## Review of Report on Evaluation of Gaseous Ozone as an Efficient Virucidal Agent in Conditions Relevant to Cruise Liners and Health-care Facilities prepared by Dr. JB Hudson and Dr. M. Sharma, Viroforce Systems Inc.

The above report was received in electronic format on 2006-04-28 from Dr. Hudson.

This comprehensive report addresses the studies undertaken by Viroforce Systems Inc. and its predecessor, Treated Air Systems. The findings in this document had in part been reviewed by me previously.

The studies explored the ability of ozone ($O_3$) to inactivate representative human viruses under well controlled laboratory conditions as well as in rooms such as would be found in health-care facilities and cruise ships. The investigations consisted of first proving that Ozone at 30 to 100 ppm could effectively inactivate greater than 99% of each representative virus when it is dried on specific surfaces such as steel, plastic and glass in a well-controlled polycarbonate chamber. The investigation then expanded into determining whether relative humidity would enhance the inactivation of viruses by $O_3$. Finally, the laboratory findings were applied to testing the ability of $O_3$ to inactivate viruses under field conditions such as representative rooms in a building. These investigations represent a logical, focused approach to determining whether $O_3$ was an acceptable agent for virus inactivation in an applied setting.

To carry out these investigations well-pedigreed stocks of the viruses to be tested were obtained and methods to measure the concentrations of viable viruses both before and after treatment were implemented. The challenge of quantitating norovirus (NV), which cannot be grown in cell cultures, was elegantly resolved by applying a quantitative the nucleic acid-based technique, RT-PCR after validating its performance with a closely related but quantifiable feline calicivirus (FCV). The methods of quantifying viruses used in these studies are well-established and in the case of NV relatively novel but scientifically sound.

The underlying premise for these studies was that viruses shed from an infected person into the environment attach to surfaces. Hence, the study measured only the inactivation of viruses dried on specific representative surfaces. This premise is realistic although viruses also spread through aerosols as well as direct contact. In the case of aerosol-borne viruses, it is likely that $O_3$ would prove a superior agent for their inactivation, although this would have to be documented in a subsequent study.

The viruses investigated include both large viruses such as influenza and herpes simplex virus (HSV) whose structures include a membrane envelope, and small viruses such as caliciviruses (FCV and NV) and picornaviruses (poliovirus and rhinovirus). These viruses represent a wide spectrum of virus structures and susceptibilities to inactivation. The virus preparations and their respective concentrations are appropriately documented in the report.

The first investigation undertaken was to establish that the viruses retained their viability after drying (Fig. 1). The findings showed that there was a loss of between 20 to 50% of viable viruses over 2 hours of drying at room temperature. Since the viruses were used in concentrations of 10e6 or greater, there was still a very substantial amount of infectious virus present by 2 hours after being dried on a surface. These findings establish that the use of viruses dried on surfaces in the subsequent inactivation studies is acceptable.

The effects of the surface on which the virus is dried were then explored (Fig. 2, 3). Influenza, HSV, murine coronaviruses and rhinovirus were inactivated to essentially the same extent and the same rate over 30 minutes exposure to $O_3$. It was assumed that the concentration of $O_3$ was the same for each virus inactivation study although this was not documented in the descriptions of these experiments. The experiments were conducted at ambient relative humidity of the room.

Norovirus (NV) is a very important agent of gastroenteritis especially on cruise ships and health-care institutions. However, the virus presents a special challenge because to date it cannot be grown in cell culture precluding the application of standard infectivity assays for its quantitiation. This has been nicely resolved by using another virus of the calicivirus family namely the feline calicivirus (FCV) which grows well in cell culture, and a quantitative RT-PCR assay. It was shown that the concentration of infectious FCV corresponds to the concentration of genomic RNA which can be quantitated by a real -time RT-PCR assay. It was then shown that exposure of FCV to 50ppm $O_3$ resulted not only in its inactivation but was reflected by the substantial decrease in the amount of genomic RNA detectable after treatment as shown in Fig. 6. This approach was applied to NV and it was shown (Fig. 7) that exposure to $O_3$ resulted in a marked decrease in the amount of genomic RNA detectable by RT-PCR. It was further determined that exposure to $O_3$ resulted in a parallel loss of infectivity and of the decrease of the RT-PCR assay signal (Fig. 8). However, it must be recognized that the inactivation of a virus by $O_3$ could either involve damage to surface proteins of the virus or the viral genome. It is likely that damage to surface components of the virus results in loss of infectivity well before damage to the viral genome as detected by the RT-PCR assay can be recognized. This likely accounts for the discrepancy in Fig. 8 between the infectivity and RT-PCR findings. However, the RT-PCR and infectivity assays, both measure damage to the virus but at different levels of sensitivity.

When stool specimen preparations containing NV were exposed to $O_3$ the amount of genomic RNA as detected by RT-PCR was substantially reduced. This indicated that $O_3$ is effective in inactivating NV under the conditions of this study. This is of course not surprising since $O_3$ very effectively inactivates FCV which is structurally nearly identical to NV, both being members of the of the *Caliciviridae* family.

The action of $O_3$ on biological materials generally occurs in an aqueous milieu. Hence, an increase in the relative humidity would likely result in a more effective action of $O_3$. However, the effects of enhancing the humidity may also have an effect on infectivity of the dried virus. This was shown to be the case (Table 1) when FCV but not influenza were subjected to high humidity alone. The concept of increasing the humidity to enhance the inactivation of viruses by ozone is an inexpensive refinement of the system. However, 99% humidity in a room setting may be difficult to achieve.

The field tests using the herpes, polio and rhinoviruses subjected to $O_3$ from a Treated Air Systems generator demonstrated a substantial inactivation of the viruses throughout the room (Table 4). Enhanced inactivation was obtained using $O_3$ from the Viroforce generator especially at a relative humidity of 70%. (Tables 5, 6)

Additional investigations showed that the presence of increased organic load through the inclusion of serum in the medium containing the viruses did not influence their inactivation by ozone. This was a relevant finding since viruses are often associated with organic material such as the case NV in stools.

**Overall, the investigations performed at Viroforce Systems Inc. showed that $O_3$, especially at elevated humidity is an effective agent for inactivation of structurally diverse viruses on dried on surfaces such as would be expected to occur in environmental contamination. The inactivation studies in field settings generated findings consistent with those obtained in the controlled laboratory setting. The $O_3$ from the Viroforce generator was produced in adequate amounts to maintain a sufficient concentration to be effective in field settings. The studies were well designed from a scientific perspective and focused on the primary aim of proving that $O_3$ is an effective agent for virus inactivation in rooms potentially contaminated with the specific viruses. Based on the findings in the above report, this aim has been achieved.**

*Review prepared on 2006-05-12 by: Martin Petric, PhD, FCCM*

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