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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

May 23, 2002

MEMORANDUM

Subject: Efficacy Review for EPA Reg. No. 5813-58 / Spruce-Ups
DP Barcode: D282372

From: Ian Blackwell, Biologist
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Through: Emily Mitchell, Team Leader 6/19/02
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Applicant: The Clorox Company

Formulation From Label:

Active Ingredient(s)	% by wt
N-Alkyl Dimethyl Benzyl Ammonium Chloride	0.145
N-Alkyl Dimethyl Ethylbenzyl Ammonium Chloride	0.145
Other Ingredient(s):	99.710
Total	100.000

I **BACKGROUND:** The Clorox Company has submitted two product efficacy studies as FIFRA 6(a)2 data. The studies were conducted by The Clorox Technical Center. The MRID Numbers are 456041-01 and -02.

A 9/18/2001 PSB/AD 6(a)2 review found that in a disinfectant test of 5813-58, two carriers were positive for growth of *Staphylococcus aureus* when tested against the product. This is considered to be a test failure. There was also one *Salmonella choleraesuis* inoculated carrier that was positive for growth (this is not a failure). The registrant was informed in a 12/2001 Antimicrobials Division letter that, due to the failed study conducted using 5813-58 against *Staphylococcus aureus*, another disinfectant study against *Staphylococcus aureus* was required. This submission includes two disinfectant studies in response to that letter.

II **Use Directions**

Spruce-Ups are pre-moistened disinfectant towelettes. They are designed to disinfect, sanitize and clean bathroom and kitchen surfaces, children's playroom and/or family dens, window blinds and other hard, non-porous surfaces.

Labeling Claims:

- 30 Second sanitization
- Kills bacteria in just 30 seconds
- Can help reduce the risk of spreading causing -or- harmful bacteria between hard, non-porous surfaces.
- Can help reduce the risk of cross contamination.
- Anti-bacterial -or- disinfecting wipes.

III **Agency Standards for Proposed Claims**

1 Test requirements. Employ the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products Test. Sixty carriers must be tested against each of S. choleraesuis, S. aureus, and Pseudomonas aeruginosa with each of 3 samples, representing, one of which is at least 60 days old. (180 carriers per sample; a total of 540 carriers.)

2 Performance requirements. To support products represented in labeling as "disinfectants", killing on 59 out of each set of 60 carriers is required to provide effectiveness at the 95% confidence level.

Concerning the choice of tests to use for disinfectant wipes, Subdivision G states: "...the AOAC Germicidal Spray Products Test appears to be the one most readily modified for this situation. Instead of spraying the inoculated surface of the glass slide, the product should be tested by wiping the surface of the glass slide with the saturated towelette, and then subculturing the slides after the specified holding time. All remaining liquid should be expressed from the used towelette and should also be subcultured."

(A) "The towelette should be removed from its container and subsequently handled with sterile gloves. One towelette should be used to wipe 60 inoculated slides. The area of the towelette used for wiping should be rotated so as to expose a maximum amount of its surface in the course of wiping a set of slides. After wiping the last slide for a particular towelette, all of the liquid remaining in the material should be expressed into an empty sterile container by squeezing the towelette; after a specified holding time (equal to the contact time stated on the product label), an aliquot from this container (ca. 0.1 ml) should be subcultured in the same manner as the slides."

However, PSB/AD policy allows one towelette to be used to wipe only **ten** carriers or slides instead of the 60 slides listed in Subdivision G.

IV Comments on the Submitted Efficacy Studies

1 MRID Number 456041-01: "Clorox Formula - F2001.0011 Pre-Saturated Towelette for Hard Surface Disinfection Test" by Denis Haire. The Clorox Technical Center. Clorox Study Number; ME-052. Study Completion Date: 2/5/2002.

This study was conducted to determine the disinfectant efficacy of the test material against hard, non-porous surfaces inoculated or contaminated with *Staphylococcus aureus* (ATCC 6538) and *Salmonella choleraesuis* (ATCC 10708). Enough Fetal Bovine Serum was added to each bacterial culture to produce a 5% soil load. Carriers measuring 25 x 25 mm were inoculated with 10µL of the working bacterial culture solutions and incubated for 35°C for 35 minutes. After drying, the carriers were wiped with 8 passes of a freshly exposed towelette pulled from the middle of the roll. A total of ten carriers were medicated per towelette. A total of 60 carriers per test organism for the batch were tested. Following a 4 minute contact time, each treated carrier was transferred to individual 20 mL containers of neutralizing broth. In addition, a 0.1 mL aliquot of the expressed solution from each wipe used was transferred to individual aliquots of neutralizing broth. The cultures were incubated for 48-54 hours at 35°C for both organisms. Two carriers per each organism are transferred to 20 mL of neutralizing broth and shaken for approximately 2 minutes. Each broth was serially diluted to 10^{-3} and aliquots from each dilution

were plated in duplicate on agar plate medium. Following incubation, the resulting colonies are enumerated and the colony forming units (CFUs) per carrier are determined.

2 MRID Number 456041-02: "Clorox Formula -2001.0011 Pre-Saturated Towelette for Hard Surface Disinfection Test" by Denis Haire. The Clorox Technical Center. Clorox Study Number ME-053. Study Completion Date 2/5/2002.

This study was conducted to determine the disinfectant efficacy of the test material against hard, non-porous surfaces inoculated or contaminated with *Pseudomonas aeruginosa* (ATCC 15442). Enough Fetal Bovine Serum was added to each bacterial culture to produce a 5% soil load. Carriers measuring 25 x 25 mm were inoculated with 10µL of the working bacterial culture solutions and incubated for 35°C for 35 minutes. After drying, the carriers were wiped with 8 passes of a freshly exposed towelette pulled from the middle of the roll. A total of ten carriers were medicated per towelette. A total of 60 carriers per test organism for the batch were tested. Following a 4 minute contact time, each treated carrier was transferred to individual 20 mL containers of neutralizing broth. In addition, a 0.1 mL aliquot of the expressed solution from each wipe used was transferred to individual aliquots of neutralizing broth. The cultures were incubated for 48-54 hours at 35°C for both organisms. Two carriers per each organism are transferred to 20 mL of neutralizing broth and shaken for approximately 2 minutes. Each broth was serially diluted to 10^{-3} and aliquots from each dilution were plated in duplicate on agar plate medium. Following incubation, the resulting colonies are enumerated and the colony forming units (CFUs) per carrier are determined.

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V Results

Table 1.

MRID Number 456041-01		
	Staphylococcus aureus	*Salmonella choleraesuis*
Working Suspension	8.5×10^8	4.4×10^8
Ave. Dry Carrier Control Count	6.9×10^6	7.0×10^4
Test Organism Positives /60 Test Carriers	1/60	0/60
Test Organism Positives /6 Towelettes Subcultured	0/6	0/6
Neutralization Controls	positive for growth	positive for growth

Table 2.

MRID Number 456041-02	
	Pseudomonas aeruginosa
Working Suspension	9.8×10^8
Ave. Dry Carrier Control Count	1.8×10^5
Test Organism Positives /60 Test Carriers	1/60
Test Organism Positives /6 Towelettes Subcultured	0/6
Neutralization Controls	positive for growth

VI Conclusions

1 MRID Number 456041-01: The submitted efficacy data supports the use of the product, Spruce-Ups Towelette, as a disinfectant when tested against *Salmonella choleraesuis* and *Staphylococcus aureus* in the presence of organic soil (5% horse serum) on hard, non-porous surfaces with a contact time of 4 minutes. There was no growth in any subculture of the towelette themselves. However, there are two problems with the study report:

A The test material was not identified as "Spruce Ups" or Registration Number 5813-58. The test material was listed as Clorox Formula - 2001.011, Clorox Disinfecting Wipes, or, CDW.
B The report did not specify the temperature of the 4-minute test material exposure.

2 MRID Number 456041-02: The submitted efficacy data supports the use of the product, Spruce-Ups Towelette, as a hospital disinfectant when tested against *Pseudomonas aeruginosa* in the presence of organic soil (5% horse serum) on hard, non-porous surfaces with a contact time of 4 minutes. There was no growth in any subculture of the towelette themselves. However, there are two problems with the study report:

A The test material was not identified as "Spruce Ups" or Registration Number 5813-58. The test material was listed as Clorox Formula - 2001.011, Clorox Disinfecting Wipes, or, CDW.
B The report did not specify the temperature of the 4-minute test material exposure.

VII Recommendations

The request to add additional label claims of Spruce Ups being a hospital disinfectant is acceptable. However, the registrant is expected to submit information clarifying the relationship between Clorox Formula - 2001.0011 and Spruce Ups, and, report the temperature at which the inoculated carriers were exposed to the test material for the tests.