**UNITED STATES ENVIRONMENTAL PROTECTION AGENCY**
**WASHINGTON, DC 20460**

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

February 6, 2007

**MEMORANDUM**

**Subject:** Efficacy Review for B-Cap® 35 Antimicrobial Agent (EPA Reg. No. 72372-1); DP Barcode: D335067

**From:** Ibrahim Laniyan, Microbiologist
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**Thru:** Tajah Blackburn, Acting Team Leader
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**To:** Karen Leavy / Marshall Swindell
Regulatory Management Branch I
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**Applicant:** FMC Corporation
Peroxygens Division
1735 Market Street
Philadelphia, PA 19103

**Formulation from the Label:**

| Active Ingredient | % by wt. |
|---|---|
| Hydrogen Peroxide | 35 % |
| Inert Ingredients: | 65 % |
| Total | 100 % |

## I. BACKGROUND

The product, B-Cap® 35 Antimicrobial Agent (EPA Reg. No. 72372-1), is an EPA-approved microbiocide for use in controlling slime and sulfate-forming bacteria in process waters, air washing systems, recirculating and once through water cooling towers and systems, and packaging and storage vessels. The product is for industrial use only. The applicant requested that EPA amend the product's registration to include a claim for effectiveness as a sterilant. Studies were conducted at Wickham Laboratories Limited, located on Winchester Road in Wickham, Fareham, Hampshire, PO17 5EU, United Kingdom; and Reading Scientific Services Limited, located at 25-27 Britten Road, Elgar Road South Reading, RG2 0AU, United Kingdom.

This data package contained a letter from the applicant's representative to EPA (dated June 30, 2006), EPA Form 8570-4 (Confidential Statement of Formula), EPA Form 8570-35 (Data Matrix), two studies (MRID Nos. 469173-01 and 469173-02), Statements of No Data Confidentiality Claims for both studies, a copy of the User Manual for the Clarus Hydrogen Peroxide Vapour Generator, the proposed label, and the last accepted label (April 30, 2001).

Note: The laboratory reports describe studies conducted for the product, FMC Durox LR 35% Hydrogen Peroxide. The letter from the applicant's representative to EPA (dated June 30, 2006) states that the tested product, Durox LR, is the product, B-Cap 35 Antimicrobial Agent, which is the subject of this efficacy report.

## II. USE DIRECTIONS

The product is a sterilant for use in conjunction with the Bioquell Clarus Hydrogen Peroxide Vapor Generator. The hydrogen peroxide vapor is intended for use as a sterilant in enclosures up to 35 cubic feet. Directions on the proposed label provided the following information regarding preparation and use of the product as a sterilant: Ensure that all surfaces are visibly clean and free from gross organic contamination. Connect the Clarus generator to the enclosure. Add the product to the generator according to the operating manual instructions. Seal the enclosure to be sterilized. Apply the product at an injection rate of 3.1 g/minute for 55 minutes. Allow vapor to remain for a minimum of 3 hours. Aerate the chamber until the concentration of hydrogen peroxide vapor is at or below 1.0 ppm.

## III. AGENCY STANDARDS FOR PROPOSED CLAIMS

**Sterilizers:** The AOAC Sporicidal Test is required for substantiating sterilizing claims. The following information applies to all products represented as sporicidal or sterilizing agents. Sixty carriers, representing each of 2 types of surfaces (porcelain penicylinders and silk suture loops), must be tested against spores of both *Bacillus subtilis* (ATCC 19659) and *Clostridium sporogenes* (ATCC 3584) on 3 product samples representing 3 different product lots, one of which is at least 60 days old (240 carriers per sample; a total of 720 carriers). Any sterilizing agent (liquid, vapor, or gas) that is recommended for use in a specific device must be tested by the AOAC Sporicidal Test in that specific device and according to the directions for use. Killing on all of the 720 carriers is required; no failures are permitted. Data to support sterilizing claims must be confirmed by tests conducted by a second, independent laboratory of the applicant's choice (other than the laboratory that developed the original data). The following minimal

confirmatory data must be developed on one sample of the product: Thirty carriers with each of the 2 types of surfaces (silk suture loops and porcelain penicylinders) against spores of both *Bacillus subtilis* and *Clostridium sporogenes* (a total of 120 carriers) by the AOAC Sporicidal Test. These Agency standards are presented in DIS/TSS-9.

## IV. BRIEF DESCRIPTION OF THE DATA

**1. MRID 469173-01 "Evaluation of Bioquell Clarus Hydrogen Peroxide Vapour Generator Using FMC 35% Durox LR Hydrogen Peroxide as Decontamination Agent By Modified AOAC Sporicidal Activity Test Method 966.04 In Accordance With EPA Requirements," by Susan Wood. Study conducted at Wickham Laboratories Limited. Study completion date – February 17, 2006. Study Number 1354 (Project CN00085180).**

This confirmatory study was conducted against *Bacillus subtilis* (ATCC 19659) and *Clostridium sporogenes* (ATCC 3584). The microorganisms used in the study were obtained from Presque Isle Cultures, Presque Isle, PA as carriers (porcelain penicylinders and polyester suture loops) inoculated with a certified spore population. One lot (lot number not provided; shipped to Bioquell on November 16, 2004) of the product, FMC 35% Durox LR Hydrogen Peroxide, was tested using a modified version of the "EPA/OPP Microbiology Laboratory ESC, Ft. Meade, MD Standard Operating Procedure for AOAC Sporicidal Activity Test (*Bacillus* species) SOP Number: MB-15-00, Date Revised: 06-25-03," which is based on the AOAC Sporicidal Activity of Disinfectants Method (Method 966.04) as described in the AOAC Official Methods of Analysis, 17$^{th}$ Edition, 2000. The product was received ready-to-use, and was a minimum of 6 months old at the time of testing. No organic load was used in testing.

Thirty (30) penicylinder carriers and thirty (30) suture loops inoculated with *Bacillus subtilis* and thirty (30) penicylinder carriers and thirty (30) suture loops inoculated with *Clostridium sporogenes* were placed in a 1 $m^3$ test chamber. The test chamber consisted of a four-glove "Pharmaflow" isolator joined to a two-glove "TPC Microflow" isolator via a lockable interface. Each isolator was connected to a Clarus C Hydrogen Peroxide Vapour Generator. Four metal wire hangers were placed at 30 pre-determined locations within the "Pharmaflow" isolator. A biological indicator of each type was placed at each of the 30 locations. Prior to the start of the test, the neutralization media were labeled and placed in the "Pharmaflow" isolator. The growth media were labeled and placed in the "TPC Microflow" isolator. The isolators were closed and the product was placed in the Clarus units. The sterilization cycle was then started. The interior of the test chamber was conditioned to achieve 40% relative humidity (conditioning phase). Next, the Clarus unit prepared to vaporize the hydrogen peroxide (pre-gassing phase). The Clarus unit injected the vaporized hydrogen peroxide at 3.1 g per minute for 55 minutes (gassing phase). The Clarus unit circulated the product around the test chamber for 3 hours (dwell phase). The Clarus unit removed the hydrogen peroxide from the test chamber by means of a catalyst, until the hydrogen peroxide concentration was less than 1 ppm as determined by Dräger tube (aeration phase). Critical parameters (results not provided) were confirmed by electronic printouts generated by the Clarus units. On completion of the sterilization cycle, the 120 biological indicators were placed in Tryptone Soya Broth (for *Bacillus subtilis*) and Fluid Thioglycollate Media USP (for *Clostridium sporogenes*). The neutralizer broths were transferred to the "TPC Microflow" isolator and the biological indicators were transferred to growth media of the same type. On completion of the transfers, the isolators were opened. The subcultures were removed and transferred to the laboratory within 5 hours. The subcultures were incubated for 21

days at 37°C. All subcultures were checked daily during the working week for a total of 21 days. Subcultures showing no growth after 21 days were heat shocked for 20 minutes at 80°C, and re-incubated for an additional 72 hours. The subcultures were checked again for the presence of growth. Controls included positive and negative controls; biological indicator enumeration to confirm spore concentrations; and confirmation of the hydrogen peroxide concentration of the product. Two positive media controls for each carrier type were set up (i.e., tubes of each media type containing a single biological indicator). Three negative media controls were set up (i.e., unopened tubes of Tryptone Soya Broth and Fluid Thioglycollate Media USP). The reported titers per inoculated carriers are: ***Bacillus subtilis* on Suture Loops $8.1x10^5$, *Bacillus subtilis* on Penicylinders $1.7x10^6$, *Clostridium sporogenes* on Suture Loops $1.8x10^6$, *Clostridium sporogenes* on Penicylinders $6.1x10^5$.**

Note: The study was conducted according to GLP standards with the following exceptions: "1. The site at Bioquell has not been inspected by GLP-MA; 2. The Clarus Vapour Generators have not been formally qualified. The Clarus Vapour Generators have been operationally qualified by annual calibrations and method specific titrations to demonstrate fitness for purpose and use."

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

**2. MRID 469173-02 "Evaluation of Bioquell "Clarus" Hydrogen Peroxide Vapour Generator in conjunction with FMC Durox LR 35% Hydrogen Peroxide against a method based on the EPA (AOAC) Sporicidal Activity Test," by Dr. Graham Pettipher. Study conducted at Reading Scientific Services Limited. Study completion date – January 26, 2006. Project Number P5-09191.**

This study was conducted against *Bacillus subtilis* (ATCC 19659) and *Clostridium sporogenes* (ATCC 3584). The microorganisms used in the study were obtained from Presque Isle Cultures, Presque Isle, PA as carriers (porcelain penicylinders and polyester suture loops) inoculated with a certified spore population. One lot (lot number not provided; shipped to Bioquell on October 13, 2003) of the product, Durox LR Hydrogen Peroxide, was tested using a modified version of the "EPA/OPP Microbiology Laboratory ESC, Ft. Meade, MD Standard Operating Procedure for AOAC Sporicidal Activity Test (*Bacillus* species) SOP Number: MB-15-00, Date Revised: 06-25-03," which is based on the AOAC Sporicidal Activity of Disinfectants Method (Method 966.04) as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. The product was received ready-to-use, and was a minimum of 6 months old at the time of testing. No organic load was used in testing.

Testing was conducted on three occasions. For each test run, sixty (60) penicylinder carriers and sixty (60) suture loops inoculated with *Bacillus subtilis* and sixty (60) penicylinder carriers and sixty (60) suture loops inoculated with *Clostridium sporogenes* were placed in a 1 $m^3$ test chamber. The test chamber consisted of a "Pharmaflow" isolator joined to a "TPC Microflow" isolator via an interface. Each isolator was connected to a Clarus C Hydrogen Peroxide Vapour Generator. Four metal wire hangers were placed at 60 evenly distributed locations within the "Pharmaflow" isolator. A biological indicator of each type was place at each of the 60 locations. The neutralization and growth media were placed within the two isolators prior to the start of each test run. The sterilization cycle was then started. The interior of the test chamber was conditioned to achieve 40% relative humidity (conditioning phase). Next, the Clarus unit prepared to vaporize the hydrogen peroxide (pre-gassing phase). The Clarus unit injected the vaporized hydrogen peroxide at 3.1 g per minute for 55 minutes (gassing phase).

The Clarus unit circulated the product around the test chamber for 3 hours (dwell phase). The Clarus unit removed the hydrogen peroxide from the test chamber by means of a catalyst, until the hydrogen peroxide concentration was less than 1 ppm as determined by Dräger tube (aeration phase). Critical parameters (results not provided) were confirmed by electronic printouts generated by the Clarus units. On completion of the sterilization cycle, the 240 biological indicators were transferred into individual tubes containing neutralization media. This transfer occurred inside the test chamber. Once all 240 biological indicators were placed into the neutralization media, the biological indicators were immediately transferred into individual tubes of secondary growth media – Tryptone Soya Broth (for *Bacillus subtilis*) and Thioglycollate with Resazurin (for *Clostridium sporogenes*). On completion of the transfers, the isolators were opened. The subcultures were removed and transferred to the laboratory within 5 hours. The subcultures were incubated for 21 days at 37°C. All subcultures were checked for the presence of growth after 7 and 21 days. Subcultures showing no growth after 21 days were heat shocked for 20 minutes at 80°C, and re-incubated for an additional 72 hours. The subcultures were checked again for the presence of growth. Controls included positive and negative controls; biological indicator enumeration to confirm spore concentrations; and confirmation of the hydrogen peroxide concentration of the product. Three positive media controls for each carrier type were set up per test run (i.e., tubes of each media type containing a single biological indicator). Three negative media controls were set up per test run (i.e., unopened tubes of Tryptone Soya Broth and Thioglycolate with Resazurin broth). The reported titers per inoculated carriers are: ***Bacillus subtilis* on Suture Loops $4.5x10^5$, *Bacillus subtilis* on Penicylinders $6.0x10^5$, *Clostridium sporogenes* on Suture Loops $5.7x10^5$, *Clostridium sporogenes* on Penicylinders $1.6x10^5$**.

Note: The study was conducted according to GLP standards with the following exceptions: "1. The site at Bioquell has not been inspected by GLP-MA. 2. Not all pieces of equipment at the Bioquell site have been qualified." The Clarus Vapour Generators have not been formally qualified; however, the generators have been operationally qualified by annual calibrations and method specific titrations.

Note: The laboratory report did not identify the neutralization media used. A May 2004 draft test protocol suggests Tryptone Soya Broth as the neutralization medium for *Bacillus subtilis* and Thioglycollate Broth as the neutralization medium for *Clostridium sporogenes*. Bioquell "believes that this neutralization step is unnecessary due to the physical removal of hydrogen peroxide from the test chamber."

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

## V. RESULTS

| MRID Number | Organism | Run | Carrier Type | No. Exhibiting Growth/ Total No. Tested | | Biological Indicator Count (CFU/ Carrier) |
|---|---|---|---|---|---|---|
| | | | | No lot number | No lot number | |
| 469173-01 | *Bacillus subtilis* | | sutures<br>penicylinders | 0/30<br>0/30 | | $8.1 \times 10^5$<br>$1.7 \times 10^6$ |
| | *Clostridium sporogenes* | | sutures<br>penicylinders | 0/30<br>0/30 | | $1.8 \times 10^6$<br>$6.1 \times 10^5$ |
| 469173-02 | *Bacillus subtilis* | 1 | sutures<br>penicylinders | | 0/60<br>0/60 | $4.0 \times 10^5$<br>$6.0 \times 10^5$ |
| | | 2 | sutures<br>penicylinders | | 0/60<br>0/60 | $4.0 \times 10^5$<br>$6.0 \times 10^5$ |
| | | 3 | sutures<br>penicylinders | | 0/60<br>0/60 | $4.0 \times 10^5$<br>$6.0 \times 10^5$ |
| | *Clostridium sporogenes* | 1 | sutures<br>penicylinders | | 0/60<br>0/60 | $5.7 \times 10^5$<br>$1.6 \times 10^5$ |
| | | 2 | sutures<br>penicylinders | | 0/60<br>0/60 | $5.7 \times 10^5$<br>$1.6 \times 10^5$ |
| | | 3 | sutures<br>penicylinders | | 0/60<br>1/60 | $5.7 \times 10^5$<br>$1.6 \times 10^5$ |

## VI. CONCLUSIONS

1. The submitted efficacy data (MRID Nos. 469173-01 and 469173-02) **do not support** the use of the product, Durox LR Hydrogen Peroxide, when used in conjunction with the Clarus Hydrogen Peroxide Vapour Generator, as a sterilant against *Bacillus subtilis* and *Clostridium sporogenes.* Conditions of the application were as follows: product injection rate = 3.1 g/minute for 55 minutes; exposure time of 3 hours; 1 $m^3$ (i.e., 35 $ft^3$) enclosure.

**Basic testing was conducted on 1 product lot on three separate occasions. Registration of a product as sterilant requires testing on three (3) different product lots.** The product was tested in the specific device recommended for use. The product lot tested was at least 60 days old at the time of testing. It is unclear whether both the neutralizer and secondary subculture tubes were incubated and examined for growth, in accordance with SOP MB-15-00. [Results were reported for one set of culture tubes only.] Biological indicator counts were at least $2 \times 10^5$ CFU/carrier, which is the EPA-recommended level, except for *Clostridium sporogenes* penicylinders (at $1.6 \times 10^5$). [The laboratory indicated that the *Clostridium sporogenes* penicylinders counts were within method uncertainty values (i.e., 0.2-0.5 log units).] The laboratory report did not discuss whether neutralization confirmation testing was conducted (other than by secondary subculturing). The positive controls (i.e., viability) were positive for growth. The negative controls (i.e., sterility) did not show growth. *Clostridium sporogenes* test spores (suture loops and penicylinders) showed resistance to acid for ≥2 minutes, in both primary and secondary subcultures. *Bacillus subtilis* test spores (penicylinders) showed resistance to acid for ≥2 minutes, in primary subcultures only. *Bacillus subtilis* test spores (suture loops) showed resistance to acid for ≥2 minutes, in both primary and secondary subcultures.

Confirmatory testing was conducted on 1 product lot, by an independent laboratory. The product was tested in the specific device recommended for use. Killing was observed in the subcultures of the required number of carriers (i.e., 30 carriers/2 types of surfaces) tested against the required number of product lots (i.e., 1). Biological indicator counts were at least 2 x $10^5$ CFU/carrier, which is the EPA-recommended level. **The laboratory report did not discuss whether neutralization confirmation testing was conducted** (other than by secondary subculturing). The positive controls (i.e., viability) were positive for growth. The negative controls (i.e., sterility) did not show growth. *Clostridium sporogenes* test spores (suture loops and penicylinders) showed resistance to acid for ≥2 minutes, in both primary and secondary subcultures. *Bacillus subtilis* test spores (penicylinders) showed resistance to acid for ≥2 minutes, in primary subcultures only. *Bacillus subtilis* test spores (suture loops) showed resistance to acid for ≥2 minutes, in both primary and secondary subcultures.

## VII. RECOMMENDATIONS

1. The proposed label claims that the product, B-Cap® 35 Antimicrobial Agent is an effective sterilant, when used in conjunction with the Clarus Hydrogen Peroxide Vapour Generator, for treating enclosures up to 35 $ft^3$. A product injection rate of 3.1 g/minute for 55 minutes and exposure time of 3 hours are specified. As noted in the Conclusions Section of this efficacy report, **efficacy of the product has not been demonstrated. The applicant must remove sterilant claims from the proposed label, or provide efficacy data that fully meet DIS/TSS-09 requirements**.

2. With the acceptance of the confirmatory data (one lot run, MRID No. 469173-01) and acceptance of the submitted data (considered as one lot run, MRID No. 469173-02), **the applicant must identify the product lot used for these studies.**

3. **The registrant must conduct additional runs using two (2) lots of the product different from the one used in those studies** (MRID Nos. 469173-01 and 469173-02).

4. Neutralization confirmation control for each of the two lots of product must be conducted by adding **low microorganism titers** to neutralization media tubes containing uncontaminated carriers from sterilization cycle.

**Note**: The proposed label does not identify the types of surfaces/objects intended for treatment, as required by DIS/TSS-15.