



Waste Management  
ENVIRONMENTAL QUALITY

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October 24, 2016

Dan Eisenman, Ph.D., RBP, SM(NRCM) CBSP  
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 Dan Eisenman:

This is in response to your letter requesting approval of the chemical treatment of *Dengue virus*, *Zika virus*, and the attenuated strains of Japanese Encephalitis and Yellow Fever virus using sodium hypochlorite as a 10% bleach 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 alternative chemical treatment of the organisms listed above and described in the procedures for treatment which was submitted with your letter of September 21, 2016, is approved.

A written plan must be placed at the facility and units of the facility to ensure consistent procedures are used to treat the waste.

Should you have any questions regarding this matter you may contact me at (919) 707-8280 or Bill Patrakis at (919) 707- 8290.

Sincerely,

Martin A. Gallagher  
Environmental Programs Manager



**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 Daniel Eisenman, Biological Safety Officer. 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.**

**Laboratories of Aravinda de Silva and Hellen Lazear  
Burnett-Womack, 9<sup>th</sup> Floor (9004, 9005, 9006, 9007, 9008, 9009, 9010, 9026)  
Marsico Hall, 6<sup>th</sup> floor (6223C, 6229F, 6230E)  
Microbiology and Immunology Department, UNC-CH**

**I. Description of infectious waste**

- a. **Describe waste to be treated (i.e. cultures, cell lines):** Cultures of infected C6/36 mosquito cells, Vero monkey cells, U937 human monocyte cells. Wash buffer from immunoassays.
- b. **Organism present:** Dengue virus, Zika virus, Japanese Encephalitis virus attenuated vaccine strain, Yellow Fever virus attenuated vaccine strain.
- c. **Estimated concentration/titer of organism:**  $<10^4$  virus particles/ml
- d. **Other materials present in the waste (i.e. other organic material):** 10% fetal bovine serum, sodium bicarbonate, borate buffer, phosphate saline buffer, carbonate buffer.
- e. **Volume of waste and frequency:**  $< 1$  liter/wk

**II. Description of treatment procedures**

- a. **Summarize proposed procedures for treating waste:** Liquid containing the flaviviruses listed above will be collected from culture vessels and the multi-well plates by aspirating or pipetting into a rigid waste container or Nalgene beaker containing 1/10 equivalent volume of Ultra Clorox regular bleach, which is 5.25%-6.15% sodium hypochlorite.
- b. **Disinfectant to be used (please attach MSDS):** Household Bleach, freshly diluted 1:10 to achieve 0.5% sodium hypochlorite (This results in about 5,250-6,150 ppm of available chlorine)
- c. **Disinfectant concentration:**  $>0.5\%$
- d. **Ratio of disinfectant (ml) to liquid waste (ml):** 1:10
- e. **Contact time of disinfectant with liquid waste prior to disposal:** 20 min.
- f. **Small variations in temperature, time, pH, concentration and state of dispersion. Penetrability reactivity of organic material may result in large differences in the effectiveness of disinfection. List the factors that may affect disinfection:** Temperature is fairly constant at 22-25°C. No solids are placed in the waste container and liquids are well dispersed. Very little organic material is present compared to the volume of bleach and buffers. The organic material is fetal bovine serum in the culture media which is  $<1\%$  of the waste volume.



### 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 envision for the proposed studies. Reports of these studies should be provided with this document in support of your request.**
  - i. Our methods dilute virus and culture media by 10 fold in a mixture of bleach and diluted buffer (phosphate buffered saline), and <1% fetal bovine serum. 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. However, there is extensive evidence of sodium hypochlorite as a potent virucidal agent against enveloped viruses including flaviviruses.
  - ii. Attached are references to support the proposed procedure.
    1. Rutala, W. 1996. APIC guideline for selection and use of disinfectants. 4(4):313. (Well established virucidal effect of household bleach).
    2. Rutala W. and Weber, D. and the Healthcare Infection Control Practices Advisory Committee (HICPAC). 2008. CDC – Guideline for Disinfection and Sterilization.
    3. MSDS for Zika and other flaviviruses showing the susceptibility to 0.5% sodium hypochlorite as well as other common disinfectants.
    4. Muller J et al., 2016. “Inactivation and environmental stability of Zika Virus”, by Muller J. and collaborators. Journal Emerging Infectious Diseases, vol 22(9):1685, September 2016. [In this article, the authors show that a Zika virus stock containing 2.5%, 10%, 40% and 90% fetal calf serum were completely disinfected (TCID50 below level of detection) after treatment with 1% hypochlorite for 1 minute.]
    5. Clinical Virology, ASM Press, 2002. (References inactivation of viruses by chemical agents including chlorine).
    6. Klein M, DeForest A. 1963. The inactivation of viruses by germicides. Chem. Specialists Manuf. Assoc. Proc. 49:116-8. (Virus were inactivated in 10 minutes with 200 ppm available chlorine).
- 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 concentrations of organism, organic material present, type of waste, organisms). Publications attached.**