UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION,
PESTICIDES
AND TOXIC
SUBSTANCES

September 3, 2009

MEMORANDUM

Subject: Protocol Review for Dispatch Hospital Cleaner Disinfectant Towels with Bleach (EPA Reg. No. 56392-8); DP Barcode: 368150

From: Tajah L. Blackburn, Ph.D., Microbiologist
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)
9/3/09

Thru: Michele Wingfield, Chief
Product Science Branch
Antimicrobials Division (7510P)

To: Emily Mitchell PM 32/ Wanda Henson
Regulatory Management Branch II
Antimicrobials Division (7510P)

Applicant: Caltech Industries, Inc.
4520 E. Ashman Road, Suite C
Midland, MI 48642

Formulation from the Label:

Active Ingredient(s)	% by wt.
Sodium Hypochlorite	0.52%
Other Ingredients	99.48%
Total	100.00%

12	Add *Neutralization Control for Expressed Liquid*	See Discussion in Cover Letter
13	Identify plating media and number of carriers for *Carrier Population Control*	Added specific media under Test Method (page 3 of the protocol). Added number of carriers used in *Carrier Population Control* (page 5 of the protocol).
14	Add incubation duration/temperature for neutralization	Added incubation duration/temperature in *Neutralization Confirmation Control* (page 5 of the protocol).
15	The *Verification of Spore Cells on Test Carriers* is required.	Added criteria for *Verification of Spore Cells* (page 5 of the protocol).
16	Insert an acceptance criterion for the under Test Criteria	Added criteria for *HCl Resistance Control* (page 5 of the protocol).
17	Add number of lots required/age under Test Criteria	Added to *Test Criteria* (page 6 of the protocol).
Wetness Determination Protocol		
1	State number of carriers that wiped by a single towel	Inserted clarification in *12" x 12" Carrier Visual & Wetness Method* (page 6 of the protocol) that one wipe treats one 12" x 12" carrier
2	Provide a video of the wetness testing	Videography was added to *12" x 12" Carrier Visual & Wetness Method* (page 6 of the protocol)
3	Increase carrier size to 12" x 12"	The increased carrier size was added to *12" x 12" Carrier Visual & Wetness Method* (page 6 of the protocol)
4	Add a wetness observation of the efficacy test slides and provide an acceptance criterion	See Discussion in Cover letter
5	State number of towels, lots, wiping protocol, and criterion for *Weight Verification of Wetness*	Inserted clarification and criteria in *12" x 12" Carrier Visual & Wetness Method* (page 6 of the protocol).

The registrant's representative provided rebuttal for several issues for which the Agency requested revision. Briefly,

Efficacy & Wetness Protocol

Agency's Initial Request: In Carrier Population Control section, the proposed acceptance criterion was $1 \times 10^5 - 1 \times 10^6$ spores/carrier. For sporicide claims, the Agency's proposed minimum requirement for carrier population control was at least 1×10^6 CFU/carrier. This correction is required in the section Preparation of Test Organism, in the statement "the final suspension will be prepared to achieve at least 1×10^8 spores/ml which will result in at least 1×10^5 spores/carrier.

Registrant's rebuttal: The Memorandum requests that the study be conducted with dried carriers containing 1×10^6 spores/carrier. The protocol has not been changed to this level and remains at $1x\ 10^5$ to 1×10^6 spores/carrier. This stance is based upon EPA's interim guidance for qualitative methods, *Guidance for the Efficacy Evaluation of Products with Sporicidal Claims against Clostridium difficile*, which states:

As these claims are novel for this type of formulation, the required carrier count minimum ensures stringency within the test system.

Evaluation of Expressed Liquid

Agency's Initial Request: The registrant must include a test for residual expressed liquid (post exposure) from used towelette, with adequate neutralization. The expressed liquid step must include a timeframe (mins) for expression of the residual liquid from the used towelette for neutralization purposes. It is recommended that the registrant determine in advance the approximate volume expressed to determine the neutralization capacity of the 40 ml neutralizing medium.

Registrant's Rebuttal: Following treatment of the carriers, the Memorandum requests that the remaining liquid in the towelette be expressed and evaluated for survivors. This evaluation and related neutralization confirmation have not been included in the revised protocol.

The product label clearly instructs users to "discard the used towel" and "not to reuse soiled cloths." The used towel is part of the waste stream. Adding this to the method adds significant cost with no value added for the user or public health.

The AOAC Germicidal Spray Test (961.02) modified for towelettes per EPA DIS/TSS is a stringent test that simulates that use of a single wipe to treat numerous different surfaces. Each wipe tested is used to sequentially treat 10 carriers. Thus, if organisms were being transferred to the expressed liquid of the towel during use and re-deposited to a new surface, then the current serial manner of testing 10 sequential surfaces/carriers would simulate this re-deposition pattern and any survivors would arise as growth in the test. The current EPA approved test method evaluates the potential for re-deposition across 10 successive surfaces and thus demonstrates a products' effectiveness against the initial dried test organisms as well as re-deposited test organisms, thus it is not necessary to add an additional evaluation of the expressed liquid for this purpose.

We expect that the above reasons are part of the EPA's rationale for omitting expressed liquid testing for all other microorganism claims for towelettes. Caltech is in agreement with this EPA position and requests that EPA maintain this stance for all organisms, including *C. difficile*.

Agency's Final Comments: As the protocols and claims are novel, the Agency is requesting testing of the expressed liquid from the tested towelette. In the initial stages, the Agency reviews protocols, and subsequent data, with heighten scrutiny to ensure that efficacy is achieved within a stringent test system. Testing of the expressed liquid is a requirement for *C. difficile* towelette testing.

ATS has determined the higher 80°C temperature will inactivate surviving spores; however, the lower temperature will not have this deleterious effect.

Agency's Final Comments: This is acceptable.

II SYNOPSIS OF REVISED SPORICIDAL TOWELETTE PROTOCOL

Protocol was revised July 24, 2009

Title: Pre-Saturated Towelettes for Hard Surface Sporicidal Disinfection

Test Organism: *Clostridium difficile* (ATCC 700792)

Product Type: Towelette

Test Purpose: This assay is to determine the efficacy of a pre-saturated towelette as a hard surface sporicidal disinfectant. The test procedure is to simulate the way in which the product is intended to be used. Due to the inability to raise sufficiently high titer spore crop and dried carrier counts with *C. difficile* in the AOAC Sporicidal Test for the towelette form of the product, the AOAC Germicidal Spray Test modified for towelettes was selected to assess *C. difficile* sporicidal activity.

Test Principle: A film of spores dried on a surface of glass carriers is wiped with the towelette and exposed to the test substance for a specified contact time. After exposure, the glass carriers are transferred to vessels containing neutralizing subculture media and assayed for survivors. Appropriate viability, carrier population and neutralization confirmation controls are run. The current version of Standard Operating Procedure CGT-4240 reflects the methods which shall be used in this study.

Growth Medium: Brain Heart Infusion Broth

Subculture Medium:
Primary Test Carrier Subculture: Letheen Broth 0.5% Sodium Thiosulfate
Secondary Test Carrier Subculture: *C. difficile* Broth
Recovery Agar: Brucella Agar
Primary HCl Resistance Subculture: Modified Fluid Thioglycollate Medium
Secondary HCl Resistance Subculture : Modified Fluid Thioglycollate Medium

Carrier Preparation: Glass slides (1" x 3") will be placed in a vessel and sterilized. Individual sterile plastic Petri dishes will be empty. One sterile glass slide will be transferred into each of the Petri dishes.

Preparation of Spore Suspension: From stock, inoculate one 10 ml of BHI broth and incubate for > 24 hours at 35-37°C under anaerobic conditions. Following incubation, transfer ≥ 10µl aliquots (≥ one 4 mm i.d. loopful) of the broth culture to sufficient 10 ml tubes containing BHI broth and then incubated for 5-12 days at 35-37°C under anaerobic

subculture the Petri dish holding the carrier. For products containing concentrated acid, alkali, or other antimicrobial agents that are difficult to neutralize, carriers may be transferred into secondary subculture bottles containing 40 ml neutralizing broth ≥ 30 minutes after the first transfer.

Incubation and Observation: All subcultures bottles will be incubated for 21 days at 35-37°C under anaerobic conditions. All plates are incubated for 48±4 hours at 35-37°C under anaerobic conditions. If necessary, subcultures can be stored for up to 3 days at 2-8°C prior to examination.

If the subculture tubes do not demonstrate growth, they are heat shocked for 20 minutes at 50±2°C. The heat shocked tubes will be reincubated for 72±4 hours at 35-37°C under anaerobic conditions.

Following incubation, the subculture bottles and control plates will be visually examined for growth.

Representative subculture bottles demonstrating growth (positive bottles) will be subcultured onto appropriate agar for confirmation of the test organism.

Test Controls

Purity Control: A "streak plate for isolation" will be performed on the organism culture on Brucella agar and incubated for 48±4 hours at 35-37°C under anaerobic conditions. Following incubation, the plates will be examined in order to confirm the presence of a pure culture. The acceptance criterion for this study control is a pure culture demonstrating colony morphology typical of the test organism.

Initial Suspension Population Control: The prepared suspension will be appropriately serially diluted in Butterfield's Buffer and 0.1 ml aliquots of the 10^{-4}, 10^{-5}, and 10^{-6} dilutions will be plated on Brucella agar and incubated for 48±4 hours at 35-37°C under anaerobic conditions. Following incubation, the organism plates will be observed to enumerate the concentration of the test organism inoculated onto the test carriers. There is no acceptance criterion for this study control.

Carrier Sterility Control: A representative uninoculated carrier will be added to the secondary carrier subculture medium. The subculture medium containing the carrier will be incubated for 21 days at 35-37°C under anaerobic conditions and examined for growth. The acceptance criterion for this study control is lack of growth.

Neutralizing Subculture Medium Sterility Control: A representative sample of uninoculated primary and secondary subculture medium will be incubated for 21 days at 35-37°C under anaerobic conditions and visually examined. The acceptance criterion for this study control is lack of growth.

Viability Control: A representative inoculated carrier will be added to the secondary carrier subculture medium and Modified Fluid Thioglycollate Medium.

Test Criteria

Test Substance Performance Criteria: The EPA efficacy performance requirements for label claims state that the disinfectant must kill the test organism on 60 out of the 60 inoculated carriers. EPA requires that 3 lots (one of which is at least 60 days old) must be tested to support a *C. difficile* sporicidal claim.

Control Performance Criteria:

The study controls must perform according to the criteria detailed in the study controls description section. If any of the control acceptance criteria are not met, the test may be repeated under the current protocol number.

Sporicidal Efficacy Data Analysis

Carrier Population (spore/carrier):

$$\frac{\text{(Average number of colonies/plate @ dilution) x dilution factor x volume neutralizer}}{\text{(Number of carriers tested) x (volume plated in ml)}}$$

Initial Suspension Population Control Calculation

Initial Suspension Population, spore/ml=

$$\frac{\text{(average number colonies/plate @dilution) x (dilution factor)}}{\text{(volume plated)}}$$

Spore Purity

$$\text{Spore Purity} = 100\% \times \frac{\text{Average number spores/field}}{\text{Averaged number of vegetative cells/field + Average number of spores/field}}$$

IV SYNOPSIS OF WENTESS DETERMINATION PROTOCOL

Title: 12" x 12" Carrier Visual & Weight Verification of Wetness Method

Product Type: Towelette

Preparation of Test Surface Tiles: The test carriers will be composed of new Formica (Black Matte Finish, e.g. style 90-58) coupons of a 12 inch by 12 inch size.

WETNESS DETERMINATION CRITERIA

Test Substance Performance Criteria

The EPA requires evidence of the surface wetting coverage over the required contact time. The surface must be wet based on visual and weight verification.

Wetness Determination Calculation

Remaining Test Substance Weight=
Final Wet Test Surface Weight – Initial Dry Test Surface Weight

V AGENCY'S CONCLUSIONS AND RECOMMENDATIONS

1. The Agency's final comments provided in response to the registrant's rebuttals must be addressed (pages 3-5).

2. The Agency recommends at least 90% spores for spore purity. The registrant proposed 60%. Is the registrant's proposal the result of preliminary data?