*** CERTIFICATION OF ANALYSIS ***

Sunshine Makers, Inc.
16771 Pacific Coast Highway
Sunset Beach, California 90742

Test Report No. 1-3-11026
Date Received: 4/01/86
Date Initiated: 6/23/86
Date Completed: 6/28/86
P.O. Number: Bruce FaBrizio

Type of Examination: MICROBIAL LETHALITY STUDY

Test Article: One (1) one-gallon plastic container of Simple Green Lot No. G55.

Purpose: To determine whether 25, 50, 100, 500 and 1000 ppm concentrations of Simple Green (without preservative) have a bacteriostatic or fungistatic effect on five (5) tester microorganisms.

Summary and Conclusion:

Growth media was prepared with Simple Green (without preservative) added to produce final concentrations of 25, 50, 100, 500, and 1000 ppm. The media was inoculated individually with the bacteria Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa and the fungi Aspergillus niger and Candida albicans. The cultures were examined for microbial and fungal growth for five (5) consecutive days and compared with control cultures without the sample. The sample did not demonstrate bacteriostasis or fungistasis(i.e., the growth of the microorganisms or fungi tested was not inhibited by the specified concentrations of Simple Green without preservative).

Experimental Design:

Growth media were prepared with Simple Green added to produce final concentrations of 25, 50, 100, 500 and 1000 ppm of the test material. The media were inoculated individually with the bacteria Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa and the fungi Aspergillus niger and Candida albicans. The inoculum concentration was approximately fifty (50) microorganisms per container. On each of the succeeding five (5) days, the cultures were examined for microbial growth and compared macroscopically with control cultures without the test sample. Positive and negative controls were prepared. (BTP 9-2-001-86)

Reason for Exposure Level Selection:
The 25 ppm level is the proposed concentration of use.

Material and Methods:

A. Test Material: Simple Green without preservative, Lot No. G55

Material and Methods (cont'd)

B. Tester Microorganisms: Staphylococcus aureus ATCC 6538, Escherichia coli ATCC 8739, Pseudomonas aeruginosa ATCC 9027, Aspergillus niger ATCC 16404, Candida albicans ATCC 102311.Reason for Selection: The microorganisms selected include representatives of major taxonomic groups: a gram-positive, a gram-negative fermentor, a gram-negative non-fermentor, a yeast and a mold.

2. Supplier: American Type Culture Collection, Rockville, Maryland 20852.

C. Growth Medium Preparation

1. Test Sample

a) On the day of test initiation, stock sample solutions containing 250, 500, 1000, 5000 and 10000 ppm of the sample were prepared using sterile deionized water as the diluent.

b) Twenty-five (25) containers of 90 mL soybean casein digest (SCD) broth were prepared, autoclaving at 121°C for thirty (30) minutes.

c) Ten (10) mL of each stock sample solution were aseptically filtered into individual 90 mL SCD broth containers, five (5) containers for each level. These represented 25, 50, 100, 500 and 1000 ppm of the sample in the growth medium.

2. Negative Controls: A total of five (5) containers were prepared as described above in C. 1. c), preparing a single container at each sample concentration level.

3. Positive Controls: A total of five (5) container were prepared as describe above in C. 1. c), using deionized water instead of a stock sample solution.

D. Preparation of Inocula

1. Preparation of Cell Suspensions

a) Liquid cultures of S. aureus, P. aeruginosa, E. coli, and C. albicans were prepared by inoculating 20 milliliter aliquots of soybean-casein digest broth. The bacteria were incubated at 35-37°C for twenty-four(24) hours and the yeast was incubated at 35-37°C for forty-eight(48) hours.

Material and Methods (cont'd)

b) The cell cultures were enumerated using plate count methodology (BTP 2-2-002).
c) The cultures were stored at 2-8°C.
d) On the day of the test, the cultures were diluted in order to achieve a working stock concentration of approximately 5.0 x 10^3 cells per milliliter.

2. Preparation of Spore Suspension
a) Petri dish cultures of A. niger were prepared on Sabouraud dextrose agar and incubated at 20-25°C for seven (7) days.
b) The spores were harvested with saline containing 0.05% Tween 80.
c) The spore concentration was enumerated using plate count methodology (BTP 2-2-002).
d) The spore suspension was stored at 2-8°C.
e) On the day of the test, the suspension was diluted in order to achieve a working stock concentration of approximately 5.0 x 10^3 per milliliter.

E. Sample Inoculation and Incubation
1. The test sample cultures were inoculated individually with 0.1 milliliter of the test organisms as indicated in the results tables below.
2. The positive control cultures were inoculated as indicated in the results tables.
3. The negative control cultures were not inoculated.
4. The bacterial cultures were incubated at 30-35°C for five (5) days.
5. The fungal cultures were incubated at 20-25°C for five (5) days.

F. Reading Test Results
1. Each day of incubation, the test sample cultures were compared macroscopically with the positive and negative controls.
2. The test results tables below were completed.

G. Interpretation
1. Negative controls should show no growth.
2. Positive controls should show heavy growth.

Materials and Methods (cont'd)

3. A sample culture with growth density comparable to the respective positive control is considered to demonstrate that the sample does not cause stasis activity.

H. Results

1. Negative controls showed no growth.
2. Positive controls showed heavy growth.
3. The sample cultures showed a growth density comparable to the respective positive control. This demonstrates that the sample did not cause stasis activity at any of the concentrations mentioned above.
4. The results are tabulated in Tables 1 to 5.

Special Circumstances: None.

Archives: The remainder of the test article will be returned to the sponsor after issuance of the final report. The raw data and final report will be retained in archives of Bio-Technics Laboratories at 1127 Crenshaw Boulevard, Los Angeles, California 90019.

Study Personnel:

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JUL 30, 1986

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Table 1
Day 1

Microorganism	Sample Concentration (ppm) 25	50	100	500	1000	Controls Pos	Neg
S. aureus	I/+	I/+	I/+	I/+	I/+	I/+	N/0
P. aeruginosa	I/+	I/+	I/+	I/+	I/+	I/+	N/0
E. coli	I/+	I/+	I/+	I/+	I/+	I/+	N/0
A. niger	I/-	I/-	I/-	I/-	I/-	I/-	N/0
C. albicans	I/0	I/0	I/0	I/0	I/0	I/0	N/0

Table 2
Day 2

Microorganism	Sample Concentration (ppm) 25	50	100	500	1000	Controls Pos	Neg
S. aureus	I/+	I/+	I/+	I/+	I/+	I/+	N/0
P. aeruginosa	I/+	I/+	I/+	I/+	I/+	I/+	N/0
E. coli	I/+	I/+	I/+	I/+	I/+	I/+	N/0
A. niger	I/+	I/+	I/+	I/+	I/+	I/+	N/0
C. albicans	I/-	I/-	I/-	I/-	I/-	I/-	N/0

Table 3
Day 3

Microorganism	Sample Concentration (ppm) 25	50	100	500	1000	Controls Pos	Neg
S. aureus	I/+	I/+	I/+	I/+	I/+	I/+	N/0
P. aeruginosa	I/+	I/+	I/+	I/+	I/+	I/+	N/0
E. coli	I/+	I/+	I/+	I/+	I/+	I/+	N/0
A. niger	I/+	I/+	I/+	I/+	I/+	I/+	N/0
C. albicans	I/+	I/+	I/+	I/+	I/+	I/+	N/0

Legend
I = inoculated
N = not inoculated
+ = heavy growth
- = light growth
0 = no growth observed

index = (not) inoculated/growth density

Table 4
Day 4

Microorganism	Sample Concentration (ppm) 25	50	100	500	1000	Controls Pos	Neg
S. aureus	I/+	I/+	I/+	I/+	I/+	I/+	N/0
P. aeruginosa	I/+	I/+	I/+	I/+	I/+	I/+	N/0
E. coli	I/+	I/+	I/+	I/+	I/+	I/+	N/0
A. niger	I/+	I/+	I/+	I/+	I/+	I/+	N/0
C. albicans	I/+	I/+	I/+	I/+	I/+	I/+	N/0

Table 5
Day 5

Microorganism	Sample Concentration (ppm) 25	50	100	500	1000	Controls Pos	Neg
S. aureus	I/+	I/+	I/+	I/+	I/+	I/+	N/0
P. aeruginosa	I/+	I/+	I/+	I/+	I/+	I/+	N/0
E. coli	I/+	I/+	I/+	I/+	I/+	I/+	N/0
A. niger	I/+	I/+	I/+	I/+	I/+	I/+	N/0
C. albicans	I/+	I/+	I/+	I/+	I/+	I/+	N/0

Legend
I = inoculated
N = not inoculated
+ = heavy growth
- = light growth
0 = no growth observed

index = (not) inoculated/growth density