UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES
Antimicrobials Division

September 18, 2001

MEMORANDUM:

Subject: 6(a)(2) Efficacy Review EPA Reg. No. 5813-58 Spruce-Ups
DP Barcode 271157, 271158, 271163
Case No. 061904

From: Nancy Whyte, Microbiologist
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Thru: Emily Mitchell, M.S., Team Leader Emily Mitchell 9/20/01
Efficacy Evaluation Team
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Thru: Michele E. Wingfield, Chief
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Applicant: The Clorox Company
PO Box 493
Pleasanton, CA 94566-0803

Formulation Label: **% by wt.**

Active Ingredient(s)

n-Alkyl (60% C_{14}, 30% C_{16}, 5% C_{12}, 5% C_{18}) dimethyl benzyl ammonium chloride	0.145%
n-Alkyl (68% C_{12}, 32% C_{14}) dimethyl ethylbenzyl ammonium chloride	0.145%
Inert ingredients	99.710%
Total	100.000%

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I. Background:

Three separate reports of failed efficacy studies were reported to the Agency. The first report concerned testing done by ViroMed BioSafety Laboratories in April 2000. The second report was of testing conducted at the Clorox Technical Center in California in July 2000. The third set of tests was also conducted by the Clorox Technical Center in August 2000. The product was tested initially at ViroMed against *Salmonella choleraesuis,* ATCC 10708 and *Staphylococcus aureus,* ATCC 6538. Subsequent testing at ViroMed and one of two at Clorox tested effectiveness only against *Staphylococcus.* None of the studies followed Good Laboratory Practices. All studies used 60 carriers for each lot of product tested.

II. Summary of Submitted Studies:

The first study at ViroMed tested six lots of product were tested against *Salmonella choleraesuis,* ATCC 10708 and *Staphylococcus aureus,* ATCC 6538. The study was not conducted using Good Laboratory Practices. Of the six samples tested, one showed growth of *Salmonella choleraesuis* on one carrier. Glass slides were used as carriers, and were wiped with the towlette. Liquid was expressed following the wiping procedure into broth tubes. Two other samples showed growth of *Staphylococcus aureus,* one carrier in one sample, and two carriers in another (failure). Repeat of the testing by the same lab using the failed lot showed that the product was effective against *Staphylococcus aureus* when no growth was observed in 59 out of 60 carriers exposed for 10 minutes in the presence of a 5% organic soil load.

Two additional studies were conducted by Clorox using the same conditions, except that glass cover slips were used as carriers rather than slides. Different lot numbers of the product were used. In the first test, using Sample No. 7432-146-3 only *Staphylococcus aureus,* ATCC 6538 was tested. Results showed that 5 carriers out of 60 showed growth following a 10 minute exposure time in the presence of a 5% organic soil load. The second test in September 2000 used both organisms tested two product batches There was no growth of *Salmonella choleraesuis* in either batch, but Batch B was not effective against *Staphylococcus aureus,* demonstrating growth in 2 carriers out of 60. Therefore the sample was not efficacious.

III. Comments and Recommendations:

Clorox has stated in the letters accompanying the failure reports that because GLP were not followed, the company believes that improper sample preparation and storage led to the failure, and that corrective measures had been taken to assure the probability of this occurring again.

A review of the original efficacy testing done at the time the product was registered by American Cyanamid in 1988 indicates that the requirements for efficacy testing mandated by the Agency for registration were not followed in the studies submitted. Only 10 carriers were tested against each organism and only one batch was used. Complete efficacy testing should have been done when the product registration was transferred to Clorox. The recent failure reports have focused on the need to verify product effectiveness at this time.

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Therefore, the registrant must submit to the Agency, within ninety (90) days for review and approval, the complete efficacy testing data required by DIS-TSS-1 for Hard Surface Disinfectants using the AOAC Germicidal Spray Method and according to the protocol for Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfectants (see attached). If label claims for additional organisms are desired, they may be included as confirmatory data at this time. No approval for any amendments to the registration of this product will be given by the Agency until the efficacy data has been accepted.

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EFFICACY DATA REQUIREMENTS

Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection

These products represent a unique combination of antimicrobial chemical and applicator pre-packaged as a unit in fixed proportions. Therefore, the complete product, as offered for sale, should be tested according to the directions for use to insure its effectiveness in disinfecting hard surfaces. Please refer to the DIS/TSS-1 enclosure, items (a), (b), and (c), for the basic efficacy data requirements for a hard-surface disinfectant. The test microorganisms indicated and the minimum number of carriers and samples specified are required regardless of the modifications which may be employed to simulate the way in which the product is actually intended to be used.

(1) Single-Use Towelette. The product is intended to be removed from the package, used immediately, and discarded after use. In this case, the testing suggested in (a), (b), and (c) below is conducted on towelettes from freshly opened packages (including 60-day shelf life). No simulated re-use protocol is required.

(a) Simulated-Use Test. The standard test methods available for hard-surface disinfectants (AOAC Use-Dilution Method and AOAC Germicidal Spray Products Test), if followed exactly, would not closely simulate the way the product is used. Of these methods, 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 a specified holding time. Liquid expressed from the used towelette should also be subcultured.

(b) Additional Procedural Suggestions. The towelette should be removed from its container and subsequently handled with sterile gloves. One towelette should be used to wipe a number of 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, some of the liquid remaining in the material should be expressed into an empty, sterile container; after a specified holding time, an aliquot from this container (ca. 0.1 ml) should be subcultured in the same manner as with the slides.

(c) Additional Test Modifications. Please refer to the DIS/TSS-2 enclosure for additional test modifications which may be necessary depending on the intended label claims and directions for use (e.g. exposure period, organic soil, etc.), as well as for documentation of neutralization. Also, please refer to the DIS/TSS-3 enclosure for guidance in reporting the tests.

(2) Multiple-Use Towelette. The product is intended to be unpackaged and used repeatedly for an extended period until a specified end-point is reached (as determined, for example, by a visible indicator in the product). When a product is intended for a pattern of repeated use, a protocol must be designed which simulates, to the extent possible, the conditions under which it is re-used and to which it could be exposed, including periodic microbiological challenge, for the duration of its intended use-life. At this point the product is tested to insure its effectiveness in disinfecting hard surfaces.

(a) Simulated Re-Use Protocol. The simulated re-use protocol must include, but is not limited to, the following basic elements:

(i) The cloth must be moistened (in the case of a dry impregnated towelette) and applied to representative type(s) of surfaces as recommended on the label and according to the directions for use. The cloth should then be allowed to partially or completely dry; and the wet-wipe-dry cycle should be repeated until the claimed use-life or specified end-point is reached. These cycles must include periodic challenge with microbiological "bioburden" (viable test bacteria dried onto surfaces/carriers which are wiped). The minimum bioburden load should be approximately equivalent to one glass slide contaminated with at least 10^6 viable bacteria (i.e. Staphylococcus aureus, Salmonella choleraesuis, Pseudomonas aeruginosa) per each 5 ml of use solution produced in wetting the towelette.

(ii) Periodic chemical monitoring of active ingredient in the use solution produced in the cloth should be performed to show the adequacy and consistency of the concentration provided. In lieu of chemical monitoring, microbiological assay of the surfaces/cloth solution exposed to the bioburden must be performed and found to meet the criteria for acceptable disinfection (DIS/TSS-1 enclosure).

(iii) The use of hard water or organic soil in the re-use protocol would not be required unless label claims are made for use of product in hard water or in the presence of soil ("one-step" cleaning and disinfecting). If such claims are made, the re-use protocol must be conducted with water of the claimed hardness and/or with at least 5% blood serum added to the bacterial inoculum employed as bioburden as well as to the water.

(iv) The specific end-point of the use-life of the towelette is critical, and must be clearly defined on the label for the user and in the protocol for the tester. A comfortable margin of effectiveness must be allowed between the end-point as perceived by the user and the time at which the product is no longer effective as claimed.

(v) At the completion of the simulated re-use protocol, the used towelettes are tested at the specified end-point of their use-life for effectiveness as disinfectants as indicated in (1)(a), (1)(b), and (1)(c) above.