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Short Communication

Tetranitromethane as a surface antiviral disinfectant

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Abstract

Tetranitromethane, a protein nitrating agent, was tested for its ability to disinfect surfaces from viruses. Different surfaces on commercially available pocket calculators were pretreated with either the Indiana strain of vesicular stomatitis virus or the Herts' strain of Newcastle disease virus. The calculators surfaces were then sprayed with either tetranitromethane or control solutions. The calculators were incubated for 30 min at ambient temperature, and then the surfaces were wiped with sterile swabs. The swabs were placed into test tubes containing phosphate-buffered saline. Samples of the phosphate-buffered saline were then titered on appropriate cell lines by plaque assay. The results indicated that the amount of vesicular stomatitis virus and Newcastle disease virus recovered from the tetranitromethane-treated surfaces was dramatically decreased compared to the amount of virus recovered from control-treated surfaces. These data suggest that tetranitromethane may be useful to disinfect surfaces from both enveloped and non-enveloped RNA viruses.

Keywords: Disinfection; Tetranitromethane; Broad spectrum disinfectant

Disinfection of surfaces from viruses and other microbiota without destroying those surfaces remains a serious difficulty. It has become an even more serious problem as more sophisticated computer and electronic equipment has been developed. In order to be effective. the disinfectant would have to not require large amounts of water, so as not to damage electronic circuits. The disinfectant would also have to leave little to no residue, and be non-toxic to animals, especially humans (Block, 1983).

Tetranitromethane (TNM) is a common nitrating agent for proteins that appears to fulfill the above criteria. TNM chemically modifies tyrosine side chains (Riordan et al., 1966; Sokolovsky et al., 1966). It is volatile and can be used with volatile buffers at a

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variety of temperatures. It also has been shown to be toxic to animals only when used at very high concentrations for prolonged periods of time (Glover and Landsman, 1964; Sax, 1979; Sutton, 1967). TNM has also been shown to be a potent disinfectant for bacterial spores, vegetative bacteria, and fungi (Singh and Doyle, 1986). Included among the organisms killed when TNM was applied to surfaces contaminated with them were spores of *Bacillus spp.* and vegetative forms of *Bacillus anthracis, Clostridium sporogenes, Escherichia coli, Legionella pneumophila, Listeria monocytogenes, Pseudomonas aeruginosa, Streptococcus pneumonia,* and *Candida albicans* (Singh and Doyle, 1986). Therefore, TNM has been shown to be a broad-spectrum antibacterial and antimycotic, with the ability to disinfect surfaces from bacterial spores.

There would be an obvious advantage if TNM also has antiviral activity as well as antibacterial and antimycotic activity. TNM could then be used as a general surface disinfectant. The present study was designed to determine if TNM could disinfect surfaces from representative RNA viruses: vesicular stomatitis virus, Newcastle disease virus. Surfaces on commercially available pocket calculators were used to determine if the calculators would still function normally after being exposed to TNM. The TNM was prepared in different buffers and applied to the surfaces by aerosol methods.

The following were the methods use for this study. TNM was obtained from the Aldrich Chemical Co., Milwaukee, WI. For sterilization of surfaces prior to experimentation, 10 μl of neat TNM was placed in 1 ml of 50% ethanol. For disinfection experiments, TNM was added to 0.05 M phosphate-buffered saline (pH 7.3) (PBS) to a final concentration of 10 μm/ml. The solution was vortexed, and allowed to stand for 10 min, The top aqueous layer was removed and used as the disinfectant. For disinfection studies with TNM in tris(hydroxymethyl)aminomethane (Tris) buffer (Fisher Scientific, Pittsburgh, PA), a similar protocol was followed. TNM was added to 0.05 M Tris buffer (pH 9.0) to a final concentration of 10 μl/ml. The solution was vortexed, and allowed to stand for 10 min. The top aqueous layer was then removed, placed in a commercially available Aerosol Sprayer (Fisher Scientific, Pittsburgh, PA), and used as the disinfectant. Laboratory biosafety level 2 procedures were followed for all experiments.

TI-35 II calculators were purchased from Texas Instruments, Fort Worth, TX. The calculators were disinfected prior to experimental use by coating them with TNM in ethanol. This was to ensure that the calculators were free of contaminating bacteria and viruses prior to application of the test viruses. The calculators were incubated overnight in a sterile container at 37°C and then any residual TNM and ethanol was removed by swabbing the surfaces with sterile water. The calculators were tested for bacterial and fungal contamination by swabbing the calculators with a sterile swab and streaking onto a nutrient agar plate. No detectable bacterial or fungi were observed after completion of the viable counts.

The Indiana strain of vesicular stomatitis virus (VSV) and the Herts' strain of Newcastle disease virus (NDV) were taken from frozen stocks maintained in the authors' laboratory originally obtained from Dr. Thomas C. Merigan of the Division of Infectious Diseases, Stanford University School of Medicine. L-929 cells for titration of VSV and HeLa cells for titration of NDV, were obtained originally from the American Type Culture Collection (Rockville, MD) and maintained in the authors' laboratory in

Minimum Essential Medium (MEM, MA Bioproducts, Walkersville, MD) supplemented with 10% heat-inactivated fetal bovine serum, sodium bicarbonate, penicillin and streptomycin, fungizone, gentamycin, and L-glutamine (Langford et al., 1981). The viruses were titered by means of a plaque assay on the appropriate cells line (Langford et al., 1981) prior to experimental application for disinfection studies. Titers were expressed as plaque forming units/ml (PFU/ml).

Virus concentrations were determined prior to use and a swab was used to apply the same amounts of virus to measured areas (14–18 cm^2) on the surface of the calculators. Sample sizes were standardized to insure equivalent application of control and experimental samples. The dimensions of the surface of the calculators used was 7.5 × 14 cm. The calculators were allowed to dry. TNM in the appropriate buffer or buffer alone was applied by aerosol (sprayed) on to different surfaces containing virus on the same calculator. The calculators were sprayed until disinifectant coverd the test surface area, as determined visually. The calculators were then incubated for 30 min at ambient temperature. The measured surfaces on the calculators were then wiped with sterile swabs which were placed into test tubes containing PBS; the swabs were saturated with PBS to remove any virus. All control and test samples were treated in exactly the same fashion. The suspension containing the virus samples was diluted 10-fold, and then 0.5 ml of the solution was used for a plaque assay (VSV on L-929 cells and NDV on HeLa cells) (Langford et al., 1981). Application of TNM-treated suspensions to cell cultures did not affect cell viability, the ability of cells to replicate, and the attachment and replication of non-TNM treated viruses.

The evaluation of the results was as follows. When calculator surfaces were coated with VSV and then were sprayed with TNM, there was no detectable virus recovered (Table 1). There was no difference in results whether the buffer used was PBS or Tris. Substantial amounts of virus were recovered from other equivalent surfaces that received the same titer of virus but were sprayed with buffer only (Tables 1 and 2).

In two different studies, calculator surfaces coated with NDV and then sprayed with TNM showed substantial decreases in recoverable virus compared with surfaces coated with virus and sprayed with buffer only (Table 3). In one experiment, when lower concentrations of virus were applied, no detectable virus was recovered. When higher concentrations of virus were applied, a decrease in titer of 10^4 was observed (Table 3).

Fifteen to twenty consistent mathematical maneuvers were carried out on each calculator before and after TNM was applied. There were no differences in the ability of

Table 1
Recovery of vesicular stomatitis virus from calculator surfaces treated with TNM in phosphate-buffered saline

Calculator #	Surface	Vesicular stomatitis virus titer (PFU)		
		Stock before application	PBS-treatment only	TNM-treatment
1	A	3.35×10^7/ml	5.10×10^5/ml	ND [a]
	B	3.35×10^7/ml	3.20×10^5/ml	ND
2	A	4.25×10^7/ml	8.40×10^3/ml	ND
	B	4.25×10^7/ml	3.40×10^4/ml	ND

[a] ND = not detectable in this assay. All values are an average of three titrations.

Table 2
Recovery of vesicular stomatitis virus from calculator surfaces treated with TNM in tris buffer

Calculator #	Surface	Vesicular stomatitis virus titer (PFU)		
		Stock before application	Tris-treatment only	TNM-treatment
1	A	3.75×10^{6}/ml	2.24×10^{2}/ml	ND [a]
	B	3.75×10^{6}/ml	1.78×10^{2}/ml	ND
2	A	2.10×10^{7}/ml	3.04×10^{3}/ml	ND
	B	2.10×10^{7}/ml	6.21×10^{3}/ml	ND

[a] ND not detectable in this assay. All values are an average of three titrations.

the calculators to carry out the maneuvers before and after TNM application. In fact, only after 10 applications of TNM any alteration in calculator function observed. This was true when calculators were treated with TNM or with buffer only and was due to seepage or leakage into the battery chambers. This was remedied by wiping the battery chamber and allowing the circuits to dry. Function was then restored.

The need for surface disinfectants has continued to grow over-time. TNM, a common nitrating agent, has been shown to be an effective antibacterial and antimycotic agent (Singh and Doyle, 1986). TNM will even kill or inactivate bacterial spores (Singh and Doyle, 1986). The results of the current study have shown that TNM can successfully disinfect surfaces from samples of both enveloped and non-enveloped RNA viruses. The titer of virus on TNM-treated surfaces was decreased substantially compared to controls. In many cases there was no detectable virus obtained from TNM-treated surfaces.

Additionally, TNM treatment did not functionally alter pocket calculators, a sample of complex electronic equipment. Function was not affected even after repeated applications of TNM. TNM has been shown to be non-toxic to animals except the animals were exposed to high doses for an extended time interval (Glover and Landsman, 1964; Sax, 1979; Sutton, 1967).

TNM was examined for its disinfectant properties because it is a nitrating agent, and that it probably nitrates critical enzymes needed for growth (Riordan et al., 1966; Sokolovsky et al., 1966). Many infectious agents possess hydrophobic enzymes on coats or membranes (Doyle et al., 1984). TNM may be able to seek out hydrophobic domains and nitrate critical enzymes, making It impossible for viruses to replicate.

Table 3
Recovery of Newcastle disease virus from calculator surfaces treated with TNM in phosphate-buffered saline

Calculator #	Surface	Newcastle disease virus titer (PFU)		
		Stock before application	PBS-treatment only	TNM-treatment
1	A	2.89×10^{7}/ml	4.00×10^{4}/ml	ND [a]
	B	3.60×10^{7}/ml	4.69×10^{4}/ml	ND
2	A	6.50×10^{9}/ml	2.30×10^{7}/ml	3.26×10^{3}/ml
	B	5.01×10^{8}/ml	2.59×10^{6}/ml	9.00×10^{2}/ml

[a] ND = not detectable in this assay. All values are an average of three titrations.

TNM is volatile and can be used in volatile buffers so that it can be eliminated from treated surfaces very soon after application. This makes it ideal for application as a disinfectant. It is broad spectrum, and not only disinfects viruses, but also most other microbial organisms.

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