stock virus on the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 43% relative humidity. A 2.0 ml aliquot of the use solution was added to the virus films for 5 minutes at 20.0°C. After the contact period, the virus-disinfectant mixture was scraped from the surface of the dish with a cell scraper to re-suspend the contents. The virus-disinfectant mixture was passed through a Sephadex column, using a syringe plunger. This initial dilution was considered the 10^{-1} dilution. A 0.2 ml aliquot of the test virus was resuspended in 2.0 ml of test substance, which equals a 1:10 dilution. Ten-fold serial dilutions were then prepared, using Minimum Essential Medium containing 5% heat-inactivated fetal bovine serum, 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B. CRFK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 ml of each dilution. The cultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 8 days for the presence or absence of specified cytopathic effects (i.e., small, rounding of the cells, with a slight granular look), cytotoxicity, and viability. Controls included those for input virus count,

dried virus count, neutralization, cytotoxicity, and data consistency. The log_{10} reduction in infectivity was calculated using EPA's Most Probable Number (MPN) method.

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

5. MRID 466708-05 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Feline Calicivirus as a surrogate for Norwalk and Norovirus" for F2001.0126, by Karen M. Ramm. Study conducted at ATS Labs. Study completion date – May 27, 2005. Project Number A02913.

This study was conducted against Feline calicivirus (Strain F-9; ATCC VR-782) using Crandel Reese feline kidney cells (CRFK cells; ATCC CCL-94; propagated in-house) as the host system. Two lots (Lot Nos. A8500514 and A8501309) of the product, F2001.0126, were tested according to ATS Labs Protocol No. CLO17011405.FCAL.1 (copy not provided). A use solution was prepared by adding 2 ml of the product to 48 ml of 100 ppm AOAC synthetic hard water (titrated at 105 ppm; a 2400 ppm solution). [The sodium hypochlorite concentration of each use solution was confirmed to be 2769.9-2877.3 ppm.] The stock virus culture contained 5% fetal bovine serum as the organic soil load. Two glass carriers were tested for each product lot against the target virus. Films of virus were prepared by spreading 0.2 ml of stock virus on the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C and a relative humidity of 40-43%. For each lot of product, 2.0 ml of the use solution was added to the virus films for 5 minutes at 20.0°C. After the contact period, the virus-disinfectant mixture was scraped from the surface of the dish with a cell scraper to re-suspend the contents. The virus-disinfectant mixture was passed through a Sephadex column, using a syringe plunger. This initial dilution was considered the 10^{-1} dilution. A 0.2 ml aliquot of the test virus was resuspended in 2.0 ml of test substance, which equals a 1:10 dilution. Ten-fold serial dilutions were then prepared, using Minimum Essential Medium containing 5% heat-inactivated fetal bovine serum, 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B. CRFK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 ml of each dilution. The cultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 7 days for the presence or absence of specified cytopathic effects (i.e., small, rounding of the cells, with a slight granular look), cytotoxicity, and viability. Controls included those for input virus count, dried virus count, neutralization, cytotoxicity, and data consistency. The log_{10} reduction in infectivity was calculated using EPA's Most Probable Number (MPN) method.

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

6. MRID 466708-06 "AOAC Use-Dilution Method, Test Organism: *Streptococcus pneumoniae* (ATCC 6305)" for F2001.0126, by Sally Nada. Study conducted at ATS Labs. Study completion date – July 27, 2005. Project Number A02901.

This study was conducted against *Streptococcus pneumoniae* (ATCC 6305). Two lots (Lot Nos. A8500514 and A8501309) of the product, F2001.0126, were tested according to the AOAC Use-Dilution Method as described in the AOAC Official Methods

of Analysis, 15th Edition, 1990. Testing was conducted on May 12, 2005, June 17, 2005, and July 14, 2005. A 2400-ppm use solution was prepared using 100 ppm AOAC synthetic hard water (titrated at 99 ppm). [The sodium hypochlorite concentration of each use solution was confirmed to be 2796-2857 ppm.] 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 2-3 day old suspension of the test organism, at a ratio of 1 carrier per 1.0 ml broth. The carriers were dried for 40 minutes at 25-30°C and a relative humidity of 65%. Each carrier was exposed to 10 ml of the use solution for 2 minutes at 20±1°C. After exposure, individual carriers were transferred to 10 ml of Brain Heart Infusion Broth containing 0.1% sodium thiosulfate to neutralize. Within at least 30 minutes after the first transfer, carriers were transferred to secondary subculture tubes containing 10 ml of Letheen Broth with 0.07% Lecithin and 0.5% Tween 80. All subcultures were incubated for 48±4 hours at 35-37°C. The subcultures were stored for 1-2 days 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 purity, sterility, viability, neutralization confirmation, and carrier population.

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

Note: The applicant provided the data for a failed trial set up on May 12, 2005. In that trial, no growth was shown in the neutralization confirmation control for 1 product lot (i.e., Lot No. A8500514), and insufficient growth was shown in the neutralization confirmation control for the other product lot (i.e., Lot No. A8501309). Testing was repeated. See Attachment I of the laboratory report.

7. MRID 466708-07 "AOAC Use-Dilution Method, Test Organism: Vancomycin Resistant *Enterococcus faecalis* (ATCC 51299)" for F2001.0126, by Sally Nada. Study conducted at ATS Labs. Study completion date – May 24, 2005. Project Number A02900.

This study was conducted against Vancomycin Resistant *Enterococcus faecalis* (ATCC 51299). Two lots (Lot Nos. A8500514 and A8501309) of the product, F2001.0126, were tested according to the AOAC Use-Dilution Method as described in the AOAC Official Methods of Analysis, 15th Edition, 1990. A use solution was prepared by adding 26.0 ml of the product to 624.0 ml of 100 ppm AOAC synthetic hard water (titrated at 105 ppm; a 2400 ppm solution). [The sodium hypochlorite concentration of each use solution was confirmed to be 2854-2861 ppm.] 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 the 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 37.7% relative humidity. Each carrier was exposed to 10 ml of the use solution for 2 minutes at 20±1°C. After exposure, the carriers were transferred to 10 ml of Letheen Broth containing 0.1% sodium thiosulfate to neutralize. All subcultures were incubated for 48±4 hours at 35-37°C. The subcultures were stored for 2 days 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 purity, sterility, viability, neutralization confirmation, carrier population, and antibiotic resistance.

Note: Antibiotic resistance of Vancomycin Resistant *Enterococcus faecalis* (ATCC 51229) 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 Vancomycin Resistant *Enterococcus faecalis* to vancomycin (Page 9 and Table 6 of the laboratory report).**

8. MRID 466708-08 "AOAC Use-Dilution Test Using *Clostridium difficile*" for Ultra Clorox Liquid Bleach, by Angela L. Hollingsworth. Study conducted at MicroBioTest, Inc. Study completion date – July 29, 2005. Laboratory Project Identification Number 320-359.

This study was conducted against *Clostridium difficile* (ATCC 9689). Two lots (Lot Nos. A8500514 and A8501309) of the product, Ultra Clorox Liquid Bleach, were tested using the AOAC Use-Dilution 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 24 ml of 100 ppm AOAC synthetic hard water (titration results not provided; a 2400 ppm solution). [The sodium hypochlorite concentration of each use solution was confirmed to be 2698-2726.4 ppm.] Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Sixty (60) stainless steel penicylinder carriers were immersed in a 48-54 hour old suspension of the test organism, at a ratio of 20 carriers per 20.0 ml broth. The carriers were dried for 20-40 minutes at 37±2°C. Each carrier was exposed to 10 ml of the use solution for 2 minutes at 21°C. After exposure, the carriers were transferred to tubes of Reinforced Clostridial Medium containing 0.1% sodium thiosulfate to neutralize. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for sterility, viability, neutralizer effectiveness, carrier counts, and confirmation of the challenge microorganism.

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

9. No MRID Number Issued. "Influence of Selected Surface Disinfectants, Fungicides, and Temperature on Seed germination and Initial Growth of Southern Seaoats (*Uniola paniculata*)" by Tyler Burgess, Frank Blazich, David Nath, and Betsy Randall-Schadel. Study conducted at North Carolina State University, Raleigh, NC.

Seeds of southern seaoats (*Uniola paniculata*) were removed from storage at 4°C (39°F) and treated with the following selected surface disinfectants, funigicides, and combinations of these chemicals: nontreated (control), 1.3% sodium hypochlorite, 2.6% sodium hypochlorite, RTU® (12.6% thiram +0.34% thiabendazole), RTU®-PCNB (24% pentachlronitrobenzene), 1.3% sodium hypochlorite and RTU®, or 2.6% sodium hypochlorite and RTU®-PCNB. Following treatment, seed were germinated at an 8/16 hr thermoperiod if 35/20°C (95/68°F). The seed treatments and germination thermoperiod utilized were based on three preliminary trials that investigated the influences of selected surface disinfectants, fungicides, and temperature on seed germination of the species. Germination was recorded every 3 days for 30 days. Seed treatment was highly significant (*P=0.0001*) for both total percentage germination and total percentage of decayed seeds. Germination of nontreated seeds was 45% , and four treatments resulted in germination >80% [RTU®-PCNB (81%), 2.6% sodium

hypochlorite and RTU® (83%), 1.3% sodium hypochlorite and RTU® (87%), and 1.3% sodium hypochlorite and RTU® (89%)]. A subsequent experiment investigated the effects of the aforementioned treatments with the exception of 1.3% sodium hypochlorite and RTU®, both used alone, on initial seedling growth of the species. Following treatment, seeds were sown in containers filled with a peat-based medium and the containers placed in a growth chamber maintained at an 8/16 hr thermoperiod of 35/20°C (95/68°F) with long day conditions. Emergence data were recorded every 3 days for 45 days. After 45 days, the study was terminated and additional data recorded to include plant height (height of main stem), leaf number, length and width of the two longest leaves, and top and root dry weights. Surface disinfectant, fungicide, and combination treatments were highly significant (*P=0.0004*). Percentage emergence of nontreated seeds was 35% and five of the seven treatments resulted in emergence ≥ 75% [2.6% sodium hypochlorite (75%), 1.3% sodium hypochlorite and RTU® (75%), 1.3% sodium hypochlorite and RTU® (76%), 2.6% sodium hypochlorite and RTU®-PCNB (81%), and 2.6% sodium hypochlorite and RTU® (83%)] with negligible effects on seedling growth. There were significant treatment differences regarding some of the variables used to evaluate seedling growth. In most cases these differences were due to seedlings from nontreated seeds having lower values for each measured variable than values for the same variables from treated seeds. Results of both experiments demonstrate the potential value of chemical seed treatment during product pf seedling transplant of *U. paniculata*. Seedling transplant of *Uniola paniculata* (southern seaoats) are in great demand for beach and sand dune restoration and stabilization. However, seed decay reduces germination and seedling emergence during production of transplant. Results herein demonstrate the importance of chemical seed treatment of the species and identify particular treatments that will inhibit decay and permit emergence ≥75% without adverse effects on initial seedling growth.

Note: In this study, the term chlorine bleach is analogous to Clorox containing 5.25% sodium hypochlorite.

V RESULTS

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested				Carrier Population (CFU/ carrier)
		Lot No. A8500514		Lot No. A8501309		
		Run 1	Run 2	Run 1	Run 2	
466708-06	*Streptococcus pneumoniae*					
	Test Date: 6/17/05	1°=0/60 2°=0/60	---	1°=0/60 2°=3/60	---	5.0×10^5
	Test Date: 7/14/05	---	---	1°=0/60 2°=0/60	1°=0/60 2°=0/60	1.65×10^6
466708-07	Vancomycin Resistant *Enterococcus faecalis*	1/60		1/60		7.6×10^5
466708-08	*Clostridium difficile*	1/60		0/60		6.1×10^6

MRID Number	Organism	Results			Dried Virus Control
			Lot No. A8500514	Lot No. A8501309	
466708-01	Avian Influenza A virus	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{5.25}$ $TCID_{50}$/0.1 ml
		$TCID_{50}$/0.1 ml	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
466708-02	Bovine viral diarrhea virus	10^{-1} to 10^{-4} dilutions	Complete inactivation	---	4.37983 log_{10}MPN
		log_{10}MPN	0.0	---	
466708-03	Bovine viral diarrhea virus	10^{-1} to 10^{-4} dilutions	Complete inactivation	Complete inactivation	4.37983 log_{10}MPN
		log_{10}MPN	0.0	0.0	
466708-04	Feline calicivirus	10^{-1} to 10^{-4} dilutions	Complete inactivation	---	>7.14187, 6.37983 log_{10}MPN
		log_{10}MPN	0.0	---	
466708-05	Feline calicivirus	10^{-1} to 10^{-4} dilutions	Complete inactivation	Complete inactivation	6.21133, 6.11244 log_{10}MPN
		log_{10}MPN	0.0	0.0	

VI CONCLUSIONS

1. The submitted efficacy data support the use of a **2400-ppm use-solution** of the product, **CPPC Ultra Bleach 2** (also known as F2001.0126 and Ultra Clorox Liquid Bleach), as a **disinfectant** 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 **contact time of 2 minutes**:

Clostridium difficile	MRID No. 466708-08
Streptococcus pneumoniae	MRID No. 466708-06
Vancomycin Resistant *Enterococcus faecalis*	MRID No. 466708-07

Killing was observed in the subcultures of at least 59 out of 60 carriers tested against the required number of product lots. [Note that initial testing of Lot No. A8501309 against *Streptococcus pneumoniae* showed unacceptable growth (i.e., growth in 3/60 subcultures); however, repeat testing of this lot in duplicate showed acceptable killing (i.e., growth in 0/60 subcultures.] Carrier population counts were at least 10^4. Neutralization confirmation/ neutralizer effectiveness testing showed positive growth of the microorganisms. The viability controls were positive for growth. When reported, purity controls were reported as pure. The sterility controls did not show growth.

2. The submitted efficacy data **support** the use of a **2400-ppm use solution** of the product, **CPPC Ultra Bleach 2** (also known as F2001.0126), as a **disinfectant with virucidal 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 **contact time of 5 minutes**:

Avian Influenza A virus	MRID No. 466708-01
Bovine viral diarrhea virus	MRID Nos. 466708-02 and -03
Feline calicivirus	MRID Nos. 466708-04 and -05

Recoverable virus titers of at least 10^4 were achieved. Complete inactivation (no growth) was indicated in all dilutions tested. Cytotoxicity was not observed. Confirmatory studies against Bovine viral diarrhea virus and Feline calicivirus were performed under different study directors and provided similar results with apparent product efficacy. The confirmatory studies used one lot of product not the standard two.

3. *According to the submitted research paper, the use-dilutions of 1.3% and 2.6%* sodium hypochlorite at 15 minutes were effective in reducing seed decay. As hypothesized initially, the 2.6% use-dilution was far more effective in attenuating seed decay. The observed effectiveness did not necessarily correspond to increased germination (75%).

VII RECOMMENDATIONS

1. The proposed label claims are acceptable regarding the product, CPPC Ultra Bleach 2, as a disinfectant on hard, non-porous surfaces against the following microorganisms at a 2/3 cup per gallon dilution (i.e., 2400 ppm available chlorine) for the contact times listed:

Clostridium difficile	2 minutes
Streptococcus pneumoniae	2 minutes
Vancomycin Resistant *Enterococcus faecalis*	2 minutes
Avian Influenza A virus	5 minutes
Human Hepatitis C virus (as bovine viral diarrhea virus)	5 minutes
Norwalk virus (as Feline calicivirus)	5 minutes

The label does not specifically claim that the product is effective as a disinfectant in the presence of light to moderate soil loads. However, because label directions do not specify a pre-cleaning step (only a pre-wash-with-water step); it is presumed that product efficacy has previously been demonstrated in the presence of light to moderate soil loads. Product efficacy against the microorganisms listed above was demonstrated in the presence of a 5% serum load and, for this reason, data provided by the applicant support these new claims.

2. The proposed label claim is acceptable regarding the product, CPPC Ultra Bleach 2, as a disinfectant on Southern seaoat seeds(*Uniola paniculata*) against mitigating plant disease-causing bacteria and fungi at a solution of 1 part product to 1.28 parts water (26,250 available chlorine) for 15 minutes. Carl Grable, PhD (Agency Botanist), agreed that this label claim is supported by the submitted research, and this use concentration will not pose any deleterious environmental issues.

3. In a letter to the applicant (dated September 16, 2005 and accompanying the last accepted label), the Agency requested that the following be deleted from the front panel of the label: the word "CORROSIVE" and the "first aid" statement. Please remove these items as instructed.

4. The label now includes information regarding NSF registration of the product [see page 2 of the proposed label]. The label identifies the NSF registration number as 122438. According to the NSF webpage (www.nsf.org), NSF Reg. No. 122438 is assigned to the product, CPPC Commercial Solutions Ultra Clorox Germicidal Bleach I, which means that this product has met the requirements of NSF registration. Based on information from the Clorox Professional Product Division webpage (http://www.cloroxprofessional.com/products/ucg_bleach.shtml), the product, CPPC Commercial Solutions Ultra Clorox Germicidal Bleach I, is the product, CPPC Ultra Bleach 2, which is the subject of this efficacy report. Label information regarding NSF registration appears to be accurate.

5. The directions for disinfecting [see page 4 of the proposed label] state to wash, wipe, or rinse surfaces with water prior to applying the use solution. In accordance with DIS/TSS-15, the directions should also include a statement to remove gross filth/heavy soil.

Note: Directions for disinfecting applications in the Service Bulletin section of the label generally (but not always) include a statement to remove gross filth.

6. The label indicates that the product may be used on ceramic and porcelain surfaces [see page 4 of the proposed label]. These surfaces are porous. The applicant must remove all general references to ceramic and porcelain from the label. The applicant may indicate on the product label that the product may be used on glazed ceramic and glazed porcelain surfaces.

7. The applicant may want to make the following revision to the label, as appropriate:

- On page 5, change "patio furniture" to read "patio furniture (except cushions and woodframes)."