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Corexit 9500 Inactivates Two Enveloped Viruses of Aquatic Animals but Enhances the Infectivity of a Nonenveloped Fish Virus

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The effects of Corexit 9500, a dispersant used to clean up oil spills, on invertebrates, lower vertebrates, birds, and human health have been examined, but there is a significant lack of study of the effect of this dispersant on aquatic viruses. In this study, the effects of Corexit 9500 on four aquatic viruses of differing structural composition were examined. Corexit 9500 reduced the titer of the enveloped viral hemorrhagic septicemia virus (VHSV) at all concentrations (10% to 0.001%) examined. The titer of frog virus 3 (FV3), a virus with both enveloped and nonenveloped virions, was reduced only at the high Corexit 9500 concentrations (10% to 0.1%). Corexit 9500 was unable to reduce the titer of nonenveloped infectious pancreatic necrosis virus (IPNV) but enhanced the titer of chum salmon reovirus (CSV) by 2 to 4 logs. With the ability to inactivate enveloped viruses and possibly enhance some nonenveloped viruses, Corexit 9500 has the potential to alter the aquatic virosphere.

Recently, chemical dispersants have been introduced into aquatic environments in large quantities because of their use in the cleanup of oil spills. Of the estimated 38 major accidental oil spills since 1970 (1), approximately 20 have been from oil tankers (2). These spills are often treated with dispersants, such as Corexit 9500. The exact composition of Corexit is proprietary, but in general terms, the ingredients include anionic surfactants, nonionic surfactants, and organic solvents (3–5). The dispersants function by breaking down large oil pools into small oil droplets, allowing them to sink into the water column. The small oil particles are diluted by waves into very minute concentrations (6). The Deepwater Horizon oil spill in the Gulf of Mexico resulted in, historically, the largest volume of dispersant ever used in one incident; approximately 7.57 million liters (mainly Corexit 9500) was used to disperse the oil, exposing the immediate and surrounding biological environment to the dispersant (7).

With the increase in the volumes of dispersant used, more attention is being given to the impact of dispersants on the health of humans and the environment. For human health, the research has ranged from clinical assessments (8) to studies of cell lines (9). For the environment, the possible influence of dispersants on a range of metazoans, including corals, crustaceans, mollusks, fish, and birds (7, 10, 11), has been investigated. Even effects on the microbial world are being examined. For example, Corexit might interfere with the capacity of bacteria from beaches to remediate spills (12). Perhaps the one component of aquatic environments that has never been studied for interactions with Corexit is viruses.

Viruses are the most abundant biological entities in the aquatic environment and are tremendously important in ecological processes, as well as being the causative agents of many diseases (13, 14). All the types of viruses in water constitute the virosphere. The most common viruses target bacteria. These bacteriophages or phages influence the microbial loop and ultimately global biogeochemical cycles (15). Although greatly outnumbered by phages, viruses that infect algae and aquatic animals are also in the sea and freshwater (16, 17). Whether from prokaryotes or eukaryotes, viruses have common structural themes. All viruses have capsids that enclose either RNA or DNA genomes. Viruses that additionally have a lipid membrane (envelope) are referred to as enveloped viruses, whereas those that have just the capsid are naked or nonenveloped viruses. Of these two groups, Corexit might be expected to impact enveloped viruses because surfactants have been reported to inactivate some enveloped viruses (18).

In this work, the effects of Corexit 9500 on four aquatic viruses that infect fish but differ in structure were studied. These are the rhabdovirus viral hemorrhagic septicemia virus (VHSV), the birnavirus infectious pancreatic necrosis virus (IPNV), the reovirus chum salmon reovirus (CSV), and the iridovirus frog virus 3 (FV3) (17). VHSV is an enveloped virus with an RNA genome. IPVN and CSV are naked viruses with RNA genomes. FV3 is a DNA virus, but the viral particles (virions) can be either naked or enveloped. The viruses were exposed to Corexit 9500 for 24 h, and titers of exposed viruses were determined on the appropriate fish cell line. Corexit inactivated VHSV and FV3, depending on the dispersant concentration, but had no effect on IPNV. Surprisingly, Corexit activated CSV. These results suggest that Corexit has the potential to alter the aquatic virosphere.

MATERIALS AND METHODS

Cell line propagation. Two cell lines used in this study were EPC, recently reclassified as being from fathead minnow (19), and CHSE-214. Both cell lines were grown in 75-cm^2 flasks (BD Biosciences, Fisher Scientific) using Leibovitz's L15 medium (HyClone; Fisher Scientific, Mississauga, ON, Canada) supplemented with 10% fetal bovine serum (FBS) (PAA Laboratories, VWR International, Mississauga, ON, Canada) and 1% penicillin-streptomycin (PS) (HyClone; Fisher Scientific, Mississauga, ON, Canada).

Virus propagation and quantification. Four viruses used in this study were viral hemorrhagic septicemia virus (VHSV), infectious pancreatic necrosis virus (IPNV), chum salmon reovirus (CSV), and frog virus 3 (FV3). VHSV and IPNV were obtained from J. Lumsden and R. Stevenson, respectively, at the University of Guelph, Guelph, Ontario, Canada, FV3 from C. Brunnetti at Trent University, Peterborough, Ontario, Can-

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ada, and CSV from the American Type Culture Collection (ATCC). VHSV and FV3 were propagated and titers were determined on the EPC cell line. IPNV and CSV were propagated and titers were determined on the CHSE-214 cell line. Propagation was done on confluent monolayers of either EPC or CHSE-214 in 75-cm^2 flasks in L15 with 2% FBS. IPNV and CSV were propagated at 18°C, VHSV at 14°C, and FV3 at 26°C. At approximately 7 days postinfection (p.i.), the supernatant and cells were collected and centrifuged at 4,000 × *g* for 5 min to pellet cellular debris. The supernatant was collected and syringe filtered through a 0.2-μm filter (Pall Corporation, Fisher Scientific, Mississauga, ON, Canada) and stored at −80°C. The Karber method of 50% tissue culture infectious dose ($TCID_{50}$/ml) was used to determine viral titers (20).

Determining effects of Corexit 9500 on cell lines. The cytotoxic effects of Corexit 9500 on EPC and CHSE-214 were determined using two methods: the fluorometric resazurin assay and the colorimetric crystal violet assay. Resazurin is an oxidation-reduction indicator dye that can be reduced by living cells in the process of cellular metabolism. The reduction of resazurin produces a fluorescent product that can be measured in relative fluorescent units (RFU). Healthy cells produce higher RFU than dead or dying cells (21). Crystal violet is a DNA-staining dye that can be used to visualize the adherence of cells to multiwell cell culture plates. For cells that have adherent growth characteristics, loss of adherence to the growth surface can be an indicator of cell death (21). In both assays, the cells were seeded into a 96-well plate at approximately 40,000 cells per well and allowed to adhere overnight. Corexit 9500 was serially diluted 10-fold in L15 with 2% FBS to 10^{-6} and added to columns of cells in the 96-well plate. For the crystal violet assay, the cells were incubated with Corexit 9500 dilutions for 24 h at 14°C before the wells were washed once with Dulbecco's phosphate-buffered saline (DPBS) (Lonza, VWR International, Mississauga, ON, Canada). Cells were fixed with cold 100% methanol for 10 min. The methanol was removed, and the cells were incubated in the crystal violet stain (0.5% crystal violent in 25% methanol) for 10 to 15 min. After the removal of the stain, the wells were washed many times in deionized water to remove excess stain before the plates were dried and imaged using a desktop personal computer scanner. For the resazurin assay, the alamarBlue commercial preparation of resazurin was used (Life Technologies Inc., Burlington, ON, Canada). Cells in the 96-well plates were incubated with the serial dilutions of Corexit 9500 for 4 days at 14°C before the wells were washed once with DPBS. Cells were incubated with 5% alamarBlue solution for 1 h before being measured in a CytoFluor fluorescent plate reader (PerSeptive Biosystems). To determine the percent viability of Corexit 9500-exposed cells, the RFU of exposed wells was divided by the RFU of control wells without Corexit, with the result multiplied by 100.

Corexit 9500 preparation and static suspension exposure with viruses. Corexit 9500 (Nalco Energy Services, Sugar Land, TX) was serially diluted (vol/vol) to the following working concentrations in DPBS: 1:5, 1:50, 1:500, 1:5,000, and 1:50,000. For virus exposure, 100 μl of stock virus was mixed with 100 μl of diluted Corexit (1:5, 1:50, 1:500, 1:5,000, and 1:50,000) to make final Corexit concentrations of 1:10 or 10%, 1:100 or 1%, 1:1,000 or 0.1%, 1:10,000 or 0.01%, and 1:100,000 or 0.001%. In control experiments, 100 μl of stock virus was mixed with 100 μl of DPBS. VHSV mixtures were incubated to specific time points at 14°C, CSV and IPNV at 18°C, and FV3 at 20°C. At specified time points, 100 μl of each mixture sample was collected, and the titer of virus was determined. Viral titer reduction or increase was determined by dividing the titer of virus exposed to Corexit with the titer of control virus exposed only to DPBS. The resulting value was log transformed and plotted using the GraphPad Prism 4.0 software program. To measure pH, viruses and Corexit were mixed at each of the above concentrations in a total volume of 1,000 μl, and the pH was measured using a Toledo pH meter (Mettler Toledo, Mississauga, ON, Canada).

Measurement of titer produced by enhanced CSV (eCSV). One hundred microliters of stock CSV was mixed with 100 μl of diluted Corexit (1:5) to make a final Corexit concentration of 1:10 or 10% and incubated for 24 h. After incubation, 100 μl of the CSV-Corexit mixture was removed and serially diluted with 900 μl of DPBS at each dilution from 10^{-1} to 10^{-4}. The 10^{-4} dilution of the CSV-Corexit mixture was added to confluent monolayers of CHSE-214 for 2 h in a 12-well plate. After 2 h, the mixture solution was removed and the cells were washed three times with DPBS. After removal of wash solution, 1 ml of L15 with 2% FBS was added to each well. Immediately upon addition, 100 μl was collected from each well for quantification of the residual amount of CSV that remained after washing (day 0). One hundred microliters of fresh L15 with 2% FBS was added to each well, to bring the volume back to 1 ml, before the plate was incubated at 18°C for 7 days. At day 7, 100 μl was collected from each well for quantification of the CSV titer.

RESULTS

Determining Corexit 9500 cytotoxicity and detection limit for $TCID_{50}$ assay. One common issue that arises during the study of the effects of chemical disinfectants on viruses is the simultaneous effects of those disinfectants on cell cultures used to report the presence of viruses. Therefore, it is important to define the lower limit of detection imposed by the toxicity of chemical disinfectants on cell cultures (22). Since Corexit 9500 has been shown to be cytotoxic to mammalian (9) and fish (23) cells in culture, the effect of the oil dispersant on the viability of the two fish cell lines, EPC and CHSE-214, that were used to monitor the viruses after their exposure to Corexit 9500 was examined. Corexit 9500 at a starting concentration of 10% (vol/vol) was serially diluted in a mock $TCID_{50}$ assay from 10^{-1} to 10^{-6}; the 10^{-1} $TCID_{50}$ dilution corresponded to a Corexit 9500 concentration of 1%, 10^{-2} to 0.1%, 10^{-3} to 0.01%, 10^{-4} to 0.001%, 10^{-5} to 0.0001%, and 10^{-6} to 0.00001%. Exposure of cells for up to 4 days to Corexit 9500 at $TCID_{50}$ dilutions of 10^{-3} (a 0.01% Corexit 9500 concentration) to 10^{-6} (0.00001%) were not cytotoxic to either cell line as determined by the alamarBlue and crystal violet assay (Fig. 1). For fish cell cultures receiving $TCID_{50}$ dilutions of 10^{-3} to 10^{-6} or higher, any destruction of cell monolayers would be due to the cytopathic effect (CPE) of the viruses, and the viral titer could be determined. However, exposure of cells to $TCID_{50}$ dilutions of 10^{-1} (a 1% Corexit 9500 concentration) and 10^{-2} (0.1%) resulted in cytotoxicity (Fig. 1). Therefore, the lowest limit of virus titer detection in the $TCID_{50}$ assay with a 10% initial Corexit 9500 concentration was 1.58×10^3 $TCID_{50}$/ml, and that with a 1% initial Corexit 9500 concentration was 1.58×10^2 $TCID_{50}$/ml.

Determining pH of virus-Corexit 9500 mixtures. To ensure that any potential effects of Corexit 9500 on the viruses were not due to extreme acidic or basic conditions, the pHs of all virus-Corexit mixtures were measured and determined to fall in the range of 7.32 to 8.16. Therefore, any effects of Corexit 9500 on the examined viruses were not due to extremely acidic or basic pH.

Effect of Corexit 9500 on a virus with a single protein capsid, IPNV. The titer of IPNV was not greatly altered by Corexit 9500 at any of the concentrations examined, even after 24 h of exposure (Fig. 2).

Effect of Corexit 9500 on a virus with a single protein capsid and an envelope, VHSV. Exposure of VHSV IVb to dilutions of Corexit 9500 reduced the viral titer. At dispersant concentrations of 10% and 1%, VHSV was inactivated immediately after the virus was mixed with the dispersant at the 0-h time point, and at 0.1%, complete inactivation was observed by 1 h postexposure (Fig. 3). At the 0.01% concentration, a steady reduction in VHSV was observed over 6 h, with complete inactivation by 24 h (Fig. 3). However, at the most dilute concentration tested, 0.001%, reduction in

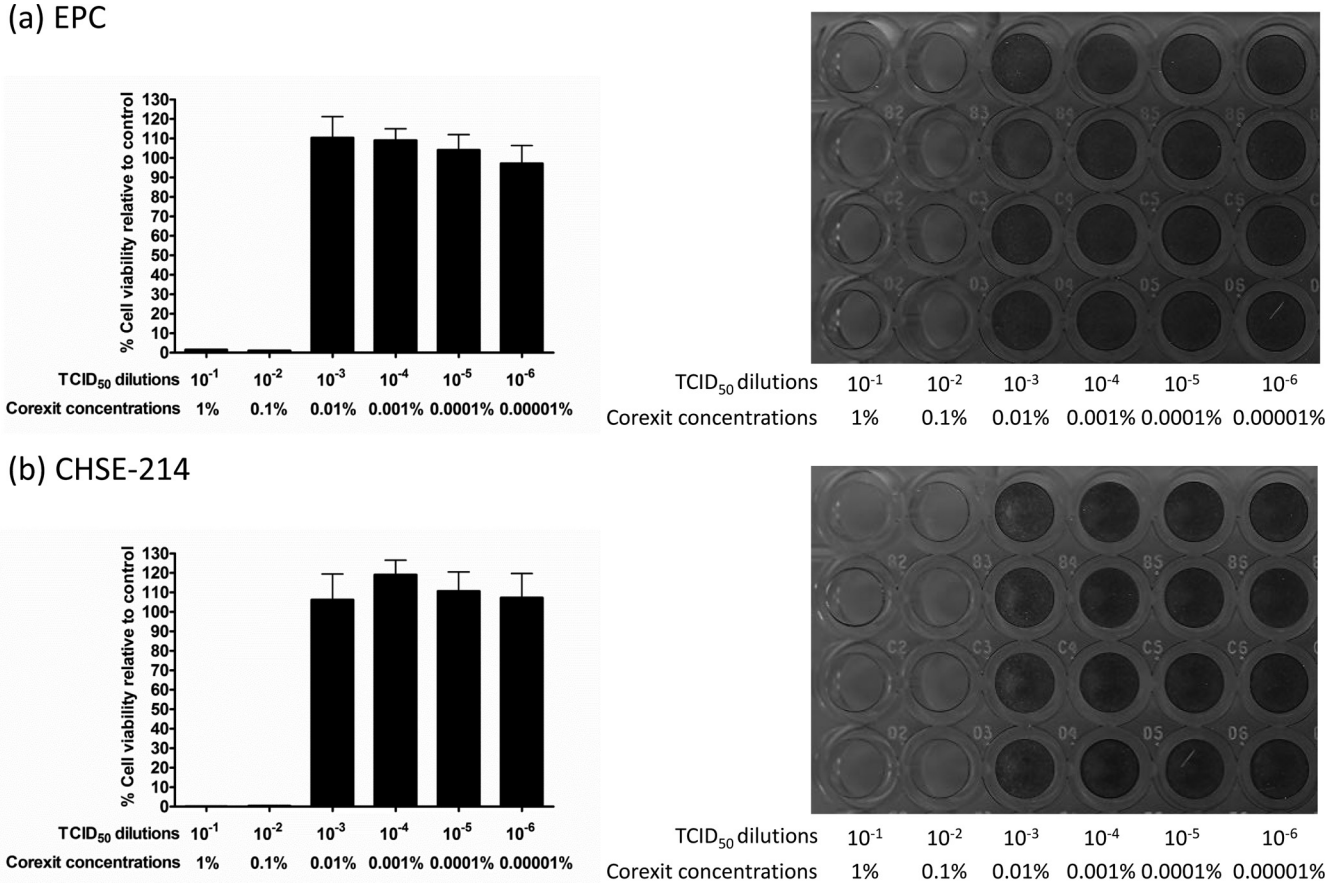

FIG 1 Cytotoxic effect of Corexit 9500 on EPC and CHSE-214. EPC (a) or CHSE-214 (b) cells were exposed to 10-fold serial $TCID_{50}$ dilutions, up to 10^{-6}, of Corexit 9500 (10%, initial concentration) in L15 with 2% FBS before cell viability was determined using the alamarBlue resazurin assay or the crystal violet assay. In the alamarBlue assay (left panel of each figure), the cells were incubated with Corexit 9500 dilutions for 4 days. The points on the graph represent means ± SD; $n = 4$. In the crystal violet assay (right panel of each figure), the cells were incubated with Corexit 9500 for 24 h.

the VHSV titer was not observed up to the 6-h time point, but by 24 h, there was almost a 3-log reduction in the viral titer compared to findings for the control; however, complete inactivation was not observed (Fig. 3). At the two highest Corexit 9500 concentrations examined, 10% and 1%, the virus titer was reduced beyond the lower limits of detection imposed by Corexit 9500 cytotoxicity; therefore, the log reduction value for the virus titer can be estimated by subtracting the log value of the lowest detectable titer (as defined under "Determining Corexit 9500 cytotoxicity and detection limit for $TCID_{50}$ assay" above) from the log value of the measured virus titer in the control and is underestimated for these two concentrations. This is also the reason why Corexit 9500 at the 0.1% concentration showed greater viral inactivation than at the 10% or 1% concentration in the graph in Fig. 3.

Effect of Corexit 9500 on a virus with a single protein capsid with or without an envelope, FV3. Exposure of FV3 to some concentrations of Corexit 9500 reduced the viral titer. At dispersant concentrations of 10% and 1%, FV3 was inactivated immediately after the virus was mixed with the dispersant at the 0-h time point (Fig. 4); this is similar to what was observed with VHSV in Fig. 3. At the 0.1% concentration, reduction in the FV3 titer was observed at the 1-h time point, but a slightly higher than 1-log reduction of the FV3 titer was also observed at the 0-h time point (Fig. 4). However, at 0.01% and 0.001%, FV3 was not inactivated by more than 1 log even after 24 h of exposure. Therefore, at more dilute concentrations of Corexit 9500, FV3 appeared to be more resistant to inactivation than VHSV as shown in Fig. 3. Similar to the VHSV results, Corexit 9500 at 10% and 1% concentrations reduced FV3 titer to beyond the lower limits of detection; therefore, the reported log reduction values are underestimated for these two concentrations.

Effect of Corexit 9500 on a virus with a double protein capsid, CSV. Exposure of CSV to Corexit 9500 produced a surprising result. The two lowest concentrations, 0.001% and 0.01%, had no effect on the CSV titer after 24 h of exposure (Fig. 5a), similar to the case with IPNV as shown in Fig. 2; however, at a 0.1% concentration and higher, an increase of 3 to 4 logs in the CSV titer was observed in Corexit 9500-exposed virus compared to results for control virus exposed to only DPBS (Fig. 5a). Treatment of CSV with Corexit 9500 appeared to enhance the infectivity of the virus. To determine whether the virus would eventually be inactivated if kept in Corexit 9500 longer, CSV was exposed to Corexit 9500 at the highest concentration of 10% for 7 days; inactivation of CSV did not occur, since virus infectivity remained enhanced even after 7 days in the dispersant (Fig. 5b). Furthermore, an increase in the CSV titer, by approximately 2 logs, occurred immediately after the virus was mixed with the dispersant, and a maximal increase of nearly 4 logs occurred by 1 h (Fig. 5b). The increases in titer could be most straightforwardly

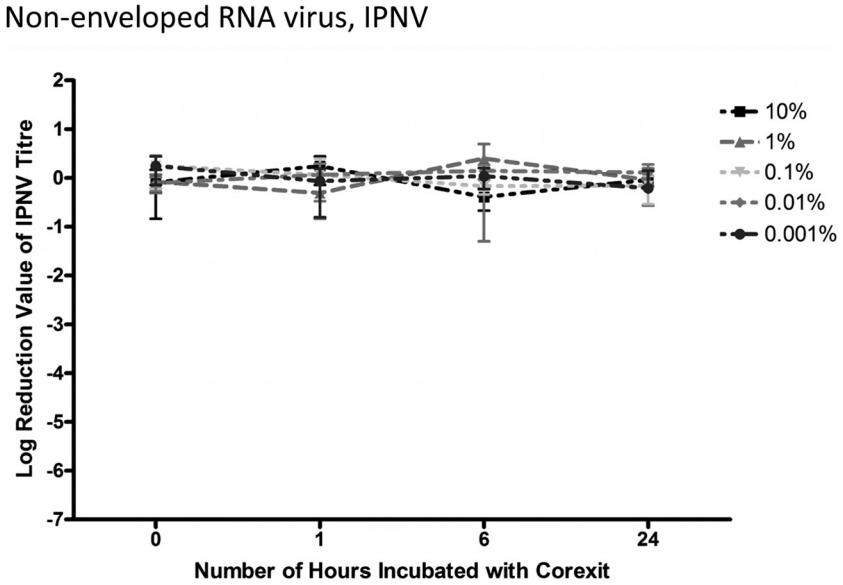

FIG 2 Effect of Corexit 9500 on the titer of a birnavirus, IPNV. IPNV was exposed to various dilutions of corexit 9500 in suspension tests over 24 h at 18°C. As a control, the viruses were exposed to the same volume of DPBS in parallel. Immediately (time zero) and 1, 6, and 24 h after the Corexit exposure began, the mixture was sampled, the titer of virus was determined, and the log reduction value was calculated as described in Materials and Methods. The points on the graph represent means ± SD; $n = 4$.

explained by Corexit 9500 activating the CSV to a more infective form, referred to as Corexit-enhanced CSV (eCSV).

Next, the ability of eCSV to produce infectious viral progenies was examined. CSV was mixed with Corexit 9500 at a 10% final concentration for 24 h (the enhancement step); afterward, the mixture was serially diluted 10-fold, to 10^{-4}. The 10^{-4} dilution was used to infect CHSE-214 cells, and the titer produced was measured at days 0 and 7 postinfection. The titer of CHSE-214 exposed to eCSV was observed to increase from approximately 10^2 $TCID_{50}$/ml at day 0 to nearly 10^5 $TCID_{50}$/ml by day 7 (Fig. 5c), similar to the case of CHSE-214 cells infected with normal CSV (data not shown). The 10^{-4} dilution of eCSV was used instead of 10^{-1}, 10^{-2}, or 10^{-3} to avoid any possible toxic effect of Corexit 9500 on CHSE-214. Therefore, Corexit 9500 can enhance the infectivity of CSV, and eCSV can fully replicate and produce viral progenies. To determine whether the progeny virions produced from eCSV (day 7 in Fig. 5c) can also be enhanced, the virions were mixed with either DPBS or Corexit 9500 (10%, final concentration) for 24 h before titers were determined on CHSE-214. Virions that were mixed with DPBS resulted in a titer of approximately 5.16×10^4 $TCID_{50}$/ml, while those that were mixed with Corexit 9500 showed a higher titer of 2.17×10^6 $TCID_{50}$/ml

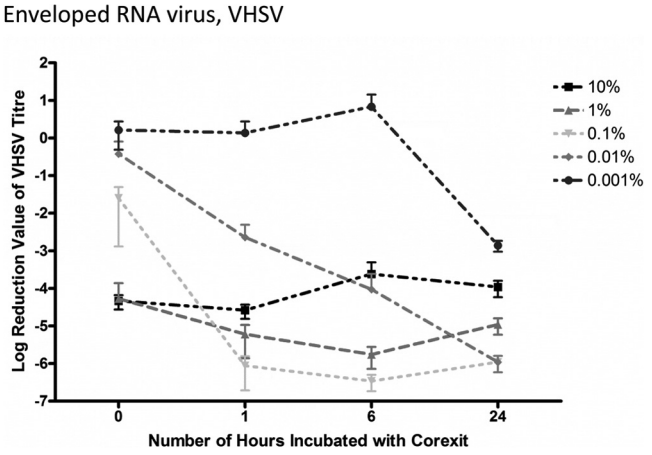

FIG 3 Effect of Corexit 9500 on the titer of a rhabdovirus, VHSV. VHSV IVb was exposed to various dilutions of corexit 9500 in suspension tests over 24 h at 14°C. As a control, the viruses were exposed to the same volume of DPBS in parallel. Immediately (time zero) and 1, 6, and 24 h after the Corexit exposure began, the mixture was sampled, the titer of virus was determined, and the log reduction value was calculated as described in Materials and Methods. The points on the graph represent means ± SD; $n = 4$.

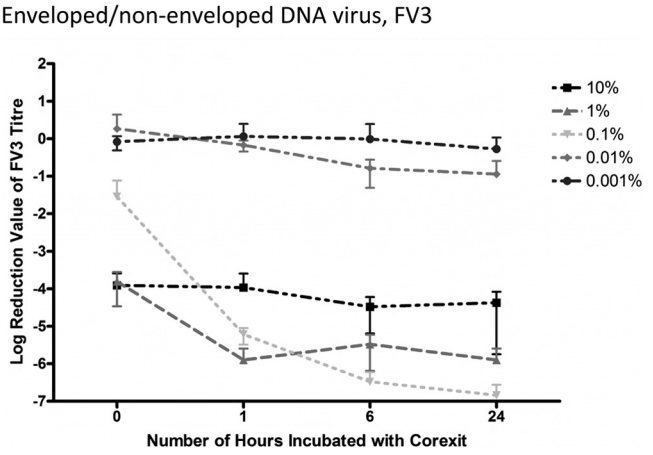

FIG 4 Effect of Corexit 9500 on the titer of an iridovirus, FV3. FV3 was exposed to various dilutions of corexit 9500 in suspension tests over 24 h at 20°C. As a control, the viruses were exposed to the same volume of DPBS in parallel. Immediately (time zero) and 1, 6, and 24 h after the Corexit exposure began, the mixture was sampled, the titer of virus was determined, and the log reduction value was calculated as described in Materials and Methods. The points on the graph represent means ± SD; $n = 4$.

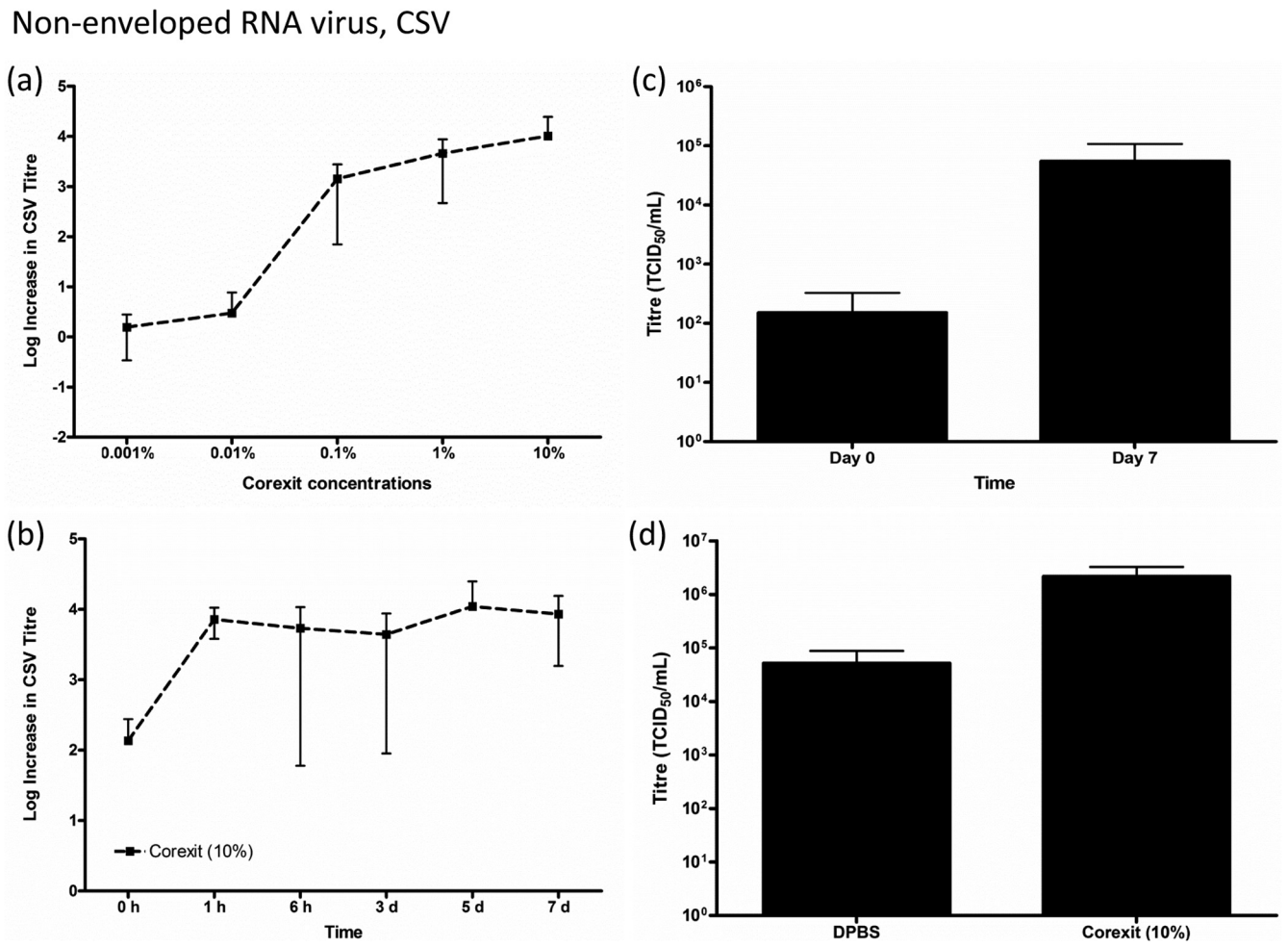

FIG 5 Effect of Corexit 9500 on the titer of a reovirus, CSV. (a) Exposure of CSV to increasing concentrations of Corexit was done for 24 h at 18°C using the same standard procedures and controls as described for the other viruses. The points on the graph represent means ± SD; $n = 4$. (b) CSV was incubated at a single high Corexit 9500 concentration of 10% for increasing times for up to 7 days. At the 0-h time point, the mixture was sampled for titer immediately after CSV and Corexit 9500 were mixed. Log-increase values were calculated as described in Materials and Methods. The points on the graph represent means ± SD; $n = 4$. (c) CSV was treated for 24 h with Corexit 9500 (10% concentration), which is referred to as eCSV. The virus-Corexit 9500 mixture was diluted to 10^{-4} and added to CHSE-214 cultures. The virus titer produced from the cultures receiving the 10^{-4} eCSV was measured at day 0 and day 7 postinfection (p.i.). The points on the graph represent means ± SD; $n = 6$. d The progeny virions of eCSV collected at day 7 p.i. in panel c were exposed to either DPBS or Corexit 9500 (10% concentration) for 24 h before titration on CHSE-214 to determine if the infectivity of progeny virions of eCSV can also be enhanced by Corexit 9500. The points on the graph represent means ± SD; $n = 3$.

(Fig. 5d). Therefore, Corexit 9500 can enhance the infectivity of CSV that had been produced from cultures initially infected with eCSV.

DISCUSSION

The effects of Corexit 9500 on four aquatic viruses with differing structural properties were examined. Corexit 9500 was able to inactivate the viruses with envelopes, VHSV and FV3, but had no effect on the nonenveloped virus, IPNV. In contrast, Corexit 9500 activated the virus with a double protein capsid, CSV. Although the complete formulation of Corexit 9500 is not available, the components include alcohols, such as 1-propanol, and nonionic and anionic surfactants which are used in detergents (3–5, 24). The interaction of these components with the four viruses is discussed below, followed by a consideration of the environmental implications of the results.

Effect of Corexit 9500 on a virus with a single protein capsid, IPNV. Corexit 9500 at all concentrations examined did not reduce the IPNV titer beyond 1 log. Relative to enveloped viruses, naked viruses have generally been found to be more resistant to inactivation by alcohol-based disinfectants (25–27). In addition, the surfactant Sorbitan, mono-(9Z)-9-octadecenoate, poly(oxy-1,2-ethanediyl), a component of Corexit 9500 (24) and a major component of Tween 80, had been previously demonstrated to enhance nonenveloped poliovirus yield instead of reducing the titer (28).

Effect of Corexit 9500 on a virus with a single protein capsid and an envelope, VHSV. The inactivation of VHSV IVb by Corexit 9500 is likely mediated through the components of the dispersant, such as alcohols and surfactants, acting on the lipids and proteins of the viral envelope, because these classes of compounds have been found to inactivate other enveloped viruses. Several studies found that alcohols inactivated rhabdoviruses, including one from mammals, vesicular stomatitis virus (VSV), as well as VHSV. 1-Propanol at concentrations of 40% or higher

abolished the infectivity of VHSV absorbed onto polystyrene surfaces (27). Ethanol, 1-propanol, and 2-propanol at less than 50% concentrations were shown to reduce the VSV titer by greater than 6 logs after 1 min of exposure in suspension tests (29); higher concentrations, 75% of combined 1-propanol and 2-propanol and 80% or higher ethanol, can reduce the titers of six different mammalian enveloped viruses by greater than 4 logs within 15 s of contact time (30). The lipid membrane of enveloped VHSV and FV3 could undergo structural disorder upon exposure to alcohols (31), and this change in membrane structure may prevent proper exposure of viral surface proteins, such as the glycoprotein of VHSV, blocking interaction with cellular receptors and viral entry into the cells. Surfactants also have a significant inactivating effect on enveloped viruses. Less than 1% concentrations of nonionic Triton X-100 and anionic sodium dodecyl sulfate (SDS) reduced the VSV titer by greater than 6 logs after 5 min of exposure in suspension tests (29). Benzalkonium chloride, a cationic surfactant, at a 1% or lower concentration can reduce the titers of enveloped herpes simplex virus and human immunodeficiency virus by 4.51 and 1.87 logs, respectively after 1 min of exposure (26). The mechanism of reduction of the enveloped virus titer by surfactants is most likely due to solubilization of membrane lipids, releasing membrane-bound proteins, which separates these components from the core of the virus, thus preventing the delivery of the viral genome into cells (32).

Effect of Corexit 9500 on a virus with a single protein capsid with or without an envelope, FV3. Surfactants and alcohols in Corexit 9500 likely also inactivated FV3 by targeting the envelope, but FV3 was more resistant than VHSV to inactivation. Surfactants have been found to inactivate at least one other ranavirus. The titer of Singapore grouper iridovirus (SGIV) was reduced by approximately 2 logs when treated in suspension tests for 1 h with three different surfactants: 0.1% Triton X-100 and 0.08 mM *n*-octyl-β-D-glucopyranoside (OG) and *n*-dodecyl-α-D-maltoside (DDM) (33). At concentrations of 0.01% and 0.001%, Corexit 9500 had a less inactivating effect on FV3 than on VHSV. This could be due to the simultaneous existence of two virion populations in FV3, both enveloped and nonenveloped virions; the reduction in the FV3 titer is likely due to inactivation of enveloped virions, since they are also more infectious than nonenveloped virions (34). As mentioned for IPNV, Corexit 9500 had no effect on a virus with just a capsid.

Effect of Corexit 9500 on a virus with a double protein capsid, CSV. Two mechanisms, working independently or synergistically, likely explain how Corexit 9500 enhanced the infectivity of CSV. One mechanism revolves around aggregation: the dispersant either prevented aggregation or broke apart aggregates of CSV. Reoviruses can aggregate, especially in solutions at pH 5 or lower (35). The dissociation of aggregated virus particles had been shown to increase viral titers in cell culture (36), and detergents can disperse aggregated viruses (37). The second mechanism involves the second capsid of reoviruses. Corexit 9500 exposure could be removing the outermost capsid of CSV, generating infectious subviral particles (ISVP). Treatment of reoviruses, including CSV, with proteases, such as trypsin and chymotrypsin, generates a smaller ISVP, through digestion of the outermost capsid protein, which leads to an enhancement in the infectivity of these viruses (38–40). Therefore, Corexit 9500 may act in a duel manner to enhance CSV infectivity.

Corexit 9500 and the virosphere. The results suggest that oil dispersants could modulate the virosphere and in doing so alter fundamental ecological processes, which in turn could have broad environmental impacts. Corexit 9500 inactivated the two enveloped viruses but left the nonenveloped viruses either unharmed or activated. The sea likely has enveloped viruses as well as nonenveloped viruses. Most aquatic bacteriophages are nonenveloped, but the ocean also appears to have abundant RNA viruses that infect eukaryotes (41). Some of these are enveloped (16). If the pattern of results from this study with four fish viruses extends to all viruses, the addition of Corexit 9500 to an aquatic environment might be expected to reduce enveloped viruses while allowing nonenveloped viruses to endure or be activated. Changing viral assemblages in this way might impede or enhance the important roles of viruses in ecological processes, such as the microbial loop, and change the environment. On the other hand, altered viral assemblages would likely be transitory because the sheer number of viral particles in the sea would allow some to remain and be capable of restoring the original viral populations, and of course, the use of dispersants would be temporary. Exploring these ideas in the future would be interesting but likely daunting because of the diversity of viruses and the complexity of ecological interactions.

For viruses of metazoans, the actions of oil dispersants might influence the prevalence of viral diseases. The inactivation of VHSV by Corexit 9500 is particularly intriguing, since some of the most important viral diseases of fish are caused by rhabdoviruses (42). Situations can be envisioned where Corexit 9500 might be suggested as a way to halt the spread of economically damaging diseases like viral hemorrhagic septicemia and infectious hematopoietic necrosis. However, the activation of CSV by this dispersant would be one virological argument against such a use.

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