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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION,
PESTICIDES
AND TOXIC
SUBSTANCES

September 1, 2009

MEMORANDUM

Subject: Efficacy Review for EPA Reg. No. 1677-226, Virasept
DP Barcode: 368441

From: Tajah L. Blackburn, Ph.D., Microbiologist
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Product Science Branch
Antimicrobials Division (7510P)

Thru: Michele Wingfield, Chief
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To: Marshall Swindell PM 33/ Martha Terry
Regulatory Management Branch I
Antimicrobials Division (7510P)

Applicant: Ecolab, Inc.
370 Wabasha St
St. Paul, MN 55102

Formulation from the Label:

<u>Active Ingredient(s)</u>	<u>% by wt.</u>
Hydrogen Peroxide.....	3.130%
Octanoic Acid.....	0.099%
Peroxyacetic Acid.....	0.050%
<u>Other Ingredients</u>	<u>96.721 %</u>
Total.....	100.000 %

I BACKGROUND

The product, Virasept (EPA Reg. No. 1677-226), is a registered one-step disinfectant (bactericide, virucide, and fungicide), tuberculocide, and deodorizer for use in hospitals, nursing homes, institutions, schools, beverage/food processing plants, and daycare centers. The submitted efficacy data is to support addition of *Clostridium difficile* sporicide claims. Efficacy data was generated at Ecolab Research Center located at 655 Lone Oak Drive in Eagan, MN 55121.

The submitted data package contained a letter from the registrant (dated July 29, 2009), Statement of No Data Confidentiality Claims, one efficacy study (MRID No. 478186-01), and the proposed label.

II USE DIRECTIONS

The product is designed to disinfect floors, walls, tables, chairs, countertops, bed rails, bathroom fixtures, sinks, shelves, racks, carts, and the exterior of refrigerators composed of glazed tiles, linoleum, vinyl, glazed porcelain, plastics, metal, stainless steel, chrome, glass, and painted surfaces. Directions on the proposed label provided the following instructions for the preparation and use of the product as a disinfectant against *Clostridium difficile* spores:

Apply Virasept with a coarse trigger sprayer, cloth, mop, brush, scrubber, or by soaking to thoroughly wet surface. Virasept is effective against *Clostridium difficile* endospores after a 10 minute exposure time.

III AGENCY STANDARD FOR PROPOSED CLAIM

Sporicidal Disinfectant against *Clostridium difficile*

The Agency has established interim guidance for the efficacy evaluation of antimicrobial products (e.g., dilutable products, ready-to-use products, spray products, towelettes) that are labeled for use to treat hard, non-porous surfaces in healthcare settings contaminated with spores of *Clostridium difficile*. The effectiveness of such a product must be substantiated by data derived from one of the following four test methods: Most recent version (2006) of AOAC Method 966.04: AOAC Sporidical Activity of Disinfectants Test, Method I for *Clostridium sporogenes*; AOAC Method 2008.05: Quantitative Three Step Method (Efficacy of Liquid Sporicides Against Spores of *Bacillus subtilis* on a Hard Nonporous Surface); ASTM E 2414-05: Standard Test Method for Quantitative Sporidical Three Step Method (TSM) to Determine Efficacy of Liquids, Liquid Sprays, and Vapor or Gases on Contaminated Carrier Surfaces; or ASTM E 2197-02: Standard Quantitative Carrier Test Method to Evaluate the Bactericidal, Fungicidal, Mycobactericidal, and Sporidical Potencies of Liquid Chemical Germicides. Modifications to each test method will be necessary to specifically accommodate spores of *Clostridium difficile*. Because *Clostridium difficile* is an obligate anaerobe, testing should ensure adequate incubation conditions for the recovery of viable spores. The following toxigenic strains of *Clostridium difficile* may be used for testing: ATCC 700792, ATCC 43598, or ATCC 43599. All products must carry a pre-cleaning step, thus no organic soil should be added to the spore inoculum. Results must

show a minimum 6 log reduction of viable spores in 10 minutes or less. Control carrier counts must be greater than 10^6 spores/carrier.

IV SYNOPSIS OF SUBMITTED EFFICACY STUDY

1. MRID No. 478186-01, “Virasept Quantitative Sporicidal Three-Step Method Against *Clostridium difficile*” by Laurinda Holen. Study Completion date—July 28, 2009. Study Identification Number 0900021.

The product was tested against *Clostridium difficile* (ATCC 700792) in accordance to a modified version of AOAC 2008.05, Efficacy of Liquid Sporicides against Spores of *Bacillus subtilis* on Hard, Non-porous surface (Quantitative Three Step Method). Using two lots (Lot Nos. AT062409A-1, AT062409B-1) of Virasept, use solution of 88.2-89.1% were prepared, to render 425 ppm peroxyacetic acid, 900 octanoic acid, and 2.85% hydrogen peroxide. The test system was inoculated into four 1 L bottles of Liver Broth and incubated anaerobically at $35\pm 2^\circ\text{C}$ for 7-10 days. Following incubation, it was verified that the preparations consisted of > 95% spores as determined by observation with phase contrast microscope. Four bottles of culture were stirred using a sterile pipette to resuspend any spores that may have settled. The entire 4L were then filtered through sterile cheesecloth. Eight 50 ml centrifuge tubes were filled with 30 ml of the filtered suspension. The tubes were centrifuged at $7500 \times g$ for 20 minutes at $20 \pm 2^\circ\text{C}$. The supernatant was disposed of by pouring off and the centrifuge tubes were filled with another 30 ml of the filtered suspension. The tubes were again centrifuged at $7500 \times g$ for 20 minutes at $20\pm 2^\circ\text{C}$. The process was repeated until the entire 4 L had been centrifuged. After the last of the suspension had been centrifuged, the eight pellets were each resuspended in 5 ml of sterile Milli-Q water and combined in one tube. After centrifuging, the supernatant was disposed of by pouring off and the pellet was washed 4 times by centrifuging at $7500 \times g$ for 20 minutes at $20\pm 2^\circ\text{C}$ and resuspending in 30 ml sterile cold Milli-Q water. The final pellet was resuspended in 40 ml sterile cold Milli-Q water and stored at 2 to 8°C for less than 2 months. Prior to using for any testing, the spores were enumerated by serial dilution and plated. The spore suspension was diluted to achieve the desired count of 10^8 to 10^9 spores/ml. Carriers were inoculated with 10 μl of the spore suspension to the center of the carrier. The carriers were allowed to dry for a minimum of 1 hour in an open Petri dish, followed by a 12 hours in a dessicator at room temperature. After preparation of test substance, approximately 1.5 ml of each test substance was dispensed in microcentrifuge tubes and placed in a water bath at $20\pm 2^\circ\text{C}$. The tubes were allowed to equilibrate for 12 minutes. Three carriers were tested per test substance batch. Each inoculated carrier was transferred to a microcentrifuge tube labeled Fraction A using forceps. The Fraction A tubes were placed in a water bath at $20\pm 2^\circ\text{C}$. To each Fraction A tube, 400 μl test substance was added at 1 minute intervals. Following the 10 minute exposure period, 600 μl ice-cold Luria-Bertani (LB) broth + 0.5% sodium thiosulfate (media was kept on ice throughout testing) was added to each Fraction A tube at 1 minute intervals. After agitating the tubes to thoroughly mix the components, each carrier was transferred to corresponding Fraction B tubes. Fraction B tubes were microcentrifuge tubes that contained 400 μl ice-cold sterile water. Fraction A tubes were centrifuged for 6 minutes ± 30 seconds at $16,000 \times g$. The supernatant was removed and discarded without disturbing the pellet and 900 μl LB broth was added. The tube was again centrifuged for 6 minutes ± 30 seconds at $16,000 \times g$. This was repeated for a total of three centrifugations. Following the third centrifugation, the pellet was resuspended in 100 μl

ice-cold LB broth by pipetting up and down repeatedly. The entire 100 µl was plated on BHI agar with 5% sheep blood by spread plating. Fraction B tubes were sonicated for 5 minutes ±30 seconds using a floating microcentrifuge tube holder placed inside an ultrasonic cleaner. After sonication, 600 µl ice-cold LB broth was added to each Fraction B tube and tubes were vortexed for approximately 1 minute. Each carrier was then transferred using forceps from Fraction B tubes to corresponding microcentrifuge tubes labeled as Fraction C. Fraction C tubes contain 400 µl ice-cold LB broth. After removing the carriers, the Fraction B tubes were centrifuged for 6 minutes ± 30 seconds at 16,000 x g. The supernatant was removed and the pellet resuspended in 100 µl ice-cold LB broth. The entire 100 µl was spread plated on BHI blood agar plates. Fraction C tubes were placed on an orbital shaker at approximately 140 rpm inside an incubator for 30 ± 2 minutes at 35 ± 2°C. After incubation, Fraction C were vortexed for approximately 1 minute. The entire 400 µl was spread plated on BHI blood agar plates. All plates were incubated at 35±2°C for 72±4 hours under anaerobic conditions. The identity of a minimum of one representative colony taken from at least one plate per test substance was confirmed by Gram stain. The total number of spores per carrier was calculated by adding the total number of viable spores per fraction for A, B, and C. The log density was then calculated by taking the log₁₀ of the total number of spores per carrier. The log reduction of test carriers was determined by subtracting the log density of test carriers from log density of control carriers. The LR was calculated for each test substance batch.

Note: Protocol deviations/amendments were documented.

V RESULTS

Carrier Numbers Control

Carrier Enumeration (CFU/Plate)										
Carrier	Fraction A			Fraction B			Fraction C			
	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	
1	235	6	2	TNTC	110	24	108	14	3	
2	138	3	2	TNTC	112	11	137	16	3	
3	88	15	2	TNTC	101	17	128	23	6	
Total Carrier Count			Carrier 1 = 1.3 x 10 ⁷			Log Density			Carrier 1 = 7.11	
			Carrier 2 = 1.3 x 10 ⁷						Carrier 2 = 7.11	
			Carrier 3 = 1.1 x 10 ⁷						Carrier 3 = 7.04	
Average Total Carrier Count				1.2 x 10 ⁷						
Average Log density				7.09						

Batch Identification (AT062409A-1)

Test System	Carrier	Tube Fraction	CFU/Plate on 10 ⁰	Total Carrier Count	Log density
<i>C. difficile</i> (ATCC 700792)	1	A	0	0	0.00
		B	0		
		C	0		
	2	A	0	2	0.30
		B	1*		
		C	1*		
	3	A	0	0	0.00
		B	0		
		C	0		
Average Total Carrier Count = < 1					
Average Log Density = 0.10					
Log Reduction = 6.99					

*Colonies Gram stained to confirm growth was that of the test system.

Batch Identification (AT062409B-1)

Test System	Carrier	Tube Fraction	CFU/Plate on 10 ⁰	Total Carrier Count	Log density
<i>C. difficile</i> (ATCC 700792)	1	A	0	1	0.00
		B	1*		
		C	0		
	2	A	0	7	0.84
		B	6*		
		C	1		
	3	A	0	1	0.00
		B	1*		
		C	0		
Average Total Carrier Count = 3					
Average Log Density = 0.28					
Log Reduction = 6.81					

*Colonies Gram stained to confirm growth was that of the test system.

VI CONCLUSIONS

1. The submitted efficacy study (MRID No. 478186-01) is acceptable regarding the use of the product, Virasept, as a disinfectant with claims against *Clostridium difficile* spores on hard, non-porous surfaces for a contact time of 10 minutes. Carrier counts were greater than 10⁶ spores/carrier. Appropriate controls were included in the test system. Neutralization confirmation testing met the acceptance criterion. Purity controls were reported as pure. Sterility controls did not show growth. Acid resistance controls showed growth after 2 minutes of exposure.

VII RECOMMENDATIONS

1. The proposed label is acceptable regarding the use of the product, Virasept, as a disinfectant with claims against *Clostridium difficile* spores on hard, non-porous surfaces for a contact time of 10 minutes. These claims are acceptable as they are supported by the submitted data. Appropriate Special Label Instructions for Cleaning Prior to Disinfection against *Clostridium difficile* spores were included on the proposed label.