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BIOCIDES TECHNICAL SERVICE REPORT

ATLAS PUTTY PRODUCTS TINLEY PARK IL

CRM # 11208

DATE: 4/12/2011



OBJECTIVE

To incorporate Nuosept 95, Nuosept 91 and Fungitrol 404D into two interior paints and evaluate for in-can preservation and dry film fungal resistance following ASTM D2574-94 and ASTM D3273

SAMPLE IDENTIFICATION

1. Gray paint w/0.20% N-91/0.20% F404D ref. 18026
2. Gray paint unprotected ref. 18041
3. White paint w/0.20% N-91/0.20% F404D ref.18029
4. White paint unprotected ref.18042

CONCLUSION

All samples prepared to contain ISP biocides provided adequate in-can preservation and resistance to dry film fungal defacement.

DISCUSSION

As indicated in Table I, the samples were initially evaluated for and found to be free of microbial contamination.

The results of the in-can evaluation presented in Table II are based on ASTM 2574-94. This procedure uses a direct streak of the inoculated sample onto Tryptic Soy Agar Plates. After a period of incubation, the streaked sample is then rated on a scale of "0" to "4" where a "0" rating would indicate that the sample is resistant to microbial spoilage in the container. A rating of "1" to "4" would indicate that the sample may not be adequately preserved against the test organisms ("4" being the worse failure). Samples are normally considered adequately preserved when a "0" rating is attained within 3 to 5 days after each of the two microbial challenges.

The results of the ASTM D3273 testing are presented in Table III. Samples were applied to Leneta drawdown paperboard and suspended in an environmental chamber for a period of four weeks. A growth rating of "10" corresponds to the complete absence of surface growth and a rating of "0" corresponds to complete coverage of the surface. A rating of "7" or higher generally indicates that the coating should be resistant to defacement under actual use conditions.



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EXPERIMENTAL

Sterility:

Each sample was initially checked for sterility by direct streak onto Tryptic Soy Agar (TSA, for bacteria), Yeast Malt Extract Agar (YM, for yeast and fungi), and Potato Dextrose Agar (PDA, adjusted to pH 3.5 for fungi and yeast) plates. The plates were incubated for 24-48 hours at 32°C for the detection of bacteria, and 3-7 days at 28°C for fungal and yeast contaminants. Absence of microbial growth along the streak indicated that the corresponding sample did not contain viable microbial cells. The presence of microbial growth indicated non-sterility (sample contained viable microbial contamination). The level and type of contamination found for each sample is reported in Table I.

In-Can Preservation:

The evaluation for adequacy of in-can preservation was performed by inoculating each sample with a mixed culture of the following organisms (inoculum strength indicated in the table):

Table with 2 columns: Organism Name and ATCC Number. Rows include Pseudomonas aeruginosa, Enterobacter cloacae, Bacillus subtilis, Bacillus megaterium, and Bacillus licheniformis.

The inoculated samples were mixed vigorously and incubated at 32°C for the duration of the test. At appropriate intervals, the samples were checked for the presence of viable microorganisms by directly streaking the sample onto Tryptic Soy Agar plates with a sterile cotton swab. The plates were incubated for 48 hours at 32°C and then rated on a scale of "0" to "4" based upon the number of colony forming units observed (See Legend Tables II).

Four –Week Environmental Chamber Test:

Samples were suspended in the environmental chamber. Observations of growth were recorded weekly in accordance with the American Society for Testing and Materials, Methods D3273 and D3274.



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RESULTS

TABLE I
Sterility Evaluation for Microbial Content

<u>Sample</u>	<u>Direct Streak</u>		
	<u>TSA</u>	<u>YM</u>	<u>PDA</u>
Gray paint with biocide	-	-	-
Gray paint without biocide	-	-	-
White paint with biocide	-	-	-
White paint without biocide	-	-	-

Legend:

Growth Rating:

- = No growth (sterile)
- 1 = Trace growth
- 2 = Light growth
- 3 = Moderate growth
- 4 = Heavy growth
- B = Bacterial
- F = Fungal
- Y = Yeast

TSA: Tryptic Soy Agar

YM: Yeast Malt Extract Agar

PDA: Potato Dextrose Agar (pH 3.5)



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**TABLE II
IN-CAN PRESERVATION EVALUATION
RESISTANCE TO BACTERIAL SPOILAGE
(ASTM D2574-94)**

<u>Sample</u>	<u>Day (s)</u>	<u>Challenge I</u>				<u>Challenge II</u>			
		<u>1</u>	<u>2</u>	<u>3</u>	<u>6</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>6</u>
Gray paint with biocide		0	0	0	0	0	0	0	0
Gray paint without biocide		2	2	2	2				
w/0.10% N-91& 0.10% F-404D		0	0	0	0	2	1	0	0
w/0.10% N-91& 0.20% F-404D		0	0	0	0	0	0	0	0
w/0.20% N-91& 0.20% F-404D		0	0	0	0	0	0	0	0
w/0.30% N-91& 0.20% F-404D		0	0	0	0	0	0	0	0
w/0.20% N-91& 0.30% F-404D		0	0	0	0	0	0	0	0
w/0.10% N-95& 0.10% F-404D		0	0	0	0	2	0	0	0
w/0.10% N-95& 0.20% F-404D		0	0	0	0	0	0	0	0
w/0.20% N-95& 0.20% F-404D		0	0	0	0	0	0	0	0
w/0.30% N-95& 0.20% F-404D		0	0	0	0	0	0	0	0
w/0.20% N-95& 0.30% F-404D		0	0	0	0	0	0	0	0
White paint with biocide		0	0	0	0	0	0	0	0
White paint without biocide		2	2	2	2				
w/0.10% N-91& 0.10% F-404D		0	0	0	0	0	0	0	0
w/0.10% N-91& 0.20% F-404D		0	0	0	0	0	0	0	0
w/0.20% N-91& 0.20% F-404D		0	0	0	0	0	0	0	0
w/0.30% N-91& 0.20% F-404D		0	0	0	0	0	0	0	0
w/0.20% N-91& 0.30% F-404D		0	0	0	0	0	0	0	0



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Legend:

Growth Ratings:

- 0 = No Contamination (sterile)
- 1 = Trace of contamination (1-9 colonies per "streak-inch").
- 2 = Light contamination (10-99 colonies per "streak-inch").
- 3 = Moderate contamination (greater than 100 colonies, but still distinguishable).
- 4 = Heavy contamination (continuous smear of growth).

- 2. A "streak-inch" is representative of a one inch length of a typical streak, which may be from ¼ inch to ½ inch wide.
- 3. A rating of 1 to 4 indicates that the sample may not be adequately preserved against the test organisms.
- 5. Samples were challenged with a bacterial inoculum size sufficient to produce 1.85×10^6 colony forming units per gram (CFU/g) of sample for the first challenge and 1.88×10^7 CFU/g in the second challenge. The inoculum consisted of a five-strain broth culture containing Psuedomonas aeruginosa, Enterobacter cloacae, Bacillus subtilis, Bacillus megaterium and Bacillus licheniformis.



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TABLE III
ENVIRONMENTAL CHAMBER
FUNGAL RESISTANCE EVALUATION
(ASTM 3273)

Sample Identification	Week(s):	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
Gray paint with biocide		10	10	10	10
Gray paint without biocide		10	9	7	6
w/0.10% N-91& 0.10% F-404D		10	10	10	10
w/0.10% N-91& 0.20% F-404D		10	10	10	10
w/0.20% N-91& 0.20% F-404D		10	10	10	10
w/0.30% N-91& 0.20% F-404D		10	10	10	10
w/0.20% N-91& 0.30% F-404D		10	10	10	10
w/0.10% N-95& 0.10% F-404D		10	10	10	10
w/0.10% N-95& 0.20% F-404D		10	10	10	10
w/0.20% N-95& 0.20% F-404D		10	10	10	10
w/0.30% N-95& 0.20% F-404D		10	10	10	10
w/0.20% N-95& 0.30% F-404D		10	10	10	10
White paint with biocide		10	10	10	10
White paint without biocide		10	8	6	6
w/0.10% N-91& 0.10% F-404D		10	10	9	9
w/0.10% N-91& 0.20% F-404D		10	10	10	10
w/0.20% N-91& 0.20% F-404D		10	10	10	10
w/0.30% N-91& 0.20% F-404D		10	10	10	10
w/0.20% N-91& 0.30% F-404D		10	10	10	10
w/0.10% N-95& 0.10% F-404D		10	10	10	10
w/0.10% N-95& 0.20% F-404D		10	10	10	10
w/0.20% N-95& 0.20% F-404D		10	10	10	10
w/0.30% N-95& 0.20% F-404D		10	10	10	10
w/0.20% N-95& 0.30% F-404D		10	10	10	10

Legend:



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**Testing carried out
by:**

Name: Daniel H. Brown

Position: Sr. Microbiologist

Project Dates:

Start: 3/3/11

Finish: 3/31/11

Reference:

N.B. Ref. 12141-6 & 11