UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

May 14, 2007

MEMORANDUM

Subject: Efficacy Review for Sani-Step, EPA Reg. No. 1677-EEG; DP Barcode: D337447.

From: Ibrahim Laniyan, Microbiologist
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Thru: Tajah Blackburn, Acting Team Leader
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To: Velma Noble / Jacqueline Campbell-McFarlane
Regulatory Management Branch I
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Applicant: Ecolab Inc.
370 N. Wabasha Street
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Formulation from the Label:

Active Ingredient(s)	% by wt.
Akyl (50% C_{14}, 40% C_{12}, 10% C_{16}) dimethyl benzyl ammonium chloride	0.479 %
Octyl decyl dimethyl ammonium chloride	0.359 %
Didecyl dimethyl ammonium chloride	0.215 %
Dioctyl dimethyl ammonium chloride	0.144 %
Other Ingredients	98.803 %
Total	100.000 %

I. BACKGROUND

The product, Sani-Step (EPA File Symbol 1677-EEG), is a new product. The applicant requested to register the product as a non-food contact surface sanitizer for use on floors in dairy, food and beverage processing industries. Studies were conducted at Ecolab Schuman Campus, located at 655 Lone Oak Drive, Eagan, MN 55121.

This data package contained a transmittal document from the applicant to EPA (dated January 18, 2007), two studies (MRID Nos. 470389-09 and 470389-10), Statements of No Data Confidentiality Claims for both studies, and the proposed label. The registrant submitted two amendments to the two studies (dated May 10, 2007) to remove "Bardac 208M" from the final reports and replace with "Quat" for clarification (Bardac 208M diluted to lower certified limit of 1.017% active ingredient, 300 ppm quat). These amendments were attached to their respective studies.

II. USE DIRECTIONS

The product is designed to be used for sanitizing hard, non-porous surfaces such as floors. Directions on the proposed label provided the following information regarding use of the product as a sanitizer: After cleaning and sanitizing equipment, apply Sani-Step evenly to the floor, at a rate of 1.2 grams - 2.4 grams per square foot. Product may be re-applied as needed during the production process. Sani-Step does not become active until it comes in contact with moisture on the floor.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Sanitizer Test (for inanimate, non-food contact surfaces)
The effectiveness of sanitizers for non-food contact surfaces must be supported by data that show that the product will substantially reduce the numbers of test bacteria on a treated surface over those on an untreated control surface. The test surface(s) should represent the type(s) of surfaces recommended for treatment on the label, i.e., porous or non-porous. Products that are represented as "one-step sanitizers" should be tested with an appropriate organic soil load, such as 5 percent serum. Tests should be performed with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old against *Staphylococcus aureus* (ATCC 6538) and either *Klebsiella pneumoniae* (aberrant, ATCC 4352) or *Enterobacter aerogenes* (ATCC 13048 or 15038). Results must show a bacterial reduction of at least 99.9 percent over the parallel control within 5 minutes. These Agency standards are presented in DIS/TSS-10.

IV. BRIEF DESCRIPTION OF THE DATA

1. MRID 470389-09 "Non-Food Contact Surface Sanitizing Efficacy" for Sani-Step, by Laurinda Holen; Project number: 0600036. Study conducted at Ecolab. Study completed on December 28, 2006.

This study was conducted against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048). Three lots (Lot Nos. P03176A, P03176B, and

P05116A) of the product,Sani-Step (KX-6187), were tested. The laboratory report referenced the Sanitizer Test from DIS/TSS-10 and the Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-food Contact Surfaces (ASTM Method E1153). The product lot P03176B was at least 60 days old at the time of testing. 300ppm use solutions were prepared by adding ~24g of the product to ~976g of 500 ppm AOAC synthetic hard water (titrated at ~485 ppm). Fetal bovine serum was added to each inoculum to achieve a 5% organic soil load. Five sterile glass carriers per product lot per organism were inoculated with 0.02 ml of a 24±4 hour old suspension of the test organism. The inoculum was spread to within 1/8 inch of the edges of the carrier. The carriers were dried at 35±2°C for 30-40 minutes. Each carrier was transferred to a sterile jar and was exposed to the use solution at for 5 minutes. After exposure, 20 ml of neutralizer was added to each jar and the jars were rotated vigorously to suspend the surviving organisms. Following neutralization, 1.0 ml and 0.1 ml the solution were plated in duplicate on Tryptone Glucose Extract Agar. All plates were incubated for 48±4 hours at 35±2°C. Controls included those for carrier quantitation, inoculum count, viability, neutralization confirmation, sterility, and purity. The average log colony forming units (CFU) per Square, for each test microorganism, are as follows: ***Enterobacter aerogenes* 2.1 x 10^7, *Staphylococcus aureus* 6.55 x 10^6**.

Note: Protocol deviations and amendments reported in the study were reviewed and found to be acceptable.

Note: The applicant provided the data for one failed trial. In that trial, the use solution was prepared at 322 ppm quat while the lower certified limit is 300 ppm. Thus, the test was invalid. These data were not used to evaluate efficacy of the test product. See Appendix B of the laboratory report.

2. MRID 470389-10 "Supplemental Non-Food Contact Surface Sanitizing Efficacy" for Sani-Step, by Laurinda Holen; Project number: 0600037. Study conducted at Ecolab. Study completed on December 26, 2006.

This study was conducted against *Escherichia coli* (ATCC 11229), *Listeria monocytogenes* (ATCC 49594), and *Salmonella typhimurium*** (ATCC 13311). Two lots (Lot Nos. P03176B, and P05116A) of the product, Sani-Step (KX-6187), were tested. The laboratory report referenced the Sanitizer Test from DIS/TSS-10 and the Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-food Contact Surfaces (ASTM Method E1153). The product lot P03176B was at least 60 days old at the time of testing. 300ppm use solutions were prepared by adding ~24g of the product to ~976g of 500 ppm AOAC synthetic hard water (titrated at ~485 ppm). Fetal bovine serum was added to each inoculum to achieve a 5% organic soil load. Five sterile glass carriers per product lot per organism were inoculated with 0.02 ml of a 24±4 hour old suspension of the test organism. The inoculum was spread to within 1/8 inch of the edges of the carrier. The carriers were dried at 35±2°C for 31-37 minutes. Each carrier was transferred to a sterile jar and was exposed to the use solution at for 5 minutes. After exposure, 20 ml of neutralizer was added to each jar and the jars were rotated vigorously to suspend the surviving organisms. Following neutralization, 1.0 ml and 0.1 ml the solution were plated in duplicate. All plates were incubated for 48±4 hours at 35±2°C. Controls included those for carrier quantitation, inoculum count, viability, neutralization confirmation, sterility, and purity. The average log colony forming units (CFU) per Square, for each test microorganism, are as follows: ***Escherichia coli* 9.8 x 10^6, *Listeria monocytogenes* 8.1 x 10^7, and *Salmonella typhimurium*** 1.1 x 10^6.**

Note: Protocol deviations and amendments reported in the study were reviewed and found to be acceptable.

V. RESULTS

MRID #	Organisms	Average Number of Survivor			Initial Population	Log Reduction
		P03176A	P03176B	P05116A		
470389-09	*Staphylococcus aureus*	$< 2.7 \times 10^1$	$< 4.9 \times 10^1$	$< 2.5 \times 10^1$	$\sim 6.55 \times 10^6$	>99.9
	Enterobacter aerogenes	$< 3.9 \times 10^1$	$< 2.9 \times 10^1$	$< 1.2 \times 10^2$	$\sim 2.1 \times 10^7$	>99.9
470389-10	*Escherichia coli*		$< 2.5 \times 10^1$	$< 1.2 \times 10^1$	$\sim 9.8 \times 10^6$	>99.9
	Listeria monocytogenes		$< 2.5 \times 10^1$	$< 2.9 \times 10^2$	$\sim 8.1 \times 10^7$	>99.9
	*Salmonella typhimurium***		$< 9.0 \times 10^1$	$< 1.0 \times 10^1$	$\sim 1.1 \times 10^6$	>99.9

VI. CONCLUSIONS

1. The submitted efficacy data **support** the use of the product, Sani-Step, as a non-food contact sanitizer on floors when tested against *Enterobacter aerogenes, Staphylococcus aureus, Escherichia coli, Listeria monocytogenes,* and *Salmonella typhimurium*** 300 ppm active quat in the presence of 500 ppm hard water and 5% organic soil, for a contact time of 5 minutes.

VII. RECOMMENDATIONS

1. The proposed label claims that the product, Sani-Step, is an effective non-food contact surface sanitizer against *Enterobacter aerogenes, Staphylococcus aureus, Escherichia coli, Listeria monocytogenes,* and *Salmonella typhimurium***, **are supported** by the applicant's data.

2. The applicant must add ATCC numbers of the tested organisms to the master label.

****Please note**: The species name of this organism has been changed by ATCC. The new designation of this organism is *Salmonella **enterica***. This change is effective immediately, and should be used for all subsequent references to this organism in the future.