North Carolina Department of Environment and Natural Resources
Division of Waste Management

Pat McCrory
Governor

Dexter R. Matthews
Director

John E. Skvarla, III
Secretary

May 31, 2013

Penelope J. Padgett, Ph.D., M.P.H.
Associate Biological Safety Officer
University of North Carolina at Chapel Hill
Department of Environment, Health, Safety
1120 Estes Drive Extension
Campus Box 1650
Chapel Hill, NC 27599-1650

Dear Penelope J. Padgett:

This is in response to your letter requesting approval of the chemical treatment of *Histoplasma capsulatum* wastes using Vesphene solution, as described in the request for approval submitted to the Division.

According to 15A NCAC 13B .1207(4)(b) the Division is authorized to approve the alternative chemical treatments of microbiological wastes.

The chemical treatment of the organism listed above and described in the procedures for treatment which was submitted with your letter of May 6, 2013, is approved.
It is the responsibility of the user of this product to assure that this product is being used in a manner which is consistent with federal, state and local law.

Should you have any questions regarding this matter you may contact me at (919) 707-8245 or Bill Patrakis at (919) 707- 8290.

Sincerely,

Ellen Lorscheider

Ellen Lorscheider
Environmental Programs Manager

Cc: Bill Patrakis, Environmental Scientist

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Request for Approval

Chemical Treatment of Liquid Infectious Waste

Approval for chemical treatment of liquid infectious waste must be obtained from the NC Division of Waste Management. Please provide answers to the following questions, attach supporting documents as outlined below, and submit your request to Penelope J. Padgett, EHS, CB #1650. EHS will submit your request to the NC Division of Waste Management.

Request for approval must be substantiated by results of demonstrated effectiveness of the chemical to treat the specific microbiological agent(s) of concern for the waste disposed.

I. Description of infectious waste
   a. Describe waste to be treated (i.e. cultures, cell lines): Fungal cultures grown in Histoplasma Macrophage Media (HMM) agar plates or broth medium
   b. Organisms present: *Histoplasma capsulatum* yeast and conidia
   c. Estimated concentration/titer of organisms: 10^8 for yeast cells and 10^6 for conidia
   d. Other material present in waste (i.e. other organic material): HMM medium
   e. Volume of waste and frequency: 1 – 500 mls, 3-5 times per week

II. Description of treatment procedures
   a. Summarize proposed procedure for treating waste: Fungal cultures will be treated with Vesphene disinfectant (at a final conc. of 1:64 or 1.6%) for at least 30 minutes.
   b. Disinfectant to be used (please attach MSDS): Vesphene
      i. 2-phenylphenol [CAS#90-43-7]
      ii. p-tertiary amylphenol [CAS# 80-46-6]
      iii. Potassium hydroxide [CAS# 1310-58-3]
      iv. Sodium hydroxide [CAS # 1310-73-2]
   c. Disinfectant concentration: 1:64 or 1.6% of Vesphene
      i. 2-phenylphenol – 0.14%
      ii. p-tertiary amylphenol – 0.119%
      iii. Potassium hydroxide – 0.078%
      iv. Sodium hydroxide – < 0.03%
   d. Ratio of disinfectant (ml) to liquid waste (ml): 1:64 (≥2 -fold more disinfectant than required to kill the tested organisms, see below)
   e. Contact time of disinfectant with liquid waste prior to disposal: Yeast and conidia cultures are completely inactivated by 30 minutes (see below). To ensure inactivation, cultures will be incubated for at least 30 minutes with disinfectant.

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f. Small variations in temperature, time, pH, concentration and state of dispersion, penetrability, reactivity of organic material may make large differences in the effectiveness of disinfection. List the factors that may affect disinfection:

Temperature and culture conditions do not vary.

III. Verification of efficacy of treatment procedures

a. Submit results of experiments that verify the proposed procedures are effective.

Such studies may include attempts to recover and quantitate the agent from liquid or swab samples, or sealed patches, by animal inoculation, plaque assay, agar or broth cultivation and similar methods, following controlled decontamination under the same experimental conditions envisioned for proposed studies. Reports of these studies should be provided with this document in support of your request.

- To test the killing efficiency of various commercial disinfection products on *Histoplasma capsulatum*, yeast cultures were grown to late log/stationary phase in a defined broth media (HMM) at 37°C, 5% CO_2, with shaking. Disinfectants were added to one ml of undiluted culture so that the final concentration was at 1X the manufacturer's recommendations (Bleach – 100 µl, Amphyl – 100 µl, Vesphene – 8 µl, TB Fresh Breeze (TBFB) – 100 µl). Ethanol was also tested but at a much higher final concentration (95% Ethanol – 500 µl; ~32% final concentration). All treatments were incubated at room temperature for 30 minutes. After incubation, the entire treated sample was spread plated onto HMM agarose plates and allowed to incubate at 37°C with 5% CO_2 for up to 30 days post-treatment. Colony forming units (CFUs) of *Histoplasma* starting cultures were determined by performing ten-fold serial dilutions and plating 100 µl volumes onto HMM agarose plates which where incubated at 37°C with 5% CO_2. Yeast colonies from untreated samples were visible within 7 days.
- For testing of killing efficiency against the conidia form of *Histoplasma*, plated cultures were incubated at room temperature (without CO_2) for several weeks to induce sporulation. Conidia were then harvested and resuspend in 1X PBS. Disinfectant treatments of conidia were performed identically as stated above for the yeast phase cultures. CFUs were also determined as stated above and again were visible within 7 days.
- Results for this experiment showed no growth at 30 days post-treatment for all treatment conditions for both yeast and conidia cultures. As Amphyl is no longer being manufactured, we will be using Vesphene as our replacement disinfectant. Final concentrations of Vesphene we propose on using will be 1:64 ($\geq$ 2 fold disinfectant

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required to kill test organisms) with a minimum incubation time of 30 minutes.

b. Please attach any publications that will support the use of this disinfectant under the proposed conditions. These publications cannot be provided in lieu of the experiments described above unless the publication describes the same treatment procedures for the infectious waste described in Section I (including concentration of organism, organic material present, type of waste, organisms).

Phenolic based disinfectants (such as Amphyl, Lysol, and Vesphene) are commonly used in laboratory and hospital settings to kill gram-negative and gram-positive bacteria, mycobacterium, pathogenic fungi, and human viruses.
The following references are attached to demonstrate the effectiveness of phenolic compounds on gram-negative bacteria.

1. Wilder, A.F. & Frei, R. Decontamination, Disinfection, and Sterilization. 2007. In P.R. Murray, E.J. Baron, J.H. Jorgensen, M.L. Landry & M.A. Pfaller (Eds.), *Manual of Clinical Microbiology* (9th ed., pp. 65-96). Washington, D.C.: ASM Press. This chapter states the susceptibility of Histoplasma capsulatum to various disinfectants (1% solutions of sodium hypochlorite, 2% phenol, 2% glutaraldehyde, isopropyl alcohol, and formaldehyde)

2. Kruse, R.H., Green, T.D., Chambers, C., and Jones, M.W. Disinfection of Aerosolized Pathogenic Fungi on Laboratory Surfaces: II Tissue Phase. 1963. Applied Microbiology. Vol. 11, No. 5: p. 436-445. This paper shows the effects of several fungicides against pathogenic fungi yeast forms (including *Histoplasma capsulatum*)

3. Kruse, R.H., Green, T.D., Chambers, C., and Jones, M.W. Disinfection of Aerosolized Pathogenic Fungi on Laboratory Surfaces: II Culture Phase. 1964. Applied Microbiology. Vol. 12, No. 2: p. 155-160. This paper shows the effects of several fungicides against pathogenic fungi spore forms (including *Histoplasma capsulatum*)