# Antiviral activity of a novel composition of peracetic acid disinfectant on parvoviruses

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## Abstract

Porcine parvoviruses (PPV) are known to be particularly resistant to many disinfectants used to control other non-enveloped viruses. However, effective disinfectants used against PPV are harsh and corrosive to animal health facilities and the environment. We propose a noncorrosive "green" disinfectant that generates peracetic acid *in-situ* and is capable of inactivating PPV completely at a 1% concentration for a 10-minute contact time.

## Résumé

*Les parvovirus porcins (PVP) sont reconnus pour être particulièrement résistants à plusieurs désinfectants utilisés pour éliminer d'autres virus non-enveloppés. Toutefois, les désinfectants efficaces utilisés contre le PVP sont rudes et corrosifs pour les installations de santé animale et l'environnement. Nous proposons un désinfectant «vert» et non-corrosif qui génère de l'acide peracétique* in situ *et qui est capable d'inactiver le PVP complètement lorsqu'utilisé à une concentration de 1 % pour un temps de contact de 10 minutes.*

*(Traduit par Docteur Serge Messier)*

In the triad of infectious agents, susceptible hosts, and the environment (water, food, contaminated surfaces, aerosols), the role of the latter is the most ambiguous in infectious disease transmission. Viruses may enter the environment in enormous quantities and environmental measures to halt or reduce transmission may offer great prospects for disease control. Ultraviolet (UV) radiation from the sun, if not shielded, is the primary germicide in the environment (1). Virtually all artificial virus inactivation methods are applied locally in laboratories or hospitals in controlled conditions (2,3) since the inhibitors are harsh or cannot be applied over large areas. Notable exceptions are reported and reviewed by Weber and Stlianakis (4) and Vinnerås et al (5). Vinnerås et al (5) reported the effects of formic acid addition to ground high-risk animal by-products (ABP 1) in terms of stabilization and pathogen inactivation. Porcine parvovirus (PPV) was still fully infective after 168 d, even though a strong reaction of formic acid with the single-stranded DNA genome was expected.

Parvoviruses can resist harsh environmental conditions (3), and while surviving on surfaces for long periods they can be transmitted to susceptible hosts. In addition to chemical biocides, heat and irradiation are usually used. Porcine parvoviruses are known to be particularly resistant, since many biocides generally considered as effective against other non-enveloped viruses and used for high-level disinfection have limited inactivation potential. It is a particularly resistant surrogate for inactivation studies of other virus species.

Glutaraldehyde-based disinfectants are used not only in agricultural establishments for animal health but also in hospitals and health care facilities for human safety in order to disinfect surfaces and instruments such as flexible endoscopes (6,7). However, since glutaraldehyde poses an occupational health hazard or risk for staff, with up to 15% of United Kingdom hospitals using it as their first-choice endoscope disinfectant, there is a need for a safer alternative in a bid to reduce potential health, safety, and environmental risks (8,9).

Two percent glutaraldehyde has been the reference disinfectant for high-level disinfection, but its frequent association with adverse effects has stimulated a search for newer disinfectants. When the efficacy of 2% glutaraldehyde was compared with a 0.2% peracetic acid-based disinfectant, both products were effective germicides in 10 to 20 min; however, when organic matter was added, the 0.2% peracetic acid formulation cleaned without corrosion, while 2% glutaraldehyde fixed the matter to the scalpel, causing corrosion within 2 h (10). Also, the PPV inactivation results with 2% glutaraldehyde have been contradictory (3,11). The fixative properties of aldehydes probably decrease due to the presence of organic matter such as soil.

In addition to glutaraldehyde, oxidizers such as chlorine (sodium hypochlorite and chlorine dioxide), hydrogen peroxide, and peracetic acid are also widely used as high-level disinfectants to disinfect surfaces, equipment, and instruments in various industries (12–15). Although they are efficient in controlling pathogens, they are irritants to users (16), corrosive to surfaces, (17) and are not as environmentally friendly as chlorine-based disinfectants (18).

In this study, a patented green inactivator was assessed for its ability to inactivate or mitigate PPV in the environment. First, a sensitive method for the relative infectivity of the virus was developed followed by an assessment of the efficacy.

Porcine testes (PT) fibroblasts, derived from ST cells (ATCC CRL-1746), were grown at 37°C in Dulbecco's modified Eagle's

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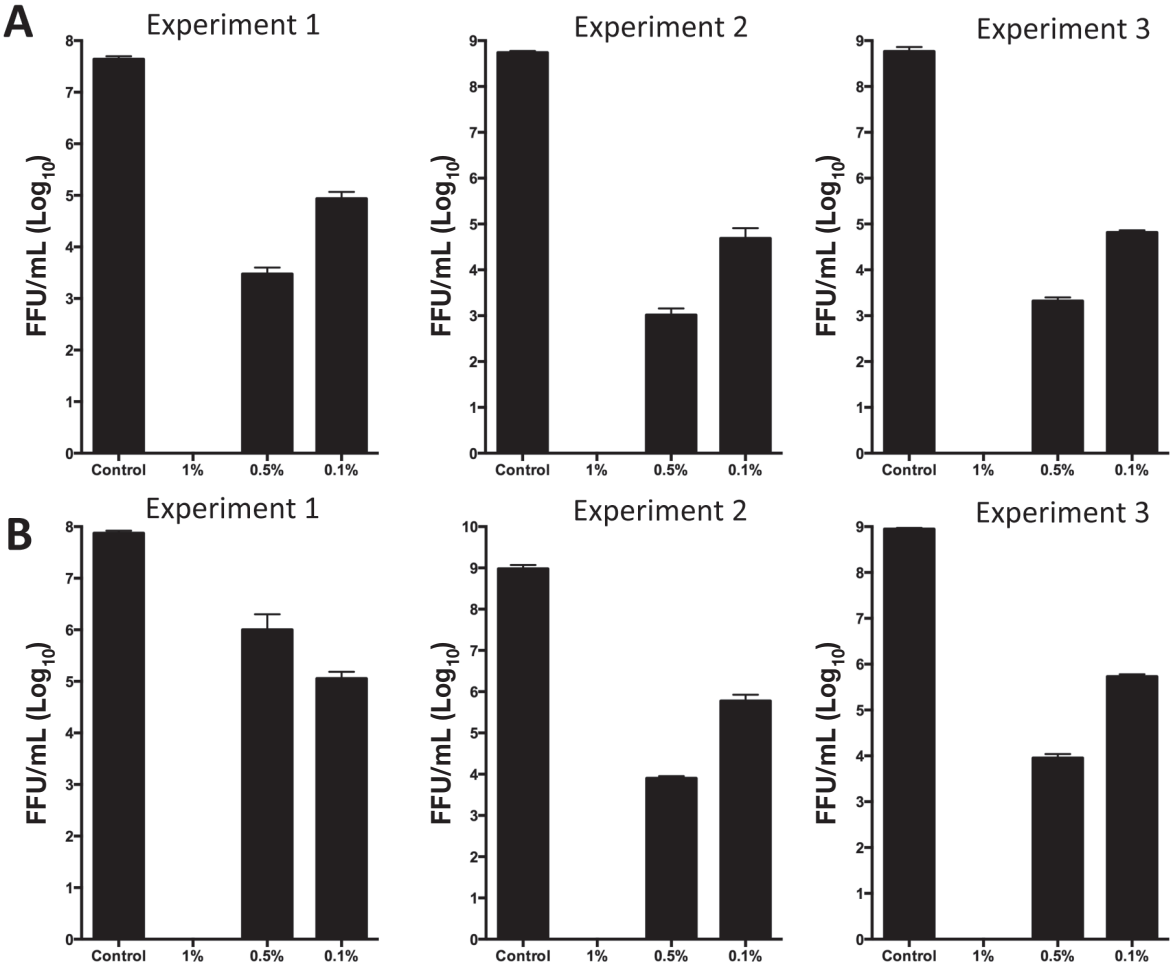


**Figure 1. Porcine parvovirus inactivation by BIOXY Enviro or BIOXY +. The PPV virus stocks were incubated with 1%, 0.5%, or 0.1% BIOXY Enviro (A) or BIOXY + (B) for 10 min. The results of repeated experiments are shown.**

medium (Wisent, Saint-Bruno, Québec, Canada) containing D-glucose and L-glutamine and supplemented with 7% heat-inactivated bovine serum (Wisent) and antibiotics Penicillin-streptomycin solution (Wisent). The porcine parvovirus (PPV, NADL-2 strain) stock was obtained by propagation in PT cell culture. The viral stocks were collected by a brief centrifugation to remove the cellular debris.

Virus inactivation was done by simply mixing the virus stocks with BIOXY Enviro or BIOXY + solution (Bioxy AFD, Montreal, Quebec, Canada). After incubation with BIOXY Enviro or BIOXY + solution, the virus stocks were mixed vigorously with an equal volume of chloroform-butanol (1:1). The viruses were then recovered by centrifuge at 10 000 rpm for 10 min as described (19). The resulting water phase was collected and loaded into a centrifugal Microcon filter unit (Millipore, Etobicoke, Ontario, Canada) by following the manufacturer's instructions, with the purpose of removing the remaining BIOXY Enviro or BIOXY + compound. The viruses that were retained by the filter were then suspended with equal volume of 1 × phosphate-buffered saline (PBS) for titration.

Viral titers were determined by immunofluorescence (IF) as described previously (20). Briefly, cells were plated at $1 \times 10^4$ per well in 96-well plates. The cells were infected 24 h later. The infected cells were proceeded for the IF assay at 20 h after infection. The cells were fixed with 3% formaldehyde solution for at least 30 min, and then were permeabilized with 3% Triton X-100 for 30 min, followed by incubation with a monoclonal mouse capsid-specific antibody (3C9-D11-H11), together with anti-mouse Alexa Fluor 488 as a secondary antibody (Thermo Fischer Scientific, Canada). The fluorescent nuclei were then scored, and the virus titers were expressed in fluorescent focus-forming units/mL (FFU/mL).

First, the PPV virus stocks were treated with 1%, 0.5%, and 0.1% of BIOXY Enviro or BIOXY + for 10 min. The experiments were repeated 3 times. It was found that 1% BIOXY Enviro or BIOXY + inactivated the viruses efficiently, while 0.5% or 0.1% of BIOXY Enviro or BIOXY + showed partial virus inactivation (Figure 1). Additionally, this concentration of BIOXY Enviro or BIOXY + did not affect the viability of the PT cell. No fluorescent signal was detected with cells treated with BIOXY Enviro or BIOXY + (data not shown).

Then, to assess the ability of the BIOXY Enviro and BIOXY + for virus inactivation over a period of 60 min, the PPV virus stock was incubated with 0.1% of BIOXY Enviro or BIOXY + for 10, 20, 30, 40, 50, and 60 min, after which the viral titers were determined. The experiments were repeated 3 times. The viral titers didn't decrease

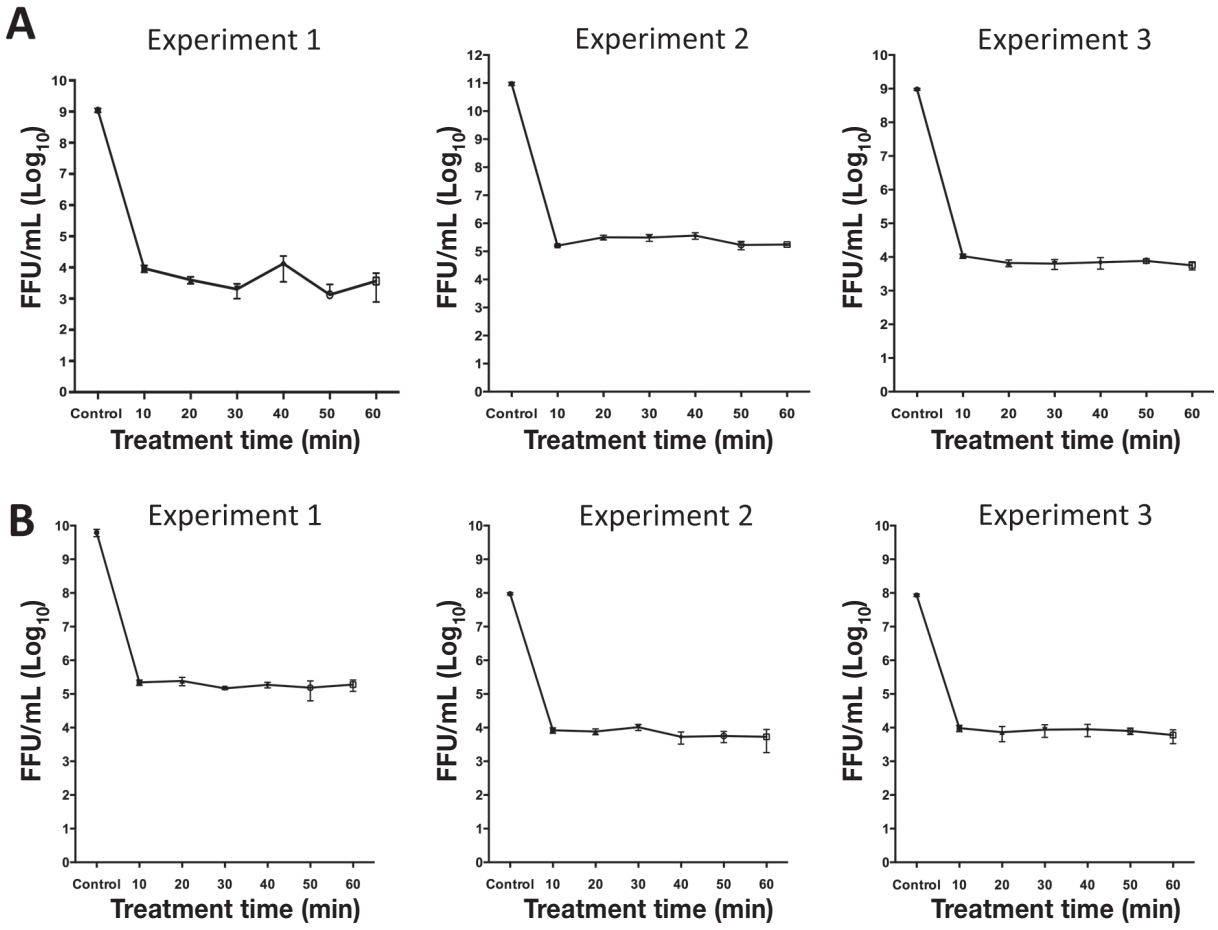


**Figure 2. Porcine parvovirus inactivation is concentration-dependent. The PPV virus stocks were treated with 0.1% BIOXY Enviro (A) or 0.1% BIOXY + (B) for 10, 20, 30, 40, 50, and 60 min. The results of repeated experiments are shown.**

dramatically after a 10-minute incubation, and the viruses were still infectious even after 60 min (Figure 2). This indicates that the virus inactivation is concentration-dependent.

The PPV were completely inactivated by 1% BIOXY Enviro or BIOXY + for 10 min; however, PPV inactivation was concentration-dependent with the best activity demonstrated at 1% concentration for both BIOXY Enviro or BIOXY +.

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