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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

August 21, 2003

MEMORANDUM

Subject: Efficacy Review for EPA Reg. No. 10466-27 / Ultra Fresh NM
DP Barcode: D289500

From: Ian Blackwell, Biologist
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510C)

Through: Emily Mitchell, Team Leader Emily Mitchell 9/23/03
Efficacy Evaluation Team
Product Science Branch
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To: Robert Brennis, PM 32 / Wanda Mitchell
Regulatory Management Branch I
Antimicrobials Division (7510C)

Applicant: Thomson Research Associates

Formulation From Label:

Active Ingredient(s)	% by wt
5-chloro-2-(2,4-dichlorophenoxy)phenol	3
Inert Ingredient(s)	97
Total	100

I **BACKGROUND:** Thomson Research Associates have submitted a set of antimicrobial efficacy studies to support the addition of labeling claims for their product. The applicant has requested an amendment to the registration of this product to include certain product performance information (regarding bacteriostatic effectiveness) against specific odor-causing microorganisms, specifically *Bacillus cereus*, *Bacillus mycoides*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus vulgaris*, as well as the fungus *Trichophyton mentagrophytes*. The product, "Ultra-Fresh NM" (EPA Reg. No. 10466-27), is an EPA-approved fabric conditioner with bacteriostatic effects for use in the manufacture of textiles. All studies were conducted at Thomson Research Associates, 95 King Street East, Toronto, Ontario, Canada M5C 1G4, although the study reports were apparently packaged by the applicant's agent, Laird's Regulatory Consultants, Inc. The primary review of these reports was conducted by DynCorp Systems & Solutions (DSS) LLC, a CSC Company. A secondary review was conducted by EET/PSB/AD.

This data package contained an EPA letter to the applicant (dated February 20, 2003), correspondence from the applicant to EPA (dated March 26, 2003), four studies (MRID Nos. 458977-01 through -04), Statements of No Data Confidentiality Claims for all four studies, the proposed Technical Data Sheet, and the last accepted label (dated January 17, 2002).

On January 17, 2002, EPA accepted the product label and Technical Data Sheet with conditions. EPA requested that the applicant delete, from the Technical Data Sheet, a table naming specific microorganisms. EPA indicated that "the organisms that have been listed are of a public health concern and cannot be disqualified by a denial of a public health claim." Subsequent to the January 17, 2002 letter, the applicant and EPA continued discussing this conditional acceptance. On February 20, 2003, EPA indicated to the applicant's agent that it was prepared to consider acceptance of the Technical Data Sheet with the list of certain human pathogens. EPA requested that the applicant provide the following:

- Data demonstrating the minimum inhibitory concentration (MIC) of the odor-causing organisms listed in the Technical Data Sheet for Ultra-Fresh NM; and
- Information regarding the odor production of organisms, which the applicant wished to list on the Technical Data Sheet as odor-causing.

At one time, this product was registered as a hard-surface disinfectant. However, in a 9/29/88 letter from the EPA, the registrant was told: "Your file record indicates that there are no data to support efficacy of this product as a hospital disinfectant and nonfood contact surface sanitizer for treating precleaned, hard, non-porous surfaces." A 3/9/95 APB/RD review stated that the registrant was deleting all claims to hard surface cleaner use.

II Use Directions

Product Label

Ultra-Fresh NM can be used to treat carpets, latexes, air filters, synthetic and cellulosic sponges, woven and non-woven general purpose cleaning clothes, pigment presscakes, dispersions, inks, and adhesives. Consult the Product Information Sheet for application parameters. Directions on the last accepted label provided the following information regarding preparation and use of the product as a bacteriostat: *For Manufacturing Use Only.* Use an application of **1-4%** (emphasis added) on weight of goods to inhibit the growth of odor-causing bacteria on textile surfaces.

Technical Data Sheet

The product is designed to be used for conferring bacteriostatic properties to cotton, wool and synthetic textiles fabrics such as fabrics used in apparel, domestics and household goods. Directions on the proposed Technical Data Sheet provided the following information regarding preparation and use of the product as a bacteriostat: *The product is to be applied during the manufacturing process.* An application of **2.0% to 5.0%** (emphasis added) based on fabric weight and durability requirements is recommended. It is recommended that all fabrics be scoured prior to treatment. The product can be applied from the pad bath, and liquor temperature has no effect on fabric pick-up of the product. Exhaustion should be done for 10-15 minutes at temperatures ranging from 100°F to 120°F (38°C to 49°C). Due to reduced substantivity, 100% polyester fibers need special application procedures to ensure durability. Please consult the corresponding Product Information Sheet for more details.

Note: The data package provided did not include the "Product Information Sheet" or a revised product label.

III Agency Standards for Proposed Claims

Antibacterial Finishes on Textile Materials - Bacteriostatic Activity

The effectiveness of antibacterial finishes on textile materials should be assessed based on the degree of antibacterial activity intended in the use of such materials. When only bacteriostatic activity (i.e., inhibition of multiplication) is intended, a qualitative procedure may be acceptable - a procedure that contrasts antibacterial activity of treated specimens with the lack of such activity by untreated specimens. Bacteriostatic claims are permitted only against

microorganisms identified as causing economic or aesthetic problems (e.g., odor-causing bacteria), and are not permitted for microorganisms of concern to public health. Bacteriostatic activity can be demonstrated by the diffusion of the antibacterial agent through agar using the "Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method," American Association of Textile Chemists and Colorists (AATCC) Method 147-1933. In this method, an agar surface is inoculated making it easier to distinguish between the test organism and contaminant organisms that may be present on the unsterilized specimen. Products may be tested against *Staphylococcus aureus*, *Klebsiella pneumoniae*, or any other species suitable for the intended end-use of the product. Testing and performance guidance for non-public health use antimicrobial agents is provided in Subseries 91B of Subdivision G. These standards are presented in Subdivision G guidelines, §91-4(e) and AATCC Method 147-1933.

Also the study may be conducted using the refer to AATCC Method 147, and, AOAC Official Method 972.04. These protocols report the conduct of bacteriostatic efficacy studies while utilizing test swatches of fabric.

Products Controlling Microorganisms of Economic or Aesthetic Significance

Algaecides, slimicides, preservatives, deodorizers, and other products expressly claiming control of microorganisms of economic or aesthetic significance not directly related to human health do not require efficacy data. However, adequate dosage recommendations and complete directions for use must be provided in labeling. These Agency standards are presented in DIS/TSS-16.

IV Comments on the Submitted Efficacy Studies

1 MRID 458977-01: "The Minimum Inhibitory Concentration Values of Ultra-Fresh DM-50, and NM-25 using *Salmonella choleraesuis*, *Proteus vulgaris* and *Shigella sonnei*," by D. Klein. Study conducted at Thomson Research Associates. Study completion date – March 31, 2003. Study Number 1904760.

This study was conducted against *Salmonella choleraesuis* (ATCC 10708), *Proteus vulgaris* (ATCC 25175), and *Shigella sonnei* (ATCC 29930) to determine the minimum inhibitory concentration (MIC). Two product formulations were tested, Ultra-Fresh DM-50 and Ultra-Fresh NM. (Testing of DM-50 will not be discussed in this review.) A working solution for each product was prepared: a 25 ppm dilution of Ultra-Fresh DM-50 and a 300 ppm dilution of Ultra-Fresh NM; lot numbers were not identified. For each product, 2 mL of the working solution was added to an empty test tube. To each of 7 remaining tubes, 1 mL of the nutrient broth was added. With a sterile pipette, 1 mL of the working solution was transferred to the first test

tube containing 1 mL of the nutrient broth. The contents of the tube were mixed thoroughly, creating the first dilution. Four more dilutions were performed by repeating this process, and a seventh tube with nutrient broth served as a control. Two sets of dilutions were prepared for each product. The cultures were incubated at 37°C for 24 hours and visually inspected for growth or no growth. The lowest concentration of product that resulted in complete inhibition of visible growth represents the MIC value.

Note: This report indicated that the study does not meet 40 CFR Part 160 requirements (Good Laboratory Practice Standards) because the "document does not contain the report of a study."

2 **MRID 458977-02: "The Minimum Inhibitory Concentration Values of Ultra-Fresh DM-50, NM and DM-25 using *Bacillus cereus, Brevibacterium epidermidis, Corynebacterium pseudodiptherium, Bacillius mycoides* and *Bacillus subtilis*," by D. Klein. Study conducted at Thomson Research Associates. Study Number 1904770. Study completion date – March 31, 2003.**

This study was conducted against *Bacillus cereus* (ATCC 11778), *Brevibacterium epidermidis* (ATCC 35514), *Corynebacterium pseudodiptherium* (ATCC 10700), *Bacillius mycoides* (ATCC 6462), and *Bacillus subtilis* (ATCC 6051) to determine the minimum inhibitory concentration (MIC). Three product formulations were tested, Ultra-Fresh DM-50, Ultra-Fresh NM, and Ultra-Fresh DM-25. A working solution for each product was prepared: a 25 ppm dilution of Ultra-Fresh DM-50, a 30 ppm dilution of Ultra-Fresh NM and a 25 ppm dilution of Ultra-Fresh DM-25; lot numbers were not identified. For each product, 2 mL of the working solution was added to an empty test tube. To each of 7 remaining tubes, 1 mL of the nutrient broth was added. With a sterile pipette, 1 mL of the working solution was transferred to the first test tube containing 1 mL of the nutrient broth. The contents of the tube were mixed thoroughly, creating the first dilution. Four more dilutions were performed by repeating this process, and a seventh tube with nutrient broth served as a control. Two sets of dilutions were prepared for each product. The cultures were incubated at 37°C for 24 hours and visually inspected for growth or no growth. The lowest concentration of product that resulted in complete inhibition of visible growth represents the MIC value.

Note: This report indicated that the study does not meet 40 CFR Part 160 requirements (Good Laboratory Practice Standards) because the "document does not contain the report of a study."

3 **MRID 458977-03: "The Minimum Inhibitory Concentration Values of Ultra-Fresh DM-50, 300DDN, NM, and DM-100, 40 and DM-25 using *Klebsiella pneumoniae* and *Escherichia coli*," by D. Klein. Study conducted at Thomson Research Associates. Study completion date – March 31, 2003. Study Number 1601310.**

This study was conducted against *Klebsiella pneumoniae* (ATCC 4352) and *Escherichia coli* (ATCC 8739) to determine the minimum inhibitory concentration (MIC). Six product formulations were tested, Ultra-Fresh DM-50, Ultra-Fresh 300DDN, Ultra-Fresh NM, Ultra-Fresh DM-100, Ultra-Fresh 40, and Ultra-Fresh DM-25. A working solution for each product was prepared: a 25 ppm dilution of Ultra-Fresh DM-50, a 10.6 ppm dilution of Ultra-Fresh 300DDN, a 30 ppm dilution of Ultra-Fresh NM, a 100 ppm dilution of Ultra-Fresh DM-100, a 40 ppm dilution of Ultra-Fresh 40 and a 250 ppm dilution of Ultra-Fresh DM-25; lot numbers were not identified. For each product, 2 mL of the working solution was added to an empty test tube. To each of 7 remaining tubes, 1 mL of the nutrient broth was added. With a sterile pipette, 1 mL of the working solution was transferred to the first test tube containing 1 mL of the nutrient broth. The contents of the tube were mixed thoroughly, creating the first dilution. Four more dilutions were performed by repeating this process, and a seventh tube with nutrient broth served as a control. Two sets of dilutions were prepared for each product. The cultures were incubated at 37°C for 24 hours and visually inspected for growth or no growth. The lowest concentration of product that resulted in complete inhibition of visible growth represents the MIC value.

Note: This report indicated that the study does not meet 40 CFR Part 160 requirements (Good Laboratory Practice Standards) because the "document does not contain the report of a study."

4 **MRID 458977-04 "The Minimum Inhibitory Concentration Values of Ultra-Fresh DM-50, 300DDN, NM, and DM-100, 40 and DM-25 using *Listeria monocytogenes, Listeria welshimeri, Enterococcus faecalis,* and Vancomycin Resistant *Enterococcus faecalis*," by D. Klein. Study conducted at Thomson Research Associates. Study completion date – March 31, 2003. Study Number 1618220.**

This study was conducted against *Listeria monocytogenes* (ATCC 7644), *Listeria welshimeri* (ATCC 43551) (MRSA), *Enterococcus faecalis* (ATCC 19433), and *Enterococcus faecalis*-VRE (ATCC 51299) to determine the minimum inhibitory concentration (MIC). Six product formulations were tested, Ultra-Fresh DM-50, Ultra-Fresh 300DDN, Ultra-Fresh NM, Ultra-Fresh DM-100, Ultra-Fresh 40, and Ultra-Fresh DM-25. A working solution for each product was prepared: a 25 ppm dilution of Ultra-Fresh DM-50, a 1.06 ppm dilution of Ultra-Fresh 300DDN, a 300 ppm dilution of Ultra-Fresh NM, a 10

ppm dilution of Ultra-Fresh DM-100, a 40 ppm dilution of Ultra-Fresh 40 and a 250 ppm dilution of Ultra-Fresh DM-25; lot numbers were not identified. For each product, 2 mL of the working solution was added to an empty test tube. To each of 7 remaining tubes, 1 mL of the nutrient broth was added. With a sterile pipette, 1 mL of the working solution was transferred to the first test tube containing 1 mL of the nutrient broth. The contents of the tube were mixed thoroughly, creating the first dilution. Four more dilutions were performed by repeating this process, and a seventh tube with nutrient broth served as a control. Two sets of dilutions were prepared for each product. The cultures were incubated at 37°C for 24 hours and visually inspected for growth or no growth. The lowest concentration of product that resulted in complete inhibition of visible growth represents the MIC value.

Note: This report indicated that the study does not meet 40 CFR Part 160 requirements (Good Laboratory Practice Standards) because the "document does not contain the report of a study."

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V Results

Table 1. From MRID Number 458977-01.

Concentration of *Ultra Fresh* NM	**Organisms Tested (positive or negative for growth)**		
ppm of active ingredient	***S. choleraesuis***	***P. vulgaris***	***S. sonnei***
0.293	No growth	No growth	No growth
0.147	No growth	No growth	No growth
0.073	No growth	No growth	No growth
0.037	Growth	No growth	Growth
0.018	Growth	Growth	Growth
0.009	Growth	Growth	Growth

Table 2. Minimum Inhibitory Concentrations of Ultra Fresh Products

MRID Number	**Organism Tested**	**MIC of Ultra-Fresh Products (in ppm)***		
		NM	**DM-50**	**DM-25**
458977-01	*Salmonella choleraesuis*	0.073	0.781	—
	Proteus vulgaris	0.037	0.391	—
	Shigella sonnei	0.073	0.391	—
458977-02	*Bacillus cereus*	0.117	0.391	6.250
	Brevibacterium epidermidis	0.469	0.391	6.250
	Corynebacterium pseudodiptherium	1.875	0.195	1.563
	Bacillus mycoides	0.938	0.391	6.250
	Bacillus subtilis	0.938	0.391	3.125

Table 3. Minimum Inhibitory Concentrations of Ultra Fresh NM in ppm

	Test Organism		
	S. choleraesuis	*P. vulgaris*	*S. sonnei*
Ultra Fresh NM	0.073	0.037	0.073

Table 4. Minimum Inhibitory Concentration of Ultra Fresh NM Against Various Species of Bacteria.

MRID Number	Organism Tested	MIC of Ultra-Fresh Products (in ppm)*					
		DM-50	300 DDN	NM	DM-100	40	DM-25
458977-03	*Klebsiella pneumoniae*	0.78	0.01	0.12	1.56	10.00	15.63
	Escherichia coli	6.25	0.04	0.06	0.78	10.00	125.00
458977-04	*Listeria monocytogenes*	0.39	0.13	2.34	1.25	10.00	7.81
	Listeria welshimeri	0.39	0.13	2.34	1.25	5.00	3.90
	Enterococcus faecalis	0.20	0.13	9.38**	2.50	>20.00	15.63
	Enterococcus faecalis-VRE	0.39	0.27	9.38**	2.50	20.00	15.63

* Values are based on the ppm of active ingredient rather than product.
** Values extracted from the table in the Results Section of the report

VI Conclusions

1 MRID Number 458977-01, 458977-02, 458977-03 and 458977-04: The applicant has not performed the particular studies that EPA describes in the Subdivision G guidelines (i.e., AATCC Method 147), rather the applicant has tested various products, including Ultra-Fresh NM, to determine minimum inhibitory concentration (MIC) values for a number of challenge organisms. AD/PSB feels that the testing facility *should* follow AATCC Method 147 for testing of bacteriostatic agents such as Ultra Fresh NM. However, in previous correspondence, the applicant was told this approach would be acceptable. It would have been best if the lab should had followed American Association of Textile Chemists and Colorists (AATCC) Method 147-1933, "Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method,", or, AOAC Official Method 972.04, "Bacteriostatic Activity of Laundry Additive Disinfectants" as a means of assessing the bacteriostatic efficacy of Ultra Fresh NM. The study and report had other problems. Problems with this study are as follows:

A The MIC data were not collected according to Good Laboratory Practices.
B The report does not state how the lab determined their colony counts.

C The report does not state that the lab used sterile solutions or sterile techniques.

D This report does not specifically state what types of media were used in this assay.

E The cover page of the report states "158.190". It is not clear what this means. The 40 CFR has a section 158.190; however, that section concerns physical and chemical characteristics of product ingredients. This would not pertain to this study.

F Concerning compliance with the 40 CFR, Part 160, the report states: "This document does not contain the report of a study and therefore does not fall under the requirements of 40 CFR, Part 160." Nonetheless, this document does give an account of a set of tests conducted on six different antimicrobial products. As such, this document is considered to contain the report of a study. If the registrant does not consider this document to report studies, then the registrant needs to more clearly inform the PM Team the reasons for the submission of such documents.

If additional studies are conducted in the future to demonstrate bacteriostatic / fungi properties of the treated textiles, the applicant must use protocol AATCC 147.

Additional Information, MRID Number 458977-04: As stated in number 1, above, this study was also not conducted in accordance with EPA guidelines. The registrant should refer to AATCC Method 147, and, AOAC Official Method 972.04. In addition to all of the issues posed above (1A - 1F), another problem with this study is that one test organism is identified as *Enterococcus faecalis*-VRE, but the lab did not submit any additional data to substantiate the vancomycin-resistance of the strain of test organisms. In addition to identifying the specific organism number and origin, scientific data must be submitted to verify the resistance results. When conducting antimicrobial efficacy studies, the testing facility must test each strain of antibiotic-resistant bacteria to prove that the strain has retained its antibiotic resistance. Data must include the following:

(1) Results of the testing, including the values for all antibiotics tested.
(2) The scientific method used to obtain the results (Kirby-Bauer agar-disk diffusion, automated MIC procedures, agar gradient diffusion). If automated procedures are used, the manufacturer of such automated procedures must be specified.
(3) Quality control procedures used to verify results.
(4) A clear link of the identity of the organisms used in the efficacy testing to those for which valid antibiotic susceptibility testing was performed.

VII Recommendations

1 The proposed Technical Data Sheet claims (as supported by MRID No. 458977-02) are acceptable regarding the use of the product, Ultra-Fresh NM, as a bacteriostatic finish on textile materials against the following, specifically-named organisms:

Bacillus cereus	MIC = 0.117 ppm active
Bacillius mycoides	MIC = 0.938 ppm active

The applicant provided MIC data for these odor-causing organisms, as requested. Although *Bacillus cereus* can cause food intoxication (the toxin forming in foods held at improper temperatures), that exposure route is unlikely to occur via treated textiles. *Bacillus mycoides* is not considered a human pathogen.

2 The proposed Technical Data Sheet claims (as supported by MRID Nos. 458977-01, -03, and -04) <u>are not acceptable</u> regarding the use of the product, Ultra-Fresh NM, as a bacteriostatic finish on textile materials against the following, specifically-named organisms:

Enterococcus faecalis
Escherichia coli
Klebsiella pneumoniae
Proteus vulgaris

Although the applicant provided MIC data as requested, the applicant failed to submit information to support its assertion that these four organisms produce odor. The applicant stated, in its letter dated March 26, 2003, that these four organisms "do have an unpleasant odor"; however, the applicant did not provide any additional information to support this statement. Furthermore, all four of these organisms are considered human pathogens. Prior to approving the Technical Data Sheet, the applicant must delete all references to these four organisms. The applicant may continue to claim that the product is a bacteriostat.

Note: The laboratory report inconsistently reports the MICs for *Enterococcus faecalis* and *Enterococcus faecalis*-VRE. Values of 9.38 ppm active are presented in the Results Section; values of 4.69 ppm are presented in the Conclusions Section of the laboratory report. The Technical Data Sheet reports a MIC value of 4.69 ppm (which could be incorrect) for *Enterococcus faecalis*.

3 The proposed Technical Data Sheet claims <u>are not acceptable</u> regarding the use of the product, Ultra-Fresh NM, as an antifungal finish on textile materials against the specifically named *Trichophyton mentagrophytes*. The applicant did not provide MIC data as requested. The applicant failed to submit information to support its assertion that *Trichophyton mentagrophytes* produces odor. Also, EPA considers *Trichophyton mentagrophytes* to be a human pathogen. Prior to the approval of the Technical Data Sheet, the applicant will have to delete all references to *Trichophyton mentagrophytes*.

4 The request to add claims of bacteriostatic efficacy against *Salmonella choleraesuis*, *Proteus vulgaris*, *Shigella sonnei*, *Bacillus cereus*, *Brevibacterium epidermidis*, *Corynebacterium pseudodiptherium*, *Bacillus mycoides*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Listeria welshimeri*, *Enterococcus faecalis*, *Enterococcus faecalis*-VRE, and, against the fungus *Trichophyton mentagrophytes* is denied.

5 Currently, the Technical Data Sheet directs the user to apply **2-5%** by weight of goods; whereas, the product label directs the user to apply **1-4%** by weight of goods. The applicant must revise the product label so that information on the label matches information on the Technical Data Sheet.

6 Prior to approving the Technical Data Sheet, the applicant must provide information and calculations demonstrating that the most conservative MIC provided in the study results for the product, Ultra-Fresh NM, (i.e., 9.38 ppm active ingredient) is no greater than the minimum effective concentration in the label's directions for use.