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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

March 4, 2002

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: Sanova Base, EPA Reg. No. 45631-20
DP Barcode: D278048

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Applicant: Alcide Corporation
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Formulation:

Active Ingredient(s)	% by wt
Sanova Base - Sodium Chlorite	31.00

I BACKGROUND

The applicant, Alcide Corporation, submitted a protocol, "Efficacy of Antimicrobial Agents to Reduce Foodborne Pathogenic Bacteria in Fruit and Vegetable Processing Waters" to the Agency on September 10, 2001. This protocol is intended to support the registration of their product, Sanova Base, as an antimicrobial agent to be used in the wash water or as a high pressure spray in a food processing facility to reduce *Escherichia coli* 0157:H7, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Shigella dysenteriae* on raw fruits, vegetables,

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seeds intended for sprouting and sprouts. The product is currently registered for use against microorganisms which may cause spoilage on fresh produce.

Included with this protocol are two data packages, MRID number 454954-01 "*Sanitizing efficacy of Acidified Sodium Chlorite Solutions on Raw Agricultural Commodities*" and MRID number 454954-02 "*Compilation of Published and Unpublished Studies: Efficacy of Acidified Sodium Chlorite Solutions on Raw Agricultural Commodities.*" The protocol and data were reviewed by the Agency's external review panel and Antimicrobials Division microbiologists.

II USE DIRECTIONS

Sanova Base: For use in the generation of chlorous acid in a food processing facility to eliminate the growth of microorganisms that cause spoilage on fruits, vegetables, seeds intended for sprouting, sprouts, red meat, and poultry. This product effectively reduces populations of *E. coli* 0157:H7, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Shigella dysenteriae* on raw fruits, vegetables, seeds intended for sprouting, and sprouts.

III 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, and the preliminary data generated with the proposed protocol. The September 1997 FIFRA Scientific Advisory Panel recommended a 99% (2 $\log_{10}$ reduction) for antimicrobial washes used in consumer household settings. However, since the microbial load in a commercial setting is likely to be greater than in a household, a 99.9% (3 $\log_{10}$ reduction) may be more appropriate for this use.

IV SUMMARY OF THE PROTOCOL

Experimental Protocols - Developed by Pure Produce, Inc., 1 Innovation Drive, Worcester, MA 01605.

The objective of this protocol was to test the efficacy of acidified sodium chlorite (ASC), at 500 ppm, as a dip application, or at 1200 ppm as a spray application, on a variety of produce items versus similar treatment with a 100-ppm chlorine solution or deionized water.

1. The produce were dipped in the microbial inoculum bath for a period of time ranging from 30 seconds to 22 hours, depending on the produce being tested.

2. The inoculated produce were then treated with the ASC or 100 ppm chlorine solution for a range of treatment times from 5 to 30 seconds. The water treatment contact time was 30 seconds. Depending on the type of produce tested, the method of application was either as a dip, high pressure spray, or hand-held spray.

3. Following treatment, the produce were subjected to a potable water rinse, employing the same application type used for the treatment, to remove any residual treatment solution.

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4. The produce were transferred to a Whirl-Pak bag with either 100 or 300 mL of Dey-Engley neutralizing solution, where the bacteria were extracted by shaking for 20 minutes.

5. Serial dilutions were made from the neutralizing solutions and plated on 3M Petrifilms. The incubation conditions (time and temperature) were not provided.

6. The produce were weighed prior to discarding.

V COMMENTS ON THE PROPOSED PROTOCOL

The proposed testing protocol is aimed at determining the efficacy of ASC in reducing foodborne pathogens when applied as a dip, high pressure spray, or hand-held spray on fruits and vegetables. The proposed protocol may be an acceptable approach for determining the efficacy of a product to reduce pathogens on a variety of produce, however, several modifications need to be made to the protocol before acceptable data can be generated and submitted for registration purposes.

1. Test Microorganisms - At a minimum, five outbreak related strains of *Escherichia coli* 0157:H7, *Listeria monocytogenes*, and *Salmonella species* are to be included in the testing protocol. Cocktails of the individual microorganisms may be used for this purpose. Complete information on the growth and characterization and maintenance of the test microorganisms must be provided. Additional foodborne related pathogens must be tested if these claims are listed on the label. The target initial microbial concentration must be stated.

2. Produce Selection - A variety of produce representing smooth, leafy, and complex surfaces should be tested. The condition of the produce (e.g., maturity, damage, presence of wax) must be documented. For residential uses, testing must be done on waxed and unwaxed produce. Background micro flora from the uninoculated produce should also be determined and documented. Testing should be conducted using the whole produce not disk segments, since the five 15 mm pieces of sample are probably too small.

3. Produce Inoculation - A complete description of the method used to inoculate the produce must be standardized and provided in the protocol. The concentration of the inoculum, inoculation contact time, inoculated fruit drying time and temperature conditions for the inoculation of the produce must be specified. The inoculum should be allowed to dry on the produce to allow for adequate attachment and to simulate use conditions. Since the performance standard is a 99.9% reduction over that achieved with water alone, the initial dried inoculum must take into account the reduction which would be achieved by the water alone. This may require an initial carrier count of 10^5 cfu/g.

4. Organic Material - Since bacterial adhesion and reduction may be influenced by the presence of organic material, and to simulate use conditions, an appropriate organic soil load should be incorporated into the protocol design. This is usually achieved through

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the addition of a 5% serum load to the inoculum which is dried on the produce.

5. Application Method - The method of product application should reflect what is being recommended on the label. If the dip application in a commercial setting requires the product to be diluted in the wash water, the type of water used must be specified in the protocol. Since water under field conditions will have a certain number of dissolved chemicals in it, water with a clearly stated level of hardness (e.g., 200, 400 ppm as $CaCO_3$) should be used. All application rates should have a specified contact time. The type of high pressure sprayer and the pressure used should be described in the protocol.

6. Water Rinse - This step may adversely bias the outcome of the study. If the potable water rinse contained any residual chlorine, it could have inactivated a certain portion of the test organisms and viable organisms could have been washed off during the potable water rinse. This step should be removed, however, if it is retained in the protocol, distilled water should be used and the rinse water tested to account for any wash off of viable organisms.

7. Neutralization Step - Proper controls to demonstrate the neutralization of the product following treatment must be specified in the protocol. The method used for removing surviving microorganisms from the produce was poorly explained and may underestimate the surviving population. The ratio of sample size to diluent volume needs to be standardized. An option could be to equate the weight of the sample with the weight or volume of the neutralizing solution.

8. Shaking Step - The shaking step needs to be standardized. If a mechanical shaker is used, the brand name of the shaker and the rpm used should be provided in the protocol. Recovery may also be enhanced by using sonication or homogenization of excised skin tissue. If either of these removal techniques are incorporated into the protocol, they must be fully described as well.

9. Microbial Enumeration - The microbial enumeration step must be fully described. The incubation temperature and time must be specified in the protocol. If the use of the 3M Petrifilm is not applicable for all microorganism types, then the appropriate plating medium must be listed in the protocol. Membrane filtration of the rinses and eluates to capture and enumerate as many viable organisms as possible in the sample fluids should be incorporated into the protocol.

10. Re-Use of the Product - Neither the protocol nor the label address the issue of re-use of the product. This is important for the dip application. Neither the protocol nor the label indicate whether a new batch of Sanova Base needs to be prepared for each batch of product or if several batches can be dipped into the same solution.

11. Number of Test Replicates - The applicant should consult with a statistician to determine the number of replicates of the individual produce items to be tested to ensure a statistically valid study.

12. Additional Microorganisms - The protocol may also be further modified to allow for

testing against fungi or viruses.

13. Method Validation - The final method must be validated either through an independent organization (such as AOAC International or ASTM) or through a peer verified method validation process conducted in two independent laboratories. The written method should provide sufficient detail such that a trained scientist is able to repeat the method without discussions with the authors. The laboratory personnel, including the study directors chosen to oversee the method validation, must be unfamiliar with the method, both in its development and in its subsequent use in determining product performance. Independent laboratory validations must be conducted un FIFRA Good Laboratory Practice (GLP) standards, as specified in 40 CFR Part 160. Consultation with the Agency is recommended prior to proceeding with the method validation of the protocol.

VI COMMENTS ON THE SUBMITTED DATA

MRID Numbers 454954-01 and 454954-02: These studies are deficient for the following reasons:

A. The studies do not meet the requirements of 40 CFR Part 160. The studies were not conducted under an approved protocol (see items 1 thru 12 above). There was not a separate Quality Assurance Unit for the testing laboratory. The study completion dates were not reported. A complete description of the laboratory conditions and apparatus used was not reported. A description of data transformations and statistical analyses was not provided.

B. The efficacy of the various concentrations of ASC were not directly compared on the same fruits or vegetables. Although the results indicate there is no significant difference in efficacy between the two concentrations when used on clean fruit, the use of clean fruit does not represent actual use conditions. The efficacy of the product must be tested in the presence of an organic soil load.

C. Actual plate count numbers achieved before and after the treatment must be reported. The $\log_{10}$ reduction is calculated over the water control.

D. Once the protocol is modified per the comments in section V, efficacy data may be generated to support the additional pathogen reduction claims on the two product labels.

VII OTHER RECOMMENDATIONS AND LABELING COMMENTS

1. The concentration of the product to be used to achieve the pathogen reduction is not stated on the label. The preliminary data suggests a 1200 ppm use since the product was not tested in the presence of an organic soil load.

2. Neither the contact time nor temperature are provided on the Sanova Base label.

3. The protocol was not used to evaluate the efficacy of ASC on seeds intended for sprouting or sprouts. These claims must be removed from the label.

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4. The submitted data does not support the use of ASC on red meat or poultry.

5. The label for Sanova Base states that the product is to be used in conjunction with the Sanova Activator and the Sanova Food Quality System. The technical bulletin should be reviewed by the Agency to determine whether the instructions are adequate for the use of the product.

VIII 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.