



NORTH CAROLINA
Environmental Quality

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June 21, 2019

Eric Lewis, Ph.D.
Biosafety Specialist
Department of Environment, Health and Safety
University of North Carolina at Chapel Hill
Chapel Hill, NC 27599

Re. Chemical Treatment of Liquid Waste Containing MMLV Based Retroviral Vectors
using 10% Bleach - Alternative Treatment Method Approval Request

Mr. Lewis,

The North Carolina Department of Environmental Quality, Division of Waste Management – Solid Waste Section has reviewed the documentation submitted to our office, received June 17, 2019. This letter is in response to your request for approval for the use of the medical waste treatment method specified above.

The Medical Waste Management rules 15A NCAC 13B .1200 through .1207, specifically Section .1207(4)(b), references the parameters applicable to the request for the use of 10 percent bleach as a regulated medical waste treatment type. The documentation submitted explains the effectiveness of the treatment type on retroviruses and considers the other factors as stated in the Section. The alternative treatment method approval request appears to meet the required parameters and is approved.

It is the responsibility of the healthcare facility to ensure that treated regulated medical waste is non-infectious prior to disposal. Documentation describing the treatment process shall be maintained.

The Medical Waste Management rules are scheduled to be updated. The public comment period for the proposed Rules ([Proposed 15A NCAC 13B .1200 - .1204](#)) began June 17th and ends August 16th. It is expected that the proposed Rules will become effective January 2020. Section .1204(e) is specific to chemical treatment. The healthcare facility is encouraged to meet the requirements as stated in the proposed rule. Section .1204(h) is specific to alternative treatment but would likely not be applicable if the parameters of Section .1204(e) are met.



If you have questions or concerns, please contact me at ph. 336.776.9673 or email john.patrone@ncdenr.gov.

Sincerely,

John Digitally signed
by John Patrone
Date: 2019.06.21
13:05:10 -04'00'
Patrone

John Patrone
Environmental Specialist II
NCDEQ, DWM - SWS

cc: Adam Ulishney, Environmental Compliance Branch Head – SWS

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 EHS Biosafety Group, 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): Human cell line cultures and primary human cell cultures containing Moloney Murine Leukemia Virus (MMLV)-based retrovirus and saline solutions for washing cells. The virus is replication deficient in the absence of packaging cells.
 - b. Organisms present: Replication-incompetent MMLV-based retrovirus
 - c. Estimated concentration/titer of organisms: $\leq 10^4$ virus particles/mL
 - d. Other material present in waste (i.e. other organic material): fetal calf serum
 - e. Volume of waste and frequency: less than 500 mL per month
- II. Description of treatment procedures
 - a. Summarize proposed procedure for treating waste: All work is conducted in a biological safety cabinet. Liquid waste is pipetted or poured into a rigid plastic bottle followed by addition of a 1/10 equivalent volume of Clorox germicidal bleach (8.25% sodium hypochlorite prior to dilution). Waste is incubated for at least 1 hour and up to 18 hours (overnight) at room temperature.
 - b. Disinfectant to be used (please attach MSDS):
 - i. Clorox germicidal bleach (8.25% sodium hypochlorite) diluted 1:10
 - c. Disinfectant concentration:
 - i. 0.825% sodium hypochlorite (10% dilution resulting in about 8,250 ppm of available chlorine)
 - d. Ratio of disinfectant (ml) to liquid waste (ml): 1:10
 - e. Contact time of disinfectant with liquid waste prior to disposal: 1 to 18 hours at ambient temperature (21-23°C)
 - f. Small variations in temperature, time, pH, concentration and state of dispersion, penetrability, reactivity of organic material may make large

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differences in the effectiveness of disinfection. List the factors that may affect disinfection:

The time of disinfection will always be at least 1 hour, and the temperature of the room is stable (21-23°C). The organic material in the waste solution includes human cells and approximately 1% fetal calf serum, which is very low compared to the volume of wash buffers and bleach. Sodium hypochlorite will be added to a final concentration of 8,250 ppm and mixed well so that it is well dispersed in the liquid waste.

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. This method dilutes the virus and culture media by 10 fold in a mixture of bleach, phosphate buffered saline and serum-free media. The final concentration of fetal bovine serum is approximately 1%. The treated waste solution cannot be used to test infectivity since 10% bleach would kill any live cells used as indicators. Diluting the waste solution to reduce the bleach concentration would make viral titers too low to detect infection. However, there is extensive research demonstrating that sodium hypochlorite is a potent virucidal agent against retroviruses.
- ii. Attached are references to support the proposed procedure. They demonstrate the effectiveness of bleach to kill retroviruses, including HIV and HTLV-III:
 1. Rutala, W. and Weber, D. and the Healthcare Infection Control Practices Advisory Committee (HICPAC). 2008. CDC Guideline for Disinfection and Sterilization. <https://www.cdc.gov/infectioncontrol/guidelines/disinfection/disinfection-methods/chemical.html>
(This is a very long document and I only included the relevant sections on chlorine and chlorine compounds, which summarizes the well-established virucidal effect of sodium hypochlorite.)
 2. Resnick, L., Veren, K., Salahuddin, S.Z., Tondreau, S and Markham, P.D. 1986. Stability and inactivation of HTLV-III/LAV under clinical and laboratory environments. JAMA 255:1877-1891. (Demonstrated that 0.5% sodium hypochlorite



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- inactivated a highly concentrated stock of infectious HTLV-III diluted in 50% human plasma within one minute.)
3. Martin, L.S., McDougal, J.S. and Loskoski, S.J. Disinfection and inactivation of the human T lymphotropic virus type III/Lymphadenopathy-associated virus. *J Infect Dis* 152:400-403. (Demonstrated that 0.1% sodium hypochlorite inactivated HTLV-III within 10 minutes.)
 4. Bloomfield, S.F., Smith-Burchenll, C.A. and Dalgleich, A.G. 1990. Evaluation of hypochlorite-releasing disinfectants against the human immunodeficiency virus (HIV). *J Hosp Infection* 15:273-278. (Demonstrated that 50 ppm sodium hypochlorite inactivates HIV in 2 minutes in the absence of other organic matter, 2500 ppm sodium hypochlorite inactivates HIV within 2 minutes in the presence of 10% plasma, and 5000 ppm sodium hypochlorite inactivates HIV in 2 minutes in the presence of 50% blood.)
 5. Aranda-Anzalso, A., Viza, D. and Besnel, R.G. 1992. Chemical inactivation of human immunodeficiency virus in vitro. *J Virol Methods* 37:71-82. (Concluded that sodium hypochlorite and Triton X-100 were the most effective agents of the ones they tested to inactivate HIV in a short period of time (1 to 2 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).

The publications listed in section a above are attached.

IV. Appendix

- A. SDS for Clorox germicidal bleach, 8.25% sodium hypochlorite