UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

September 29, 2016

Nicole Listner
Associate Regulatory Specialist II
Ecolab Inc.
370 N. Wabasha Street
St. Paul, MN
55102

Subject: Protocol Review – Efficacy protocol for sporicidal of a laundry additive
EPA Protocol Identifier: 1677-193
Application Date: June 10, 2016
Decision Number: 518432

Dear Ms. Listner:

The protocol submission referred to above, submitted in connection with registration under the Federal Insecticide, Fungicide, and Rodenticide Act, FIFRA, as amended, has been reviewed. Please see the attached review dated 09/28/2016.

Please note that the Agency's review of this protocol is considered complete. Any future submissions related to this protocol must be submitted under the appropriate PRIA category.

If you have any questions, please contact Zebora Johnson by phone at (703) 308-7080, or via email at johnson.zebora@epa.gov.

Sincerely,

Julie Chao, Product Manager Team (33)
Regulatory Management Branch 1
Antimicrobials Division (7510P)
Office of Pesticide Programs

Enclosure: Efficacy Protocol Review

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

September 28, 2016

MEMORANDUM

Subject: Protocol Review for Advacare 120 Sanitizer/Sour; EPA Reg. No. 1677-193; DB Barcode: D434653.

From: Ibrahim Laniyan, Ph.D.
Microbiologist
Product Science Branch
Antimicrobials Division (7510P)

Thru: Mark Perry, Team Leader
Product Science Branch
Antimicrobials Division (7510P)

To: Julie Chao PM33 / Zebora Johnson
Regulatory Management Branch I
Antimicrobials Division (7510P)

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

Formulation from the Label:

Active Ingredients	% by wt.
Hydrogen Peroxide	11.2 %
Peroxyacetic acid	15.2 %
Inert Ingredients:	73.6 %
Total	100.0 %

I. BACKGROUND

Ecolab, Inc. intends to assess the disinfectant efficacy of a laundry additive against spores of *Clostridium difficile* (*C. diff*). The registrant is proposing a protocol (MRID # 49945501) that is a combination of the following three methods:

1. ASTM E2406-09 Standard Test Method for Evaluation of Laundry Sanitizers & Disinfectants for Use in High Efficiency Washing Operations

2. ASTM E2839-11 Production of Clostridium difficile Spores for Use in Efficacy Evaluations of Antimicrobial Agents (for preparation of the test system)

3. US EPA Office of Pesticide Programs MB-31-03 Standard Operating Procedure for Quantitative Disk Carrier Test Method (QCT-2) Modified for Testing Antimicrobial Products Against Spores of Clostridium difficile (ATCC43598) on Inanimate, Hard, Non-porous Surfaces (for quantitative measurement of spore reduction, enumeration of control and treated samples)

II. BRIEF DESCRIPTION OF THE PROTOCOL

Title: STANDARD OPERATING PROCEDURE FOR MEASURING THE SPOROCIDAL EFFICACY OF A LAUNDRY ADDITIVE AGAINST SPORES OF *CLOSTRIDIUM DIFFICILE* ON FABRIC

Purpose: To determine the sporicidal efficacy of laundry additives against spores of *Clostridium difficile*.

Test System (Microorganism): *Clostridium difficile* (ATCC 43598), a toxigenic strain (tcdA-, tcdB+), obtained from ATCC or another reputable vendor.

Procedure:

Test system preparation:

- Test System – *Clostridium difficile* ATCC 43598
- Prepare spore crops of *C. difficile* according to ASTM E2839. The spore titer may be adjusted, as appropriate, to ensure adequate recovery of the test system. Applicable dilutions will be prepared in identical spore suspension medium (e.g. PBS + 0.1% Tween 80).
- If an organic soil load is used in the test, add the organic soil load to the organism suspension. Prepare the organism suspension/soil load mixture as follows: 340 µL organism suspension, 25 µL of BSA stock, 100 µL of mucin stock, 35 µL of yeast extract stock. Equivalent volumes of this mixture may be used as needed.

Scouring treatment of fabric:

- If not already treated, the following scouring procedure may be used on the fabric carriers and test fabric.
- Boil 300 g or less of fabric with 1.5 g of sodium carbonate and 1.5 g of a non-ionic wetting agent (such as Triton X-100) in 3 L of water for about one hour. An equivalent dilution or ratio may be prepared.

- Rinse fabric with boiling water and then cold water until all visible traces of the wetting agent (e.g. foam) are removed. Dry for a minimum of 24 hours at ambient temperature.
- After fabric is completely dry, verify the test fabric is within the weight range 15 ± 0.1g.
- Wrap weighed test fabric around spindle and fasten the loose end with a pin or other suitable method to prevent unraveling. A pocket between the 6th and 7th fold may be created by stapling along one horizontal side. Autoclave to sterilize.
- Place cut, scoured

Hard water preparation: If the product possesses a hard water claim, prepare the test substance dilution in synthetic hard water.

Test substance preparation: Prepare test substance dilution according to label. If the test substance requires dilution, use ≥ 1.0 mL or ≥ 1.0 g of test substance to prepare the use solution

Operating technique:

- Prepare six sterile carriers (1" × 1.5") for each test substance to be tested and place in a sterile petri dish. Inoculate test swatches with 0.01 mL to 0.03 mL of the prepared test system avoiding the marker, staple or pin.
- Three sterile carriers are similarly prepared as numbers controls.
- Dry the inoculated carriers inside a petri dish (lid off) in a biosafety cabinet for 30 ± 5 mins. After the inoculum has dried, place the petri dish in a desiccator connected to a vacuum line. Remove the petri dish lid. Vacuum dry the carriers at 20 – 25 in. mercury for 2 hrs.
- At the end of the drying period, remove the petri dishes from the vacuum desiccators and replace the lids.
- Carriers may be dried overnight in a desiccator without vacuum for use the next day. Use dried carriers within 24 hrs of inoculation.
- Using sterile forceps, insert three fabric carriers between the sixth and seventh folds of the fabric wound spindle (e.g. two from one side and the third from the other side). Place the spindle into the exposure chamber. Two canisters will be needed for each test substance.
- The test substance is added at the desired fabric to water ratio and product concentration. This is the start of the exposure time.
    1. A 1:5 fabric to product ratio is suggested for industrial laundry applications.
    2. A 1:10 fabric to product ratio is suggested for household laundry applications.
    3. If justified, then alternate ratios may be used.
- The exposure chamber is secured in the laundrometer within one minute and agitated at desired exposure temperature.
- Approximately one minute prior to the end of the exposure time, the chamber(s) are removed from the laundrometer.
- Following the desired exposure time, remove each of the test carriers with sterile forceps and place each one separately into 10 mL of the appropriate neutralization medium. Vortex well for 30 ± 5 seconds at high speed to recover the inoculum. Glass beads incorporated in the recovery medium may be used to assist with test system recovery.
- Filter the contents of the tubes containing neutralized treated carriers through a 0.2 µm pore size membrane.
- Remove each of the 3 control carriers with sterile forceps and place each one separately into 10 mL of diluent. Vortex well for 30 ± 5 seconds at high speed to recover the inoculum. Glass beads incorporated in the recovery medium may be used to assist with test system recovery.
- Make serial dilutions of the neutralizer containing control carriers. Filter the contents of the 10^{-3}, 10^{-4}, and 10^{-5} dilution tubes. The diluent used shall contain 0.1% Tween 80 to ensure spores are evenly distributed in the medium (e,g, PBS + 0.1% Tween 80).

- It may be necessary to squeeze the wash water from the fabric spindle, using a sterile glove, prior to neutralizing the wash water. Add concentrated neutralizer (e.g. 25 mL of 2 % sodium thiosulfate) to the wash water and mix well.
- Filter the contents of the treated canisters (wash water + neutralizer) a 0.2 µm pore size membrane.
- Make serial dilutions of the wash water in control canisters. Filter the contents of the 10^{-2}, 10^{-3}, and 10^{-4} dilution tubes.
- Aseptically remove each membrane filter and place on a BHI-HT plate and incubate under anaerobic conditions at 35 ± 2°C for 120 ± 4 h.

Performance Standard: A claim of sporicidal efficacy of *C. difficile* on fabric in a laundry process may be added to an EPA-registered antimicrobial pesticide if the following is true:

A LR of 6 $\log_{10}$/carrier of viable spores on fabric and no recovery of viable spores/canister in wash water has been obtained compared to the control.

Example claims are listed below:

- Disinfects *Clostridium difficile* on fabric in the laundering process
- Kills 99.9999% of *Clostridium difficile* on fabric in the laundering process
- Kills a minimum 99.9999% of *Clostridium difficile* on fabric in the laundering process

Method References:

1. ASTM E2839-11 Production of *Clostridium difficile* Spores for Use in Efficacy Evaluations of Antimicrobial Agents

2. ASTM E2406-09 Standard Test Method for Evaluation of Laundry Sanitizers & Disinfectants for Use in High Efficiency Washing Operations

3. ASTM E2274-09 Standard Test Method for Evaluation of Laundry Sanitizers & Disinfectants

4. ASTM E2197-11: Standard Quantitative Disk Carrier Test Method for Determining Bacterial, Virucidal, Fungicidal, Mycobactericidal & Sporicidal Activities of Chemicals

5. US EPA Office of Pesticide Programs MB-31-03 Standard Operating Procedure for Quantitative Disk Carrier Test Method (QCT-2) Modified for Testing Antimicrobial Products Against Spores of *Clostridium difficile* (ATCC43598) on Inanimate, Hard, Non-porous Surfaces

6. Guidance for the Efficacy Evaluation of Products with Sporicidal Claims Against *Clostridium difficile,* US EPA, June 2014

III. CONCLUSION AND COMMENTS

1. The submitted protocol (MRID 499455-01) **<u>is not adequate</u>** for assessing Advacare 120 Sanitizer/Sour (EPA Reg. No. 1677-193) efficacy as a laundry additive against spores of *Clostridium difficile* (*C. diff*). **Revision to include the following recommendations is necessary**:

2. Three batches diluted to the LCL must be tested in triplicates per microorganism (*c. diff*) on three different test days.

3. For each exposure chamber (canister), the method calls for "at least 3 fabric carriers and 1 fabric wrapped spindle for each active test formulation/product and control/numbers control". At step 9.1 of the proposed protocol, prepare 9 carriers and 3 fabric wrapped spindles for each batch to be tested.

4. As a reminder, internal/parallel/number controls must be used to determine log reduction. The required internal controls (at least 3: triplicate) must also contain fabric wrapped spindles. The Number control must be washed using 500ppm hard water and, just like tests, swatches and wash waters must be neutralized, diluted, filtered and plated in duplicate (modifying step 9.12 of the proposed protocol).

5. The test substance must be added at the fabric to water or product ratio of **2.5:1 or less** for all applications (see step 9.7 of the proposed protocol).

6. Hard water preparation: Product must possess a hard water claim, and the test substance must be diluted in 500 ppm synthetic hard water.

7. It is recommended that a rinse step (PBS rinse) be added after filtrations (steps 9.11, 9.13, 9.15, 9.16, 10.1, and 11.5).