UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

October 19, 2001

MEMORANDUM:

Subject: Protocol Efficacy Review of Test Method for "Efficacy of Antimicrobial Agents to Reduce Foodborne Pathogenic Bacteria in Fruit and Vegetable Processing Waters"
Product Name, "Oxy-15," EPA Reg. No. 1677-164
DP Barcodes: D278312
Case No.: 046821

From: Emily Mitchell, M.S., Team Leader Emily Mitchell 10/24/01
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510C)

Through: Michele E. Wingfield, Chief
Product Science Branch
Antimicrobials Division (7510C)

To: Laura Morris-Bailey, Special Assistant
Antimicrobials Division (7510C)

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

Formulation:

Active Ingredient(s)	% by wt
Peroxyacetic acid	15.0%
Hydrogen peroxide	11.0%
Inert Ingredients	74.0%
Total	100.0%

BACKGROUND:

The registrant, Ecolab, Inc. submitted a revised protocol,"Efficacy of Antimicrobial Agents to Reduce Foodborne Pathogenic Bacteria in Fruit and Vegetable Processing Waters" to the Agency dated September 28, 2000. The authors of the protocol are Heidi M. Hanson and John D. Hilgren, Ecolab Research Center, Sibley Memorial Highway, St. Paul, MN 55118. The product, "Oxy-15," EPA Registration No. 1677-164, contains peroxyacetic acid and hydrogen peroxide as active ingredients. This is a water additive for reducing microbial contamination on post-harvest, fresh cut and processed fruit and vegetable surfaces and in fruit and vegetable process waters. The label list the following claims: (1) Oxy-15 significantly reduces the numbers of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella javiana* suspended in fruit and vegetable processing water and on the surface of fruits and vegetables. (2) Oxy-15 significantly reduces the numbers of these pathogenic bacteria on fruit and vegetable surfaces that can occur through cross-contamination. (3) Oxy-15 also provides control of decay (and/or spoilage) causing microorganisms present in process waters and on the surface of post-harvest, fresh cut processed fruits and vegetables. Examples of process water are flumes, chill tanks and wash water systems. This is a secondary review based on the recommendations from an expert panel and Agency microbiologists.

AGENCY STANDARDS:

The Agency does not have set guidelines and/or a performance standard for products of this type. Since this is a new area for the Agency, the protocol was submitted to an expert panel for review. The panel was provided with the protocol, a label, a summary of a meeting between the Agency and registrant, a copy of a presentation made by the registrant dated August 30, 2000, and correspondences from the registrant to the Agency dated October 10 and October 17, 2000. Performance standard per SAP comments is probably 99%, a 2 log reduction.

SUMMARY OF PROTOCOL:

The bacteria used for this test method include three strains of *Listeria monocytogenes* (ATCC 49594, ATCC 19114, & ATCC 19116), three strains of *Escherichia coli* serotype O157:H7 (ATCC 43895, ATCC 35150, & ATCC 43890), and three strains of *Salmonella choleraesuis* subsp. *choleraesuis* (serotype *javiana* ATCC 10721, serotype *newport* ATCC 6962, and serotype *typhimurium* ATCC 13311).

1. Choose an agent that inactivates (neutralizes, quenches) the bactericidal properties of the antimicrobial agent but does not exert an inhibitory effect on the bacteria being tested.
2. From a stock culture, make >3 but <30 consecutive daily transfers on agar slants with incubation at 37±2°C.
3. Inoculate French slants
   a. From a 99 mL bottle of buffered peptone water (BPW), transfer 5 mL onto an agar slant containing growth of the test organism. Mix or shake the slant to suspend the growth in the BPW. Transfer the organism suspension back into the bottle of BPW. Mix the suspension well and add 2 mL to each French slant.

Tilt the slant back and forth so the 2 mL wets the entire agar slant. Remove excess liquid aseptically.

b. Incubate slants in a horizontal position at 37±2°C for 24±2 hours.

4. Harvest the culture from the slant by adding 3mL of BPW, 10-20 sterile glass beads and shaking back forth. Collect the resulting cell suspension and beads in a test tube and Vortex mix for 30 seconds. Filter the suspension through a sterile Buchner funnel containing Whatman No. 2 filter paper that has been pre-wet with 1 mL of BPW. Collect the suspension in a sterile container.
5. Combine equal volumes of each strain of *L. monocytogenes* in a sterile container. Do the same for *S. choleraesuis* and *E. coli* 0157:H7.
6. Adjust the density of the suspension to yield approximately 10^{10} organisms/mL. The percent transmittance reading needed to achieve a 10^{10} organisms/mL culture suspension must be determined prior to performing this test.
7. Dispense 99mL of the antimicrobial agent working solution into a sterile 250 mL Erlenmeyer flask. Prepare triplicate flasks for each test organism. Also prepare triplicate flasks with 99 mL of sterile BPW for determination of inoculum numbers. Place flasks into a 25°C water bath and let rest until they reach 25±2°C.
8. Vigorously swirl the test flask. While the liquid is still in motion, immerse the tip of a pipet containing 1 mL of organism suspension into the test solution midway between the center and edge of the flask. Dispense 1 mL of organism suspension into 99 mL of the test solution.
9. After a 2 minute exposure period, transfer 1 mL of the test solution/organism suspension mixture into 9 mL of inactivating agent using a sterile pipet and Vortex mix. This tube is considered a 10^{-1} dilution of the test solution.
10. For antimicrobial agent working solution test samples, plate in duplicate 1 mL and 0.1 mL from the inactivating agent tube. For the inoculum number tests, prepare 10^{-5} and 10^{-7} dilutions in BPW. Plate in duplicate 1 mL and 0.1 mL from the 10^{-5} dilution and 1 mL of the 10^{-7} dilution. Use pour plate technique with molten, tempered Trypticase Soy agar. After agar solidifies in the petri plates, invert them and incubate at 37±2°C for 48±2 hours.
11. Controls-Triplicate inactivation checks should be performed using each test organism suspension. Plate 1 mL and 0.1 mL from each control. Use pour plate technique with molten, tempered Trypticase Soy agar (or Brain Heart Infusion agar for *L. monocytogenes*.)
12. The inactivating agent effectively neutralized the working solution if the average plate count from Control C is approximately equal to Control A. The inactivating agent was not detrimental to the culture suspension if the average plate count from Control C approximately equal to Control B.

USE DIRECTIONS:

"Oxy-15" an antimicrobial water additive is intended for use in the control of microorganisms (or bacteria) which may cause decay (and/or spoilage) and that may be present in the water and on the surface of fresh-cut and post-harvest fruits and vegetable, as well as processed fruits and vegetables. Oxy-15 significantly reduces the numbers of *E. coli* 0157:H7, *L. monocytogenes*, *S. javiana* suspended in fruit and vegetable processing water and on the surface of fruits and vegetables. Oxy-15 also significantly reduces the numbers of these

pathogenic bacteria on fruit and vegetable surfaces that can occur through cross-contamination. Mix Oxy-15 with water either batchwise or continuously to no more than 533 ppm (wt/wt) (80 ppm residual peroxyacetic acid) in use solution. This can be accomplished by initially adding 53.3 grams (47.8 mls) Oxy-15 per 100 liters of water or 1.0 fluid ounces Oxy-15 per 16.4 gallons of water. The fruits and vegetables can be sprayed or submerged in the resulting solution, followed by adequate draining. Minimum contact times of a 2 minutes continuous spray application and 1 minute for submersion are recommended.

COMMENTS ON THE PROPOSED PROTOCOL(S):

The proposed testing protocol is aimed at determining the efficacy of a sanitizer on common pathogenic microorganisms in water used in the handling of raw produce. The data thus far presented to the Agency by the applicant suggests that the product is efficacious under certain selected test conditions. However, additional changes are required to the protocol in order to provide evidence of efficacy of the product.

1. Inactivating Agent - The agent used to inactivate the bactericidal properties of the anti-microbial agent should be specified rather than leaving it to the analyst to choose.

2. Tempered Agar - When plating the antimicrobial agent working solution, the temperature of the Trypticase Soy Agar or the Brain Heart Infusion should be specified in the protocol.

3. "Recycled Water" - The proposed use of "recycled water" was mentioned in the protocol. However, the registrant is required to generate data from a challenged water including a soil load. The proposed test method does not mention any soil loading to reflect that which will almost certainly be present in such water.

4. Dilution Water - Water used in the dilutions of the antimicrobial agent should be reflective of what is used in the field, not sterilized deionized water as stated in the proposed protocol. It is highly unlikely that such water is used in the field for processing raw fruits and vegetables. Any water under field conditions will have a certain number of dissolved chemicals in it and this should be reflected in the proposed test protocol by incorporating the use of water with a specified and clearly stated level of hardness. Many standard test methods require the use of water with 200-400 ppm hardness of $CaCO_3$.

5. Reducing Populations - The end-user should not imply that reducing populations of pathogens in the water will also significantly reduce populations on the product. This rather definitive statement should be changed. When referring to populations, replace the term "numbers" with a more precise concentration or population. This avoids confusion with strain numbers.

OTHER RECOMMENDATIONS AND LABELING COMMENTS:

1. The stated test organism concentrations 10^{10} are likely much higher than would be encountered in all but the dirtiest processing water. However, this concentration is reasonable for testing efficacy in that it allows the analyst to determine log or percent reductions in populations.

2. The two minute contact time would be adequate assuming that the end user would actually be expected to employ this time. If there appears to be any chance that the end user would employ a shorter time, then perhaps a shorted time should be tested and evaluated.

3. The title of the protocol should be changed to more accurately reflect its proposed application. The change would put emphasis on the process water and avoid any potential confusion with any existing methods for washing of raw fruits and vegetables.

CONCLUSIONS:

The proposed test protocol provides a basic sound scientific approach to the evaluation of the registrant's product. However, there are several specific details that require additional refinement listed above. These details above should be discussed with the Agency and worked out before the proposed method is used to generate sufficient data to demonstrate the product's effectiveness and reproducibility.