UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

December 17, 2012

MEMORANDUM

Subject: Efficacy Review EPA Reg. No. 4972-43, Protexall Sun Pac Mildewcide; DP Barcode: 407604

Evaluation of Amendment to Add New Uses: (a) fumigation/sterilization/ decontamination of pre-cleaned hard, non-porous and porous surfaces in sealed enclosures located in government, industrial, commercial and institutional microbiological settings, including human and animal research facilities, laboratory equipment, biological safety cabinets, HEPA filters and airlocks; and (b) fumigation of leafcutting bee nest materials and leafcutting bee cells.

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Applicant: Protexall Products, Inc.
73356 Highway 41
Pearl River, LA 70452

Label formulation:

Active Ingredient(s)	% by wt.
Paraformaldehyde	91%
Inert Ingredients	9%
Total	100%

I BACKGROUND

The current product, Protexall Sun Pac Mildewcide (EPA Reg. No. 4972-43), is registered solely as a mildewcide. The applicant has requested to amend the registration of this product to include (a) fumigation/sterilization/decontamination of pre-cleaned hard, non-porous and porous surfaces in sealed enclosures located in government, industrial, commercial and institutional microbiological settings, including human and animal research facilities, laboratory equipment, biological safety cabinets, HEPA filters, and airlocks, and (b) fumigation of leafcutting bee nesting materials and bee cells. These proposed uses are included in a "Laboratories Product Manual" and a "Leafcutting Bee Product Manual," respectively, which will accompany the product label.

The data package contained a letter from the applicant to EPA (dated November 7, 2012), EPA Form 8570-4 (Confidential Statement of Formula), three supporting documents (MRID No. 48994701), the proposed label, two product manuals and EPA Form 8570-4 (Confidential Statement of Formula).

II USE DIRECTIONS

The proposed new uses for the product have been separated into two product manuals—the "Laboratories Product Manual" and the "Leafcutting Bee Product Manual."

The Laboratories Product Manual states that the product may be applied only by persons who are trained in the application procedures and use of the safety equipment specified in the product manual. All personnel involved in the application must be familiar with the guidance pertaining to use of paraformaldehyde/formaldehyde for laboratory and laboratory equipment decontamination found in "Biosafety in Microbiological and Biomedical Laboratories, 5th Edition" (U.S. DHHS, PHS, CDC, NIH, December 2009) and in "NSF/ANSI Standard 49, Annex F" (2011). The laboratory product manual provides extensive use directions organized into six sections: Overview of the Application Process, User Safety Requirements, Efficacy, Fumigation Management Plan (FMP) or Standard Operating Procedure (SOP), Preparation of Enclosures and Application to Sealed Enclosures. The Appendix contains an outline for the Fumigant Management Plan that is required to be developed, approved and followed for each application site. The Laboratories Product Manual Directions describes in explicit detail how to prepare the product, heat it, neutralize it and monitor after treatment (prior to reentry). These use directions are based on well established procedures and protocols that have been developed, tested and adopted in NSF/ANSI 49 as well as at multiple federal agencies.

The Leafcutting Bee Product Manual states that the applicator must inform the owner or person in charge of the treated structure and/or space of the plans for fumigation, review the Material Safety Data Sheet (MSDS) for paraformaldehyde, ensure access to MSDS during treatments, and comply with all applicable state and local laws. The use directions call for placing nest material or bee cells in well-sealed fumigation chamber that is placed in a building set aside specifically for paraformaldehyde fumigation and not used for any other purpose. The nest material or bee cells are conditioned in the chamber for 48 hours at 20-25°C (68°-77° F) with a relative humidity of 60-70%. Fumigation with paraformaldehyde requires 1.1 lb. /1000 cubic ft. (5 g/cubic ft.) placed in heat generation unit (electric frying pan) attached to electric timer. The timer is set to heat the product for 4 hours at a heat setting of at least 400° (but no more than 475° F) in a sealed and locked chamber. After a 24 hour period, the chamber is ventilated by exhausting from

the top of the chamber and ensuring an adequate incoming flow of fresh air. Ventilation continues for 48-72 hours. Prior to reentry, the air is tested with a device or method capable of measuring 0.1 ppm formaldehyde to ensure that the formaldehyde concentration is below this level before allowing unprotected reentry. If formaldehyde is still detectable above 0.1 ppm, ventilation is required for an additional 24-48 hours. The chamber may be reentered without respiratory protection only after the air concentration of formaldehyde is less than 0.1 ppm.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

EPA's Product Performance Guidelines document (OCSPP 810.2100; March 16, 2012; hereafter referred to as "Guidelines document") does not provide specific guidance for products applied as sprays, gases or foams; this section is "reserved." However, the Guidelines document does give guidance for gas products intended to inactivate *Bacillus anthracis* spores that provides a basic framework for determining efficacy test methods and performance standards for gas fumigant/sterilant/decontaminant products. That framework consists of (a) required laboratory efficacy testing using either a qualitative or a quantitative sporicidal efficacy test method, and (b) required field efficacy testing using biological indicators inoculated with bacterial spores that are set out in a large enclosed space such as a room or other large enclosed space (such multiple rooms or a building).

Laboratory Testing and Performance Standards

Performance standards for laboratory sporicidal efficacy test methods vary according to the specific test method. For example, the performance standard for the AOAC Official Method 966.04 Sporicidal Activity of Disinfectants Test (a qualitative method) is that the product should kill the test spores on all 720 carriers on glass and porcelain penicylinder coupons inoculated with *Bacillus subtilis* and *Clostridium sporogenes* spores without any failures (e.g., growth of test organism after carrier treatment). The performance standard for an equivalent quantitative sporicidal test method is that the product must achieve a 6 log reduction against an acceptable number of coupons inoculated with specific bacterial spores.

Field Testing and Performance Standards

The effectiveness of a sterilant within a sealed enclosure may be supported by efficacy data from "in-use testing" (i.e., field testing) conducted according to an EPA-approved protocol. For fumigants/sterilants/decontaminants, the space in which efficacy will be measured should be a room or structure as large as will be allowed by the label. After the space is fumigated at the requisite concentration and contact time, and then neutralized and aerated, the biological indicators (BIs) must show no growth for specific bacterial spores. All parameters for product application (i.e., temperature, relative humidity, concentration, contact time) must be recorded and shown to be met for all phases of the treatment cycle.

IV SUBMITTED SUPPORTING EFFICACY DATA

MRID 48994701: Formaldehyde Gas Inactivation of *Bacillus anthracis*, *Bacillus subtilis*, and *Geobacillus stearothermophilus* Spores on Indoor Surface Materials. J.V. Rogers, Y.W. Choi, W.R. Richter, D.C. Rudnicki, D.W. Joseph, C.L.K. Sabourin, M.L. Taylor and J.C.S. Chang. Journal of Applied Microbiology ISSN 1364-5072. January 6, 2007.

- In this study, *Bacillus anthracis*, *Bacillus subtilis*, and *Geobacillus stearothermophilus* spores were dried on seven types of indoor surfaces (industrial carpet, bare pine wood, painted concrete cinder block, glass, white formica laminate with matte finish, galvanized metal ductwork, and painted wallboard paper).
- Each coupon was laid flat in a Biological Safety Cabinet (BSC) Class III, and inoculated with approximately 1.0 X 10^8 *B. anthracis*, *B. subtilis*, or *G. stearothermophilus* spores. For each test material, three coupons were used for decontamination, three coupons were used as controls (inoculated, not decontaminated), and two coupons were used as blanks (not inoculated). A micropipette was used to deliver spore suspensions (100 µl) to the surface of each coupon as small droplets and the coupons were allowed to dry overnight.
- The CERTEK Model #1414RH formaldehyde gas generator/neutralizer (CERTEK, Inc., Raleigh, NC, USA) was used for generating formaldehyde gas by heating and depolymerizing paraformaldehyde prills (91–93% purity; Hoechst Celanese Corporation, Dallas, TX, USA).
- The operational parameters included temperature (16–32° C; 60-90° F), relative humidity (Rh, 50–90%), paraformaldehyde concentration (10.5 g paraformaldehyde per cubic meter of treated volume; approx. 8500 ppm theoretical value), decontamination contact time (10 h), and neutralization with ammonium carbonate.
- To calculate the efficacy of the decontamination treatment, the number of viable spores extracted from the decontaminated test coupons was compared with the number of viable spores extracted from the control coupons. Efficacy for biological agents was expressed in terms of a log reduction using the following equation: Log Reduction = log(N/N') where N is the mean number of viable organisms recovered from the control coupons (i.e., those not subjected to decontamination), and N' is the number of viable organisms recovered from each test coupon after decontamination.
- In all tests, the formaldehyde concentration (as measured by the formaldehyde monitor) was maintained at approximately 1100 ppm with a Rh range of 70–75% and a temperature range of 22–23° C during the 10 h contact time.
- The formaldehyde gas exposure significantly decreased viable spores of all organisms on the tested materials. The observed log reduction in viable spores for the three organisms inoculated on all test materials evaluated were >6.0, with the exception of *B. anthracis* on painted wallboard paper and *G. stearothermophilus* on industrial carpet. These results suggest that when using the decontamination parameters outlined in this study, material porosity did not appear to affect decontamination efficacy of formaldehyde gas.

MRID 48994701: Environmental Technology Verification Program. Building Decontamination Technology Center. Test/QA Plan for Verification of Formaldehyde Vapor Technologies for Decontaminating Indoor Surfaces Contaminated with Biological or Chemical Agents. Prepared by Battelle Columbus, Ohio. GSA Contract Number GS-23F-0011L Blanket Purchase Agreement 2C-R903-NBLX. Task Order Number 1104. EPA Task Order Project Officer John C.S. Chang. November 10, 2003.

- In 1997, the EPA established the ETV Building Decontamination Technology Center, which is managed by Battelle, of Columbus, OH, under contract with EPA. Verification tests were conducted in the Center in accordance with the ETV process, under the direction of the EPA. All verification activities were subject to the Quality Management Plan (QMP) and the generic verification protocol for the Center. In performing each verification test, Battelle followed the procedures described in this document and developed a separate test/QA plan appropriate for the decontamination technology being tested. This document (MRID 48994701) is the test/QA plan for verification testing of the formaldehyde decontamination technology performed in the 2007 study cited above (MRID 48994701).

MRID 48994701: Compilation of Available Data on Building Decontamination Alternatives. Prepared by Science Applications International Corporation for the U.S. Environmental Protection Agency, National Homeland Security Research Center, Office of Research and Development. May 27, 2005.

- The National Homeland Security Research Center of the United States Environmental Protection Agency funded this study. This study evaluated the decontamination of *Bacillus anthracis*, *Bacillus subtilis*, and *Geobacillus stearothermophilus* spores on indoor surface materials using formaldehyde gas. Formaldehyde gas has been used for many years by U.S laboratories nationwide to successfully inactivate a wide range of human pathogens, including *Bacillus spp.* spores, in a wide range of sites such as biological safety cabinets, air ducts, rooms and buildings.
- Pages 151-167 of this document describe the use of paraformaldehyde for laboratory decontamination, and summarized available laboratory efficacy data conducted in small biological safety cabinets and small rooms. The document reports two significant field uses of paraformaldehyde: (1) a 2002 fumigation of mail sorting equipment at the Department of Justice's Landover Operations Center and (2) a 1998 fumigation of a large area (78,000 cubic feet) in the Hazelton Research Center in Reston, VA to inactivate suspected Ebola virus contamination. The document also reports EPA's granting of quarantine exemptions to the Department of Defense and the U.S. Department of Agriculture for use of PF to decontaminate high-containment laboratories at Plum Island, NY and Ames, IA beginning in the 1990s.
- This document indicates that paraformaldehyde may be generated either through the use of "hot plates" or with a formaldehyde generator (vaporizer).
- This document points out a wide range of concentrations of formaldehyde gas that have been reported to be efficacious (i.e., 100 to 10,500 mg/m^3) although it appears that most federal laboratories use 0.3 g/ft^3.

- This document notes that the largest space treated to date was about 100,000 cubic feet.

V ADDITIONAL EFFICACY DATA PREVIOUSLY REVIEWED BY EPA IN RELATION TO SECTION 18 REQUESTS

"Paraformaldehyde for Surface Sterilization and Detoxification" by Larry A. Taylor, Manuel S. Barbeito and Gardner G. Gremillion. 1969. Applied Microbiology, April 1969, pp. 614-618.

Bactericidal Tests

- The paraformaldehyde tested was 91% to 95% formaldehyde with 9 to 5% water.
- The powdered paraformaldehyde was depolymerized by heating on a modified hot plate or in household electric frying pan at a controlled temperature of 232°C (450° F).
- Two microbiological laboratory rooms (4,598 and 2,250 ft^3) and a mobile laboratory trailer (2,200 ft^3) were seeded with *B. subtilis* var. *niger* spores and *S. marcescens* organisms by placing suspensions at delineated sites.
- Static air condition was achieved during the test, and relative humidity was adjusted to 60% at a room temperature of 23.3°C.
- The paraformaldehyde powder (1,379 g in the 4,598 ft^3 room, 675 g in the 2,250 ft^3 room, and 330 g for the mobile trailer) was placed in the disseminators, the entrance door was sealed, and dissemination was initiated and continued for 1 hour contact time.
- The treated rooms were aerated for 1- 2 hours before re-entry, and sampling with sterile, moistened cotton swabs and subsequently streaked on agar plates. Plates were incubated for 48 hours at 37°C.
- Bacterial spores of *B. subtilis* var. *niger* at a concentration of 10^7 spores/mL and *S. marcescens* organisms at the highest concentration of 2×10^{10} organisms/ml were eliminated after treatment with 0.3 g of paraformaldehyde /ft^3 of space for a 1 hour contact time.

Virucidal Tests

- Newcastle disease virus (NDV) GB strain at a concentration of 106.5 ELD_{50}/mL was used both as an aerosol and as a suspension inoculated on filter patches, and a 2 mL amount of the virus-suspension was nebulized into a 1 ft^3 chamber and allowed to dry for 15 minutes.
- The temperature and relative humidity in the 1 ft^3 chamber cabinet were 24°C and 60%, respectively.
- The formaldehyde gas (0.3 g of paraformaldehyde) was released into the chamber and after a 1 hour contact the surfaces were sampled.
- The filter pads inoculated with NDV were placed in a Class I cabinet with a temperature and relative humidity of 24°C and 60%, respectively.
- Following dissemination of the gas into cabinet (approximately 42 ft^3) and maintenance at 30 minutes (contact time), the filer patches were aseptically transferred to sterile nutrient broth for recovery, identification and confirmation of NDV.
- NDV at a concentration of $10^{6.5}$ ELD_{50}/mL was sterilized after depolymerization of 0.3 g of paraformaldehyde/ft^3 of space at 30 minute contact time.

"The Practical Use of Formaldehyde Vapor for Disinfection" by J.R. Songer, D.T. Braymen, R.G. Mathis, J.W. Monroe. 1972. Health Lab Science. Jan; 9 (1): 46-55.

- Paraformaldehyde (91-95%) was used at rate of 0.3 gm/ft^3 of space.
- The authors stated that formaldehyde is ineffective as a gaseous disinfectant at a relative humidities below 70%, and the temperature should be 20°C or higher.
- In this study, electric frying pans were not totally satisfactory as formaldehyde vapor formed on the hot surface of the fry pan holding the bulk of the paraformaldehyde from the pan surface. The powdered form of paraformaldehyde also acted as insulation restricting the vaporizing temperature to near the hot surface. The actual data was not presented in this publication.
- To address some of the practical problems associated with paraformaldehyde in larger areas (4,300 ft^3), one generator was used for half of the studies and two generators were used for the other. Approximately 45 minutes were required to vaporize 3 pounds of paraformaldehyde using two generators; when one was used it required 68 minutes. Sixteen tests were conducted using two generators and eight using one generator. Spore strips contained at 3 x 10^7 *Bacillus subtilis* spores. In all tests, all of the *B. subtilis* spores were killed within 6 hour exposure. In every test except one, all of the spores were killed within 4 hour exposure.
- The authors concluded it is advisable to vaporize 0.3 g of paraformaldehyde/ft^3 of space within 45 minutes. Below 60% RH, formaldehyde vapor was not an effective sterilant. Furthermore, excessively high RH was also problematic (i.e., when the RH exceeded 90%, formaldehyde vapor concentration was depressed). Formaldehyde vapor was effective as a sterilizing agent at room temperature.

VI CONCLUSIONS

1. The submitted laboratory and field test data provided in the first EPA study cited above (MRID 48994701) <u>support</u> the registration of the new uses of 91% or greater paraformaldehyde for fumigating/sterilizing/decontaminating laboratories and laboratory equipment as well as leafcutter bee cells and nesting materials. In these studies, the test results for formaldehyde efficacy are acceptable to support the quantitative sporicidal performance standard by achieving at least a 6 log reduction in the number of viable spores on porous and non-porous materials for three bacterial spore types-- *Bacillus anthracis*, *Bacillus subtilis* and *Geobacillus stearothermophilus.*

2. The second study cited above (MRID 48994701), the test QA/QC plan for the first EPA study (MRID 48994701), demonstrates that the first EPA study <u>was conducted according to EPA's Good Laboratory Practices requirements</u>.

3. The third document cited above (MRID 48994701), a literature review of available efficacy testing for fumigation use of formaldehyde gas, <u>supplementally supports</u> the registration of the proposed new uses of paraformaldehyde.

4. The fourth and fifth documents cited above (Taylor et al., 1969; Songer et al, 1972), which were previously submitted by other federal agencies in support of Section 18 requests and reviewed by the Antimicrobials Division, <u>supplementally support</u> the registration of the proposed new uses of paraformaldehyde.

VII RECOMMENDATIONS

1. All of the above documentation supports an application rate of 0.3-0.6 g/ft^3 of paraformaldehyde (91% or greater) when it is heated in an electric frying pan or created by a formaldehyde gas generator and held for a contact time of at least 10 hours at 60-90% relative humidity and at 60°-90° F (16-31° C). The proposed Laboratory Product Manual specifies use directions which fall within these use parameters and are acceptable.

2. The proposed Leafcutter Bee Product Manual application rate is 5 g/ft^3 (rather than 0.3-0.6 g/ft^3 for the lab uses) and the contact time is 24 hours (rather than 10 hours for the lab uses). However, the higher application rate and contact time are acceptable since the surfaces in bee cells and nesting materials cannot be precleaned, and, therefore, the increased application rate and contact time are acceptable to assure efficacy.