



North Carolina Department of Environment and Natural Resources

Dexter R. Matthews, Director

Division of Waste Management

Michael F. Easley, Governor
William G. Ross Jr., Secretary

January 2, 2007

Donii Fox, MSPH, CIH
Biological Safety Officer
University of North Carolina at Chapel
Department of Environment, Health, Safety
1120 Estes Drive Extension
Campus Box 1650
Chapel Hill, NC 27599-1650

Dear Donii Fox:

This is in response to your letter requesting approval of the chemical treatment of *Dengue Fever* virus using bleach as described in the request for approval submitted to the Department.

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 *Dengue Fever* virus as described in the procedures for treatment which was submitted with your letter of December 18, 2006, is approved.

The test descriptions and documents which were submitted to the Department substantiate the efficacy of the treatment of the organism with bleach.

Should you have any questions regarding this matter you may contact me at (919) 508-8499 or Bill Patrakis at (919) 508- 8512.

Sincerely,

Ellen Lorscheider
Environmental Programs Manager

Cc: Bill Patrakis, Environmental Biologist



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 Donii Fox, 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): C636 mosquito cells, VERO monkey cells, U937 human monocyte cells
 - b. Organisms present: Dengue virus
 - c. Estimated concentration/titer of organisms: 10^4 virus particles/ml
 - d. Other material present in waste (i.e. other organic material): ,10% fetal bovine serum, sodium bicarbonate
 - e. Volume of waste and frequency: <1 liter/wk
- II. Description of treatment procedures
 - a. Summarize proposed procedure for treating waste: Liquid containing Dengue virus will be collected from multi well plates by aspirating or pipeting into a Nalgene beaker containing 5 equivalent volumes Ultra Clorox regular bleach=6.15%
 - b. Disinfectant to be used (please attach MSDS): 1% sodium hypochlorite (bleach)
 - c. Disinfectant concentration: >1%
 - d. Ratio of disinfectant (ml) to liquid waste (ml): 5:1
 - e. Contact time of disinfectant with liquid waste prior to disposal: 1 hr minimum up to 6 hrs. at ambient temperature
 - 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 is fairly constant at 22-25C. No solids are placed in the waste container and liquids are well dispersed. Very little organic material is present compared with the volume of bleach and buffers. The organic material is fetal bovine serum in the culture media.
- 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.
 - i. Attached is an MSDS for Dengue fever virus showing susceptibility to 1% sodium hypochlorite as well as other common disinfectants. In



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addition attached is a passage from Clinical Virology referencing Inactivation by chemical agents including chlorine.

- ii. Our methods dilute virus and culture media by at least 5 fold in a mixture of bleach and diluting buffer (phosphate buffered saline). The virus particles cannot be recovered from the treatment solution in order to test infectivity as bleach would kill any live cells used as indicators and any lower titers by increased dilution would be too low to detect infection. There is no animal model for Dengue virus.
- 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).