EEE BRANCH REVIEW

DATE: IN _____ OUT _____ IN _____ OUT _____ IN 3/6/78 OUT 6/2/78

FISH & WILDLIFE ENVIRONMENTAL CHEMISTRY EFFICACY

FILE OR REG. NO. 10466-EI

PETITION OR EXP. PERMIT NO. _____

DATE DIV. RECEIVED 11/8/77

DATE OF SUBMISSION 10/31/77

DATE SUBMISSION ACCEPTED _____

TYPE PRODUCTS(S): I, (D.) H, (F.) N, R, S Industrial Preservative

DATA ACCESSION NO(S). 232252

PRODUCT MGR. NO. 33 (Banks)

PRODUCT NAME(S) Steri-Chem DM-50N

COMPANY NAME Thomson Research Associates, Ltd.

SUBMISSION PURPOSE New application with data.

CHEMICAL & FORMULATION Liquid to be used as formulated.

Active Ingredient:

Tri-n-butyltin maleate.....25%

200.0 Introduction

200.1 Use(s)

The proposed label bears claims for use as a preservative for rendering fabrics resistant to the growth of mold and mildew, for both in-can preservation of latex paint and water based adhesives against bacteria and fungi and for protection of applied aqueous adhesive films against fungal attack. The inclusion of 0.1% will also render applied interior acrylic latex paint films resistant to fungal growth. When this product is blended with the plasticizer prior to plastic ingredient mixing fungistatic characteristics are conferred to vinyls. The label refers to the Product Data Sheet for specific directions for use.

The Product Data Sheet bears vague claims for use in rendering fabrics and water-based emulsions (latex emulsion paints, water-based adhesives and related aqueous emulsion systems) resistant to "microorganisms" or "biologically induced instability and degradation", and for manufacturing use in rendering alkyd paints, and epoxy, urethane, and vinyl coatings resistant to the growth of mildew.

The Product Data Sheet also contains a list of minimum inhibitory concentrations of the product against numerous bacteria and fungi.

200.2 Factors Affecting Amount/Type of Data Required

The only specific claims made in the labeling of this product as a preservative against bacterial deterioration which can be reviewed in terms of effectiveness data, are for in-can preservation of latex paint and water based adhesives.

Other vague and/or imprecise claims in the Product Data Sheet for rendering various materials resistant to "microorganisms", etc., cannot be evaluated in terms of efficacy and require clarification or deletion.

Claims of intrinsic broad spectrum antibacterial activity of the technical product, such as tables of minimum inhibitory concentration values, can be considered if the product is intended for formulating use and the intent and limitations of such claims are also indicated in the labeling. In this case, the claims can be evaluated on the basis of supporting data.

201.0 Data Summary

201.1.1 Brief Description of Tests

(A) In-Can Paint Preservative Test. Number 9-4. Reference 349-38. Dated 11/3/76. Report by Dr. W.G. Meathrel, Thomson Research Associates, Ltd., Toronto, Ontario, Canada.

(B) Bacteriostatic Action of Steri-Septic DM-50N Treated Fabric. Number 11-3. Reference 235-5A. Dated 4/24/75. Report by P.J. Radford, Thomson Research Associates, Ltd., Toronto, Ontario, Canada.

(C) Determining Minimum Inhibitory Levels of Steri-Septic DM-50N Germistat-II. Number 11-3. Reference 246-60. Dated 10/1/76. Report by P.J. Radford, Thomson Research Associates, Ltd., Toronto, Ontario, Canada.

201.1.2 Data Summaries

(A) In-Can Paint Preservative Test.

SAMPLE: 5 gallons of acrylic latex paint containing no preservative was obtained from Tonecraft Paints Limited, 10 Carson St., Toronto, Ontario.

TEST METHOD: "In-Can" Preservative - Various compounds, at a level of 0.05%, have been added to Tonecraft latex paint. The paint samples were inoculated with either Pseudomonas species or a mixed Bacilli species and incubated for 2-weeks at room temperature. After 2-weeks, the number of viable organisms was determined.

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TEST RESULTS: "In-Can" Preservatives (0.05%)

Inoculum: Pseudomonas: 6.0×10^5/gm. paint. Bacilli: 1.2×10^5/gm. paint.

Compound	Bacteria/gm. of paint after 2-weeks	
	Pseudomonas	Bacilli
Control	1.3×10^8	5.4×10^4
DM-40	$< 10^3$	$< 10^3$
DM-50	$< 10^3$	$< 10^3$
Dowicil 75	$< 10^6$	7.0×10^4
Amical 50	3.2×10^3	6.1×10^3
Phenyl mercuric propionate	$< 10^3$	$< 10^3$

(B) Bacteriostatic Action of Steri-Septic DM-50N Treated Fabric.

Samples: One piece of 4-oz. woven fabric, i.w.g. if Steri-Septic DM-50N.

An untreated piece of the same fabric was also included as a control.

Test Method:

Laundering

Both the treated and untreated fabrics were laundered (separately) a total of 50-times at 140°C with 0.2% "Tide" detergent. Test pieces were removed after 0, 5, 10, 20, 30, 40, and 50 washes, and bacteriologically evaluated as detailed below.

Bacteriological Evaluation (AATCC Method 90-1974)

38 mm. test discs were placed on the surface of Nutrient agar, in Petri dishes that had been seeded with either Staphylococcus aureus or Klebsiella pneumoniae. Following this, the dishes were incubated for a period of 24-hours at 37°C, and the growth patterns examined.

Five replicate test discs were used for each evaluation.

TEST RESULTS:

Sample	Test Organism	Replicate	Mm. Zone of Inhibition After Given Number of Washes						
			0	5	10	20	30	40	50
Treated	S. aureus	a	5	4	3	2	2	1	1
		b	6	3	3	2	2	1	1
		c	5	4	3	2	1	1	1
		d	6	5	3	2	2	2	1
		e	6	4	3	3	2	2	1
	K. pneumoniae	a	3	2	2	2	1	1	1
		b	4	2	2	1	1	1	1
		c	4	3	2	2	1	1	1
		d	4	3	3	2	2	1	1
		e	4	3	3	2	2	1	1
Control	S. aureua	a	0	0	0	0	0	0	0
		b	0	0	0	0	0	0	0
		c	0	0	0	0	0	0	0
		d	0	0	0	0	0	0	0
		e	0	0	0	0	0	0	0
	K. pneumoniae	a	0	0	0	0	0	0	0
		b	0	0	0	0	0	0	0
		c	0	0	0	0	0	0	0
		d	0	0	0	0	0	0	0
		e	0	0	0	0	0	0	0

(C) Determining Minimum Inhibitory Levels of Steri-Septic DM-50N Germistat-II.

Test Method: Bacteria

1. Dilution levels

Suitable dilutions of the Steri-Septic DM-50N were made in sterile distilled water. A final dilution containing 1-ml. of the water diluted Steri-Septic DM-50N in 9-ml. Nutrient broth culture medium in a sterile test tube was prepared in each case. Tests were initially made on a wide dilution range, 1,000-ppm. to 1-ppm. in 100-ppm. steps. When the approximate inhibition level had been determined against the specific organism being used, a further one or

two dilution series covering a narrower range were made, ending up with 1-ppm. steps in the concentration of Steri-Septic DM-50N to which the test organisms were exposed.

2. Preparation of Inoculum

Nutrient broth cultures were used with all the bacterial organisms. The test cultures for use as the inocula were prepared by transferring loop samples from stock cultures into 10-ml. aliquots of the Nutrient broth medium. These broth cultures were incubated at 37°C for 24-hours, then a loop sample of each transferred to a second 10-ml lot of broth, and incubated as above. This procedure was repeated for three consecutive days, by which time the various inocula were ready for use.

3. Exposure to Steri-Septic

For each test organism, a 0.03-ml. aliquot at a 1 in 50 dilution of the final broth culture was added to each 10-ml. lot of broth culture medium containing the various Steri-Septic DM-50N dilutions. The inoculated Steri-Septic DM-50N dilutions were then incubated at 37°C for 24-hours and examined for evidence of bacterial growth. The lowest concentration of Steri-Septic DM-50N that prevented bacterial growth in the broth culture was recorded for each organism.

Test Results:

Bacteria	Minimum Concentration (ppm.) Required for Complete Inhibition of Growth
A. Gram positive	
Bacillus cereus	10
Bacillus licheniformis	10
Bacillus megatherium	37
Bacillus mycoides	5
Bacillus subtilis	26
Brevibacterium ammoniagenes	8
clostridium Oroticum	150
Corynebacterium hobfanii	4
Sarcina lutea	10
Staphylococcus aureua	2
Staphylococcus (coagulase +)	33
B. Gram negative	
Azobacter vinelandii	275
Escherichia coli	334
Enterobacter aerogenes	722
Enterobacter cloaca	656
Klebsiella pneumoniae	24
Mima polymorpha	5
Proteus mirables	36
Proteus morganii	26
Pseudomonas aeruginosa	200
Pseudomonas fluorescens	1
Rhizobium leguminosarum	250
Salmonella schotmuelleri	620
Salmonella typimarium	30

202.0 Recommendations

202.1 Claims Supported by the Data Submitted

The submitted minimal inhibitory concentration tests are adequate to support a claim of intrinsic value for the product as a bacteriostat intended for formulating use only. The intent, in this case, must be indicated in the labeling of the product. There is no objection to inclusion of this data accompanied by appropriate qualifying statements in the Product Data Sheet.

This information must not be represented as having any relevance to recommended end uses, effective dosages, activity against specific microorganisms, or any other implications of effectiveness of formulated products for specific end uses.

202.2 Claims Not Supported by the Data Submitted

The submitted in-can paint preservative test does not support a claim for the product in-can preservation of latex paint and/or water based adhesives against bacterial deterioration.

The test is deficient with respect to the following:

(A) A detailed testing protocol was not provided;

(B) The test paint was not identified as to composition or whether it was an interior or exterior type of acrylic latex;

(C) Only one type of paint was tested. No water based adhesives were tested;

(D) The method of application of the product to the paint was not described nor whether the test dosage was by weight or by volume;

(E) The test bacteria were not identified as to source;

(F) It was not shown that the test bacteria caused deteriorative (physical and/or chemical) changes in the untreated control paint nor whether the treated paint was preserved from deteriorative effects, if any.

(G) It cannot be determined whether a neutralizer for the active ingredient(s) was included in bacteriological subculture media;

(H) The amount of replication, if any, cannot be determined from the test report;

(I) A two-week test is not adequate, in any case, to demonstrate in-can shelf life preservation.

202.3 Data Not Appropriate

The submitted test concerning bacteriostatic properties of treated fabric is not appropriate since no specific claims are made in labeling in this regard. In addition, the technique of demonstrating zones of inhibition around treated fabric swatches on seeded agar plates is not considered to be of any value in providing meaningful results than can be associated in-use conditions. Such qualitative data may be considered, however, as evidence of intrinsic value of the product as a bacteriostat for formulating use only (See 202.1 Claims Supported by the Data).

202.4 Additional Data Required to Support Claims

To support a claim for the product as a shelf life preservative to control bacterial deterioration of water based interior/exterior acrylic and/or polyvinyl acetate latex paint or specified water based adhesives, the following type of data must be submitted and found to be acceptable:

(A) Testing must be based upon an adequately controlled in-use or simulated-use design representing each specific type of substrate claimed and its usage for the duration of time and under the conditions anticipated for preservation of the material. For shelf life

preservatives, both test and control samples must be tested for a period of 6 months to 1 year.

(B) Documentation must be provided that the proposed test substrates are representative of an entire class of materials to be preserved, e.g. "latex paint", "water based adhesives". In lieu of supporting documentation for broad claims, label recommendations must be restricted to the specific types (chemical composition) of materials or formulations in which the product has been treated and found to be effective in the intended use pattern;

(C) Test bacteria must be those types and number that have been identified as the cause of deterioration (liquidization, putrefaction, discoloration, odor, etc.) in each specific substrate intended for treatment.

(D) A mixed culture inoculum of bacteria and fungi is not recommended. Although control of microbial deterioration of a given substrate may involve fungi as well as bacteria, fungal growth must be considered as a separate, though related, problem which should be tested separately;

(E) Specific identified (characterized as to chemical composition) substrates of each type claimed must be treated with the product according to the directions for use. Untreated control substrates must be included. Tests must be carried out in triplicate for the duration of time and in a manner which is realistic for the intended use pattern;

(F) Environmental conditions (temperature, relative humidity, etc.) employed in the study, relative to each substrate, must be reported and must be those encountered under actual conditions of use;

(G) If the substrate intended to be preserved (raw material, intermediate, finished product, or whatever) would be expected to be subjected to repeated bacterial contamination or other challenge (organic soil, aeration, heat, moisture, etc.) during the time preservation is intended, these repetitive challenges to the substrate must be incorporated into the study. The test should simulate the more severe conditions which are anticipated in the actual use situation during the entire period of intended preservation;

(H) Quantitative bacteriological assay techniques (plate counts) must be employed in the study. An appropriate neutralizer should be employed in subculture media and/or absence of bacteriostasis in subculture media must be demonstrated;

(I) The study must be designed to demonstrate inhibition of bacterial growth or metabolism in each treated substrate over that in the untreated control substrate. Data from each untreated control substrate must show that growth of the target bacterial pest(s) occurs and causes specific deterioration problems with that substrate. Data from each treated substrate must show that control of growth of the target bacterial pest(s) occurs, and development of the specific deterioration problem associated with that substrate is prevented. The development and type of deterioration must be described and documented in the study, for each substrate;

(J) You may wish to refer to the procedure "Evaluation of Latex Preservatives", F. Buono, W.J. Stewart, and M. Fairfield, Journal of Paint Technology, Vol. 45, N577, February, 1973, for the basic elements of testing and incorporate the necessary modifications indicated above.

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(K) Specific and complete details of all aspects of the study must be provided for review, including substrate preparation (test and control), inoculation/reinoculation scheduler inoculum levels and amounts, assay procedures, etc.

It is suggested that the requested information and revised protocols be submitted for review and comment prior to initiation of the tests.

(L) Clarified claims and adequate directions for use of the product as a preservative must be provided in labeling. In addition to the recommended dosage rate in terms of weight or volume (to be specified), the directions for use must indicate how, when, and where the product is to be applied, as well as the intended nature and duration of preservation for each type of substrate claimed.

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RCM 6/12/78

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June 2, 1978
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