Consumer Product Testing Co.

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MICROBIOLOGY DEPARTMENT

FINAL REPORT

Study Number M95-267

COMPARATIVE EVALUATION OF STABILITY OF ANTIMICROBIAL EFFECTIVENESS OF SURFACE CLEANER / DISINFECTANTS

SPONSOR: **BETTER WORLD DISTRIBUTORS**

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COMPARATIVE EVALUATION OF STABILITY OF ANTIMICROBIAL EFFECTIVENESS OF SURFACE CLEANER / DISINFECTANTS

OBJECTIVE: To determine the relative antimicrobial effectiveness of candidate surface cleaner / disinfectants over a four week period. This method was designed and is intended for obtaining basic information about the relative stability of the formulations tested. It is not intended for demonstration or satisfaction of official performance requirements.

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TESTING FACILITY: Microbiology Laboratory
10 Industrial Road
Fairfield, New Jersey 07004

TEST MATERIALS: Three cleaning / disinfecting solutions, coded Product A, B and C.

Identification: Test materials were identified by Consumer Product Testing Company, Inc. (C.P.T.C.) study numbers:

M95- 267.01 (Product A),
M95- 267.02 (Product B),
M95- 267.03 (Product C).

TEST DATES: The study was conducted as detailed in the procedural outline (i.e. per proposed protocol) below; the study was initiated on the week of March 13, 1995 with subsequent runs on 3/31/95 and 4/13/95 (days 14 and 28 after the initial evaluation).

DEVIATIONS: The following deviation from the original Protocol was necessary in STUDY DESIGN step 4)i):

In pre-test trials with 5 uL of inoculum alone, drying of the inoculum on the test pledgets had taken about 3 minutes. On running the test, with test product applied first, drying took ~20 minutes. This was likely due to hygroscopicity on the part of the dried test sample. Since the sponsor had requested that the sample pledgets be timed after drying, subculture was carried out at 10 and 15 minutes after apparent drying of the sample / inoculum. This practice was continuous throughout the study and, where a killing / concentration gradient was observed, a test system for obtaining comparative results was apparently achieved.

The Protocol indicates a 24 - 48 hour incubation period, however it was observed that several broth cultures were growing out after the assigned period. Positive growth from 72 and /or 96-hour incubation were therefore included in the interpretation of results. Such growth is indicative of surviving / proliferative organisms and therefore gives information about the relative antimicrobial activities of the product / time periods.

STUDY DESIGN:

The study will be conducted according to GENERAL PROCEDURE FOR DETERMINATION OF PRODUCT MINIMUM INHIBITORY AND MINIMUM LETHAL CONCENTRATIONS (previously issued to sponsor) with the following additions and amendments:

1) The test organism / strain is specified as *Pseudomonas aeruginosa* ATCC 9027.

Two consecutive 24-hour Trypticase Soy Broth (TSB) cultures will be prepared, the second diluted with (TSB) to prepare an inoculum containing approximately 10^7 cells per milliliter.

2) The test solution dilutions will be prepared using sterile deionized water; per Sponsor directive, the dilution schema will be:

Sample Code								
A:	2%	1.5%	1.0%	0.75%	0.5%	0.25%	0.125%	0.0625%
dilute:	as is	1:1.33	1:2	1:2.667	1:4	1:8	1:16	1:32
B:	2%	1.5%	1.0%	0.75%	0.5%	0.25%	0.125%	0.0625%

3) Test samples of "treated surfaces" will be prepared using Formica pledgets approximately 0.75 cm^2. Each trial solution / dilution will be applied at 50 µL onto each of six pledgets, two for each test interval, one for each time period trial (10 and 15 minutes). The solutions were allowed to air dry forming a treatment film on each pledget. Each dilution's set of 6 pledgets will be placed in a sterile petri dish, covered for the duration of the test. Bacterial challenges will be performed on pledgets in the petri dish.

4) At each time interval (Time 0, 14 and 28 days) one pledget pair of each product/dilution will be tested as follows:

i) A 5µL aliquot of the *Pseudomonas aeruginosa* suspension is placed at the approximate center of each pledget, and allowed to air dry; this takes about 3 minutes

Post - test note: See DEVIATIONS In STUDY DESIGN step 4) I) -

ii) After 10 minutes, one pledget will be aseptically transferred to a tube of Trypticase Soy Broth (TSB).

iii) After 15 minutes, the second pledget will be aseptically transferred to a second tube of Trypticase Soy Broth (TSB).

iv) The TSB / pledgets are then incubated at 30 - 35 °C for 24 - 48 hours, then examined for growth.

5) The data gathered from the study will be tallied on the attached DATA / REPORT SHEET. The relative antimicrobial activity of each product dilution at 10 and 15 minute exposure times will then be assessed and any reduction in activity over the twenty-eight day test period realized.

RESULTS: See appended Data / Report sheets

Lethality of formulation *vs P. aeruginosa* A.T.C.C. 9027; a (NEG) result indicates lethality, a " + " denotes organism growth in initial broth and / or subculture.

Time 0

Dilution	10 min.	15 min
2%	NEG	NEG
1.5%	NEG	NEG
1.0%	NEG	NEG
0.75%	NEG	NEG
0.50%	NEG	NEG
0.25%	NEG	NEG
0.125%	+	+
0.0625%	+	NEG

Day 14

Dilution	10 min.	15 min
2%	NEG	NEG
1.5%	NEG	NEG
1.0%	NEG	NEG
0.75%	NEG	NEG
0.50%	NEG	NEG
0.25%	NEG	NEG
0.125%	NEG	NEG
0.0625%	NEG	NEG

Day 28

Dilution	10 min.	15 min
2.0%	NEG	NEG
1.5%	NEG	NEG
1.0%	NEG	NEG
0.75%	NEG	NEG
0.50%	NEG	NEG
0.25%	NEG	NEG
0.125%	NEG	NEG
0.0625%	NEG	NEG

Analyst:

Reviewed / Approved by: