

EFFICACY OF PERACETIC ACID AND SODIUM HYPOCHLORITE AGAINST SARS-
COV-2 ON CONTAMINATED SURFACES

by

JULIANNA MORRIS

(Under the Direction of Malak Esseili)

ABSTRACT

SARS-CoV-2 is a respiratory pathogen that can be transmitted through fomites. Previous studies indicated that SARS-CoV-2 RNA can be detected on high-touch surfaces such as those found at hospitals and food production facilities. Therefore, there is a need for a validated disinfectant that can inactivate more than 3 log₁₀ TCID₅₀/ml of SARS-CoV-2 on common surfaces, as required by the EPA. The objective of this study was to investigate the virucidal efficacy of sodium hypochlorite (NaOCl) and peracetic acid (PAA) against SARS-CoV-2 following the ASTM protocol for in suspension and carrier assays. NaOCl at a concentration of 1,000 ppm for 10 minutes inactivated > 3 log TCID₅₀/ml SARS-CoV-2, on stainless steel or high-density polyethylene surfaces. Similarly, PAA at 200 ppm for 10 minutes inactivated > 3 TCID₅₀ SARS-CoV-2 on stainless steel and HDPE surfaces. PAA could be a safer and more environmentally friendly alternative to NaOCl to use against SARS-CoV-2.

INDEX WORDS: SARS-CoV-2, Disinfectants, Sodium Hypochlorite, Peracetic Acid,
Contaminated Surfaces

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JULIANNA MORRIS

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JULIANNA MORRIS

Major Professor: Malak Esseili
Committee: Francisco Diez-Gonzalez
Yen-Con Hung

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
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DEDICATION

This is dedicated to my parents, for their love and support throughout all my endeavors.

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TABLE OF CONTENTS

| | Page |
|---|------|
| ACKNOWLEDGEMENTS | v |
| LIST OF TABLES | viii |
| LIST OF FIGURES | ix |
| CHAPTER | |
| 1 INTRODUCTION | 1 |
| Introduction..... | 1 |
| 2 LITERATURE REVIEW | 2 |
| Human Coronaviruses..... | 2 |
| Economic Impacts..... | 4 |
| Transmission and Fomites | 4 |
| Chemical Disinfection | 6 |
| 3 MATERIALS AND METHODS..... | 18 |
| Introduction..... | 18 |
| Suspension Assay..... | 19 |
| Surface Assay..... | 20 |
| 4 RESULTS | 24 |
| Effect of NaOCl and PAA on SARS-CoV-2 in Suspension at One Minute Contact Time | 24 |
| Effect of NaOCl and PAA on SARS-CoV-2-Contaminated Surfaces at One Minute Contact Time | 25 |

| | |
|--|----|
| Effect of NaOCl on SARS-CoV-2-Contaminated Surfaces at Five- and Ten- Minutes Contact Times | 25 |
| Effect of PAA on SARS-CoV-2-Contaminated Surfaces at Five- and Ten-Minutes Contact Times | 26 |
| 5 DISCUSSION | 36 |
| 6 CONCLUSION..... | 45 |
| REFERENCES | 46 |

LIST OF TABLES

| | Page |
|---|------|
| Table 1: List of Manuscripts using NaOCl and PAA against Human Coronaviruses in Suspension. | 16 |
| Table 2: List of Manuscripts using NaOCl and PAA against Human Coronaviruses on surfaces | 16 |
| Table 3: List of Manuscripts using Various Disinfectants against Human Coronaviruses | 17 |

LIST OF FIGURES

| | Page |
|--|------|
| Figure 1: Effect of NaOCl at 5, 50 and 200 ppm on the reduction of SARS-CoV-2 infectivity (log ₁₀ TCID ₅₀ /ml) at one min contact time in the presence and absence of a soil load. | 28 |
| Figure 2: Effect of PAA at 5, 50 and 200 ppm on the reduction of SARS-CoV-2 infectivity (log ₁₀ TCID ₅₀ /ml) at one min contact time in the presence and absence of a soil load | 29 |
| Figure 3: Effect of NaOCl on the reduction of SARS-CoV-2 infectivity (log ₁₀ TCID ₅₀ /ml) at one minute contact time using 200, 400, 600 and 1000 ppm of the disinfectants on SS and HDPE surfaces | 30 |
| Figure 4: Effect of PAA on the reduction of SARS-CoV-2 infectivity (log ₁₀ TCID ₅₀ /ml) at one minute contact time using 200, 400, 600 and 1000 ppm of the disinfectants on SS and HDPE surfaces | 31 |
| Figure 5: Effect of NaOCl on the reduction of SARS-CoV-2 infectivity (log ₁₀ TCID ₅₀ /ml) at five- and ten-minutes contact times using 200 and 1000 ppm of the disinfectants on SS surfaces. | 32 |
| Figure 6: Effect of NaOCl on the reduction of SARS-CoV-2 infectivity (log ₁₀ TCID ₅₀ /ml) at five- and ten-minutes contact times using 200 and 1000 ppm of the disinfectants on HDPE surfaces..... | 33 |
| Figure 7: Effect of PAA on SARS-CoV-2 infectivity at five- and ten-minutes contact time using 200 and 1000 ppm of the disinfectants on SS surfaces..... | 34 |
| Figure 8: Effect of PAA on SARS-CoV-2 infectivity at five- and ten-minutes contact time using 200 and 1000 ppm of the disinfectants on HDPE surfaces..... | 35 |

CHAPTER 1

INTRODUCTION

SARS-CoV-2 is the causative agent of the current COVID-19 pandemic. It is primarily a respiratory pathogen that can be transmitted through both aerosols and fomites. Previous studies indicated that SARS-CoV-2 RNA can be detected on high-touch surfaces commonly found at hospitals, food production facilities, residential care facilities, and even cruise ships. In addition, in laboratory studies, infectious virus was found to survive for days on these surfaces. Due to the high transmission capabilities of this virus, it continues to pose a serious threat to public health worldwide. Along with following mitigation strategies recommended by the CDC such as wearing face coverings and receiving vaccination when appropriate, proper cleaning and disinfection methods should also be implemented. However, there is significant variability among efficacy of available and relatively low-cost disinfectants against SARS-CoV-2. Therefore, there is a need for a validated disinfectant that can inactivate more than $3 \log_{10}$ TCID₅₀/ml, as is required by the EPA to be considered an effective disinfectant, of SARS-CoV-2 on common surfaces (OPP, 2018). Very few studies have been reported using common industry disinfectants, like sodium hypochlorite (NaOCl) and peracetic acid (PAA) against SARS-CoV-2 while testing on both stainless steel and high-density polyethylene surfaces and including soil load and water hardness. Therefore, there is a gap in our knowledge regarding the efficacy of NaOCl and PAA against this virus.

CHAPTER 2

LITERATURE REVIEW

Human Coronaviruses

Human coronaviruses are mainly associated with respiratory tract illness and range in the severity of illness they can cause in humans. The *Coronaviridae* family obtains its name from the crown-like appearance that is observed on the virus surface. In the family of coronaviruses, there are seven human coronaviruses, HCoV-229E, NL63, OC43, HKU1, MERS-CoV, SARS-CoV, SARS-CoV-2 (Mulabbi et al., 2021). Coronaviruses are enveloped single stranded RNA viruses with genomes ranging from 27 to 33 kb in length. They have a high mutation rate due to their RNA dependent RNA polymerase (RdRp) being error prone (Mulabbi et al., 2021). Because of the high mutation rate, each coronavirus can have many variants and therefore be found in a multitude of hosts and break species barriers (Halawa et al., 2022).

Some coronaviruses (HCoV-229E, NL63, OC43, HKU1) are common cold viruses with symptoms often present as diarrhea and upper respiratory tract infections (Wang et al., 2020). In children and immunocompromised individuals, these common cold viruses can be more serious (Wang et al., 2020). The other three coronaviruses are highly pathogenic viruses that cause severe infections (SARS-CoV, MERS-CoV, SARS-CoV-2). SARS-CoV-2 is known as the causative agent of the COVID-19 pandemic. In the United State alone, the COVID-19 pandemic has caused over 1 million deaths (CDC, 2023). While generally associated as a respiratory illness virus, the infection and symptoms can display almost anywhere in the body. Angiotensin-converting enzyme-2 (ACE2) is the cell receptor used by SARS-CoV-2. As the heart, brain,

vessels, liver, kidney, and lungs are all organs expressing ACE2, it is theorized that these are attacked first during COVID-19 infection (Montazersaheb et al., 2022). Some of these organs have a higher expression of ACE2, leaving them at a higher risk of infection and complication than others (Montazersaheb et al., 2022). This is a possible reason why infection can cause a range of neurological (García-Grimshaw et al., 2022) to gastrointestinal disease (Brooks & Bhatt, 2021).

COVID-19 symptoms in many individuals often involve fever, cough, fatigue, shortness of breath, and even lymphopenia with symptom severity increasing in individuals with comorbidities (Wang et al., 2020). In critical cases, increased levels of proinflammatory cytokines have been found, also known as cytokine storm (Li et al., 2021). These cytokines are rapidly secreted as a response to infective stimuli. This in turn can trigger acute respiratory distress syndrome (ARDS) and lead to death (Halawa et al., 2022). Symptoms have not been limited to displaying presenting themselves only during active infection. Long-term effects post COVID-19 infection can also develop. These long-term effects include brain fog, dizziness, difficulty sleeping, digestive symptoms, blood clots, heart conditions, and depression or anxiety (Staff, 2022). One study recently investigated the long-term effects SARS-CoV (which belongs to the same species as SARS-CoV-2 and was the causative agent behind the 2002 SARS epidemic) infection had on the immunological status and lung function of individuals 15 years post infection. Their subgroup analysis revealed that 57.1% of SARS-CoV survivors who experienced severe infection encounter small airway dysfunction 15 years later (Li et al., 2021). Thus, the COVID-19 pandemic has the potential to continue to impact public health decades for decades.

Economic Impacts

The economic impacts of the COVID-19 pandemic were also profound. In the first few months of the pandemic in 2020 in the United States, tens of millions of jobs were lost with the unemployment rate at an all-time high since 1930. State spending on hospitals increased from 96.1 billion in 2019 to 103.5 billion in 2020 and public health care spending rose by 11.4% (Steven Roman, 2022). At the end of 2021, 10 million households were behind on rent, and 20 million households were reported as having too little to eat (Priorities, 2022). Supply chain interruptions were also experienced, as personal protective equipment and pharmaceutical supplies were unable to meet the increased global demand. This in turn required increased management of these resources and gave the FDA the responsibility and authority to help mitigate negative impacts due to medical device supply chain disruptions. However, disruptions were not only noticed in healthcare settings. In the United States, the food industry experienced rapid disruptions. In April and May of 2020, 239 meat and poultry facilities reported outbreaks of SARS-CoV-2 amongst employees with over 17,000 cases and 91 deaths in those two months alone (Waltenburg et al., 2020). As these industries are a major part of the US food infrastructure, ensuring the health and safety of the employees and protecting the food supply became of imminent importance. These major events point to the need of minimizing the transmission of SARS-CoV-2.

Transmission and Fomites

SARS-CoV-2 is thought to be transmitted from human-to-human by direct airborne transmission via respiratory droplets or aerosols (Rutter et al., 2021). An infected individual emits respiratory droplets of varying sizes carrying infectious virus particles, with larger droplets carrying a higher viral load (Kirubananthan et al., 2021). These droplets land on mucous

membranes like eyes, nose and mouths or are inhaled by the non-infected individual (Rutter et al., 2021). SARS-CoV-2 is also known to infect gut tissue, and viral RNA has been detected in feces of COVID-19 patients (Meng & Liang, 2021). One study found that COVID-19 patients who tested positive for RNA of SARS-CoV-2 in fecal samples continued to have positive fecal samples for a mean of 27.9 days while their respiratory samples remained positive for only a mean of 16.7 days (Wu et al., 2020). Another study found that infectious SARS-CoV-2 may persist through the gastrointestinal tract and thus the fecal-oral transmission may also be considered (Esseili et al., 2022).

Fomite transmission of SARS-CoV-2 occurs when expelled droplets or aerosols containing an infectious viral load are deposited on surfaces, like plastic or stainless steel, and remain viable until an uninfected individual encounters this surface and deposits the viral particles onto their mucous membranes (Kirubananthan et al., 2021). Fomite transmission of SARS-CoV-2 is thought to have the highest possibility of occurring in healthcare facilities and to a lesser extent in frozen-food facilities (Kirubananthan et al., 2021; Sobolik et al., 2022). As shown by environmental testing of surfaces in healthcare settings, door handles, among many other surfaces, were found to be positive for viral RNA (Elbadawy et al., 2021). However, these same surfaces that experienced more routine cleaning and disinfection were not as often contaminated.

Cruise ships that experienced widespread infection among the passengers on board were also investigated for surface contamination of SARS-CoV-2 RNA. One study swabbed the surfaces in the rooms that contained infected passengers on the Diamond Princess. Viral RNA was found in these rooms up to 17 days after the rooms were vacated. SARS-CoV-2 RNA was most often detected from the floor around the toilets and on pillowcases in these rooms. In high

traffic areas where hydrogen peroxide was regularly used to disinfect surfaces, no viral RNA was detected (Yamagishi et al., 2020). One review study of surface swabbing found that 17.3% of samples collected from hospital settings and 10.1% of samples collected from non-hospital settings were positive for SARS-CoV-2 RNA (Gonçalves et al., 2021). Surfaces such as plastic and stainless steel artificially contaminated with SARS-CoV-2 show that the virus can remain infectious for several days up to a week (Chin et al., 2020; Liu et al., 2021), supporting the role of fomites as a possible route of transmission. Additionally, surfaces used in food packaging can retain infectious SARS-CoV-2. For example, infectious SARS-CoV-2 was recovered from imported frozen cod fish packaging which was later implicated as the source for an outbreak (Liu et al., 2021). Another study showed that SARS-CoV-2 remained infectious on artificially-inoculated plastic wrap, fruit wax and cardboard takeout containers for days, showing 3 log reduction with 10 days at 4 °C and 3 days at 20 °C (Glasbrenner et al., 2023). Therefore, controlling fomite mediated transmission of SARS-CoV-2 is important for public health.

Chemical Disinfection:

Alcohol

Several studies have been conducted covering a broad spectrum of available disinfectants and their efficacy against human coronaviruses (summarized in Table 1-3). Commonly used disinfectants are alcohol-based disinfectants, quaternary ammonium compounds, and strong oxidizers like electrolyzed water, chlorine, and peroxyacetic acid. Alcohol based sanitizers are widely used as surface and hand sanitizers. Alcohol-based sanitizers are generally divided into two categories: ethanol, and isopropanol. Isopropanol is often preferred as it has a higher ability to dissolve in lipids (Singh et al., 2020). Alcohol based disinfectants are effective against many microorganisms at a wide range of concentrations, from 26% to 70% (v/v) (Singh et al., 2020).

When this disinfectant is combined with an acid, it can be even more effective, especially against microorganisms that are more resistant to this sanitizer (Golin et al., 2020).

For alcohol-based disinfectants used against enveloped viruses, disrupting the viral envelope is the main mode of action. As they are amphiphilic compounds, they can easily enter the viral envelope. The alcohol disinfectant increases the internal membranes affinity for water, destabilizing the lipid membrane (Singh et al., 2020). Disrupting the viral envelope inhibits the virus's ability for attachment and replication, ultimately making the virus inactive (Golin et al., 2020). However, non-enveloped viruses are more resistant to alcohol-based disinfectants. One study tested efficacy of alcohol-based disinfectants against non-enveloped viruses, human norovirus and human norovirus surrogates. The alcohol-based sanitizer containing 29.4% ethanol had a $>2.5 \log_{10}$ reduction on the viruses when used on stainless steel surfaces (Escudero-Abarca et al., 2022).

The effect of alcohol disinfectants against enveloped viruses differs. For example, a study using 60-80% ethanol (EtOH) and isopropyl alcohol (IPA) at contact times starting at 15 s against HCoV-229E was able to cause a $>4 \log$ reduction in viral titer when tested on contaminated ceramic tile (Meyers et al., 2021) (Table 5). However, when these two disinfectants were used at 95% (v/v) disinfectant efficacy decreased viral titer by $<2 \log$. The authors state that water may play an important role in the mechanism of this disinfectant (Meyers et al., 2021). Another study using 36.2% (v/v) ethanol for a 3 minute contact time in the presence of 0.03% bovine serum albumin (BSA) against SARS-CoV-2 while in suspension found a $>4 \log$ reduction in viral titer (Nomura et al., 2021) (Table 6). However, while alcohol can be an effective disinfectant it is also inherently volatile and comes with variability due to the raw

material. Therefore, choosing the proper formulation, volume, contact time, and concentration according to use and soil load makes this disinfectant harder to use (Singh et al., 2020).

Quaternary Ammonium Compounds (QAC)

Quaternary ammonium compounds are a highly used broad-spectrum antimicrobial. They are used against bacteria, fungi, and viruses. Since the pandemic, their use has increased even more, as they are an active ingredient in more than half of the EPA's *List N: Disinfectants for Use Against SARS-CoV-2* (Hora et al., 2020). QACs' largest antimicrobial activity is due to their carbon chain. They are amphiphilic, therefore targeting bacterial cell membranes and the lipid membrane of enveloped viruses (Hora et al., 2020). Variations in the QACs' R groups and presence of aromatic compounds can affect antimicrobial activity and dose required (Xiling et al., 2021). QACs are also relatively stable compounds. One study tested the efficacy of two QACs, MicroChem Plus and FWD (an unspecified QAC that is still in the development stage), against SARS-CoV-2. It was found that both QACs at 0.56% concentration for 15 s contact time can inactivate $>4 \log_{10}$ of SARS-CoV-2 when tested in suspension (Huang et al., 2022) (Table 3). It was also found that the disinfectant was still effective at inactivating SARS-CoV-2 even when the solution was held a room temperature for more than a week (Huang et al., 2022).

Another study also tested two QACs, Di-N-decyl dimethyl ammonium bromide (DNB) and Di-N-decyl dimethyl ammonium chloride (DNC) against SARS-CoV-2 in the presence of 3% soil load while tested in suspension. DNB and DNC at concentrations of 170 mg/L for a 1-minute contact time can inactivate $>4 \log_{10}$ when used against SARS-CoV-2 (Xiling et al., 2021) (Table 1). While both studies found that QACs are effective disinfectants, they both also acknowledged that QACs have negative environmental and microbiological impacts, and can disrupt wastewater treatment plant operations (Huang et al., 2022). QACs can be found in

wastewater and soil, as it decomposes much slower than chlorine and hydrogen peroxide-based disinfectants (Hora et al., 2020). The presence of QACs in the environment can be toxic to aquatic and soil organisms. This in turn can have negative impacts on natural microbial communities and could impact or increase antibiotic resistance (Hora et al., 2020; Huang et al., 2022). Therefore, though QACs are effective disinfectants against SARS-CoV-2, the negative impact of high-level use should be considered.

Electrolyzed Water (EW)

Electrolyzed water is an environmentally friendly disinfectant that has gained popularity in many facets, including the food industry. Electrolyzed water is produced by the electrolysis of acid or salt. It is known to be low cost and easy to produce. The efficacy of EW is dependent upon the presence and stability of hypochlorous acid (HOCl). EW can be made and divided into three categories, acidic electrolyzed water (AEW), neutral electrolyzed water (NEW), and basic electrolyzed water (BEW) (Farah & Al-Haj Ali, 2021). The free chlorine and pH level work synergistically to alter the cell structure and viral envelopes of microorganisms. Out of the three categories, NEW is most often used as the neutral pH makes it less corrosive, with longer shelf life, and a higher oxidation reduction potential (Farah & Al-Haj Ali, 2021). However, electrolyzed water can be easily affected by organic material (Chen & Hung, 2017). The free available chlorine (FAC) in the EW readily reacts with organic material leaving less available for microorganism inactivation.

Many studies have also found that pH and water hardness level can also affect EW efficacy against microorganisms (Chen & Hung, 2017; Pangloli & Hung, 2013). One study found that AEW (pH 2.5, FAC 74 ppm) held in suspension for a 1-minute contact time in the presence of 10% FBS was able to induce a >3 log reduction in SARS-CoV-2 titer (Takeda et al.,

2020) (Table 3). The authors also found that AEW efficacy decreased with increased storage time and increased concentration of FBS as FAC concentration diminished due to storage or was used by the organic material (Takeda et al., 2020). Another suspension study using super alkaline ionized water (SAIW) for 30 s contact time induced a 4.5 log reduction in HCoV-OC43 titer. This was performed in the presence of only 0.2 % FBS (Suzuki et al., 2021) (Table 3). Therefore, EW is an environmentally friendly and sustainable disinfectant that can be used to inactivate SARS-CoV-2 in suspension. However, it is highly susceptible to organic material and more research is needed to determine efficacy against viruses on contaminated surfaces.

Sodium Hypochlorite

Sodium hypochlorite (NaOCl) is a strong oxidizer disinfectant used in an abundance of settings. It is an easily accessible and cheap disinfectant that can be quickly diluted according to need. NaOCl is a disinfectant composed of different chlorine species. The pH of the disinfectant solution determines the concentration of the different species that are present. Hypochlorous acid (HOCl) is the dominant chlorine species in between pH 4-7 (McDonnell & Russell, 1999). HOCl is highly reactive and a stronger disinfectant than the other chlorine species that exist at a higher pH (Lin et al., 2020). The HOCl molecule disrupts peptide bonds and thiol groups on proteins through oxidation and chlorine exchange (Lin et al., 2020). The neutral HOCl molecule penetrates negatively charged membranes and causes an exchange of hydrogen with chlorine. This exchange causes the membrane to fall apart and inactivates microorganisms (McDonnell & Russell, 1999). Sodium hypochlorite solutions are unstable in temperature variations beyond (10-35 °C) as well as light exposure (Lin et al., 2020). When NaOCl solutions are made as 5000 ppm and pH is >9, they have a longer shelf life, in some cases up to 30 days. However, as mentioned previously, NaOCl is a more effective disinfectant when used at pH < 8 due to dominance of

HOCl species. The shelf-life of typical household bleach is estimated to be one year (Gagliardi, 2022).

Guidelines for disinfectant concentrations vary according to use, setting, and organic material presence as increased organic material decreases disinfectant efficacy. In clinical settings, sodium hypochlorite solution recommendations vary generally between 500-1000 ppm (Lin et al., 2020) and can increase to up to 10,000 ppm (CDC, 2016). For food contact surfaces, 50-100 ppm is recommended (Lynch, 2021). For use against SARS-CoV-2 in most healthcare settings, the WHO recommends 1000 ppm NaOCl solutions used at a pH <8 and made fresh daily, with a 1 minute contact time (WHO, 2020). Using NaOCl at high concentrations is corrosive to surfaces, especially stainless steel, and is harmful to human health as it irritates mucous membranes, skin, and airways (Lin et al., 2020). The byproducts formed through interactions with organic material may also be toxic (Gil et al., 2019).

Studies can be found using NaOCl against human coronaviruses or SARS-CoV-2 (Table 1 & 2). However, the composition of the disinfectant solutions as well as contact time, soil load, and use on surfaces varies greatly and is not consistent among previous literature (addressed in the discussion section). For example, a 10% NaOCl (10000 ppm) solution was found to reduce SARS-CoV-2 infectivity by 3.25 log₁₀ during a one-minute contact time, as determined by an in-suspension assay (Chan et al., 2020) (Table 1). In another study relying on an in-suspension assay, 1.5-3 ppm free chlorine from chlorinated pool water at pH 7.4 inactivated SARS-CoV-2 by 3 log₁₀ during a 30 seconds contact time (Brown et al., 2021) (Table 1). Furthermore, studies testing disinfectants on surfaces vary in their inclusion of factors such as soil load and hard water, known to affect the virucidal ability of the disinfectant. For example, 5000 ppm NaOCl tested on stainless steel surfaces inoculated with HCoV-229E in the presence of soil load showed

a $>3 \log_{10}$ reduction during a one-minute contact time (Sattar et al., 1989) (Table 2). In contrast, NaOCl at 600 ppm diluted in 380-400 ppm hard water, tested on animal coronaviruses suspended in soil load and dried on stainless steel surfaces found less than 1 \log_{10} reduction in one-minute contact time, respectively (Hulkower et al., 2011) (Table 2). Therefore, it is hard to draw meaningful conclusions based on previous studies regarding the best concentration and contact time for NaOCl to inactivate at least 3 log of SARS-CoV-2 (OPP, 2018). Because of the accessibility of this disinfectant and the lack of studies showing efficacy against surfaces while following standardized methods, this disinfectant was chosen to be included in this research project.

Peracetic Acid

Peracetic acid (PAA, CH_3COOOH) is a disinfectant that is commonly used in healthcare and food industry settings. It has been shown to inactivate spores, fungi, and viruses. PAA decomposes or dissociates to three safe by-products, acetic acid, oxygen, and water. Therefore, it is considered more environmentally friendly and less hazardous to humans compared to QAC and NaOCl. Like NaOCl, PAA is thought to act as an oxidizer. It is theorized to denature proteins, oxidize sulfhydryl and sulfur bonds, and permeabilize cell walls or viral envelopes (Zoellner et al., 2018). In comparison to EW and NaOCl, PAA efficacy is thought to be minimally affected by the presence of soil load or variation in pH between 1-8 (McDonnell & Russell, 1999). In storage, this disinfectant has a shelf-life of one year (Envirotech, 2016). However, 1% solutions has been shown to lose half of its strength in just 6 days (Choi et al., 2022). Guidelines for PAA in the food industry is 80 ppm for produce wash water and 200 ppm on food contact surfaces with variations in between (FDA, 2010).

PAA is effective at inactivating pathogens such as *L. monocytogenes*, *E. coli* O157:H7 , and poliovirus at concentrations of 12-2250 ppm (CDC, 2016). However, there are very few studies investigating the effect of PAA on SARS-CoV-2 in suspension or on surfaces (Table 1 & 2). For example, a study found that PAA can inhibit the spike protein of a pseudovirus SARS-CoV-2 variants (Yamamoto et al., 2022). One study showed that 173 ppm PAA when tested in suspension reduced SARS-CoV-2 by a range of 6.8-56.28% using a 10-minute contact time but without organic load added (Lee et al., 2023) (Table 1). Studies that have tested PAA against SARS-CoV-2 on surfaces thus far have used this disinfectant by application of dry fogging or using food as the carriers (Cutts et al., 2021; Jung et al., 2023). Disinfectant solution composed of a 0.01/0.03 % peroxyacetic acid and hydrogen peroxide solution caused a >4 log reduction in viral titer for SARS-CoV-2 (Schinköthe et al., 2021) (Table 3). Another study found when 80 ppm PAA was used on lettuce, as recommended by the FDA, a 5 min contact time was required to induce a 3-log reduction in SARS-CoV-2 titer (Jung et al., 2023) (Table 2).

Increasing the concentration to 160 ppm required a contact time of only 1-minute to induce a 4 log reduction in virus titer in lettuce (Jung et al., 2023). Previous studies used various other viruses in their investigation of PAA virucidal efficacy. For example, one study found that 200 ppm PAA diluted in hard water and held for 5 min while performed in the presence of soil load reduced HCoV-229E titer by 3.8 log (Choi et al., 2022)(Table 2). A study on the efficacy of PAA as a disinfectant for medical instruments found that a range of 400-1000 ppm was needed to inactivate enteric non-enveloped viruses such as adenovirus, murine norovirus and poliovirus (Becker et al., 2017). As PAA is a more environmentally friendly alternative to NaOCl, and there are currently no studies assessing its potential against SARS-CoV-2 on surfaces commonly found in hospitals or food establishments while following standardized methods, this disinfectant

was also chosen for further investigation. One study only compared the efficacy of NaOCl at 1000 and 2000 ppm to PAA at 2000 ppm using cloth food packaging surfaces contaminated with SARS-CoV-2 at cold chain temperatures (-20 C), showing a 4 log₁₀ reduction in virus titer at 5-minute contact time (Wu et al., 2022) (Table 2). Therefore, PAA will be investigated in comparison to NaOCl as it is the most used disinfectant that is also recommended for use against SARS-CoV-2 by the WHO.

Dry Fogging

As with the other disinfectant methods, more research is being conducted on other efficient no-touch methods of applying different disinfectants to rooms and on surfaces. Dry fogging is one often considered when no touch disinfectant application is desired. Dry fogging applies disinfectants as aerosols that do not leave objects wet post application (Urushidani et al., 2022). This could be important in spaces where materials cannot get wet. It can also be used to disinfect many hard to access areas. Many studies can be found applying sodium hypochlorite, peracetic acid, quaternary ammonium compounds, and even formaldehydes to rooms and surfaces via dry fogging. One study found that a 8700 ppm hypochlorous acid solution and a 56400 ppm hydrogen peroxide solution was able to induce a > 1.5 and 2 log reduction in SARS-CoV-2 titer (Urushidani et al., 2022) (Table 3).

Another study found that a 0.01/0.03 % peroxyacetic acid and hydrogen peroxide solution caused a >4 log reduction in SARS-CoV-2 titer (Schinköthe et al., 2021) (Table 3). However, the study noted that when SARS-CoV-2 was deposited on the surfaces in droplet form, it was less sensitive to the aerosolized disinfectant than when the virus was evenly distributed across the surfaces. The droplet may have allowed aggregates to form, shielding layers of virus particles from the disinfectant mixture. As viruses are often expelled onto surfaces in droplet

form, this poses an additional issue to dry fogging. This study concluded that surfaces should be wet wiped before application of aerosolized disinfectant to obtain proper disinfection (Schinköthe et al., 2021).

Other difficulties when using dry fogging as a disinfectant have been found. Any surfaces blocked by objects when dry fogging is conducted may not receive proper exposure to the disinfectant, negating the purpose of using disinfectants. In addition, variation in room temperature can affect the efficacy of dry fogging (Schinköthe et al., 2021). Temperature gradients within a room prevent proper distribution of the aerosols, leaving surfaces untouched. This in turn leads to reduced widespread disinfectant efficacy. Though there are studies suggesting dry fogging is effective against SARS-CoV-2, it is currently not a recommended practice by WHO or the CDC as it poses risks to human health and can affect the eyes, respiratory system and skin of individuals working in close vicinity to this method (CDC, 2008; WHO, 2020).

Table 1: List of manuscripts using PAA and NaOCl against human coronaviruses in suspension.

| Paper | Virus Tested | Disinfectant | Concentration | Contact time | Soil load | Suspension | Viral Inocula Titer | Log Reduction |
|-------------------------|--------------|------------------------------|----------------------------------|------------------|-------------------------------|------------|---|--|
| (Xiling et al., 2021) | SARS-CoV-2 | NaOCl QACS: DNB DNC | 1000 ppm 170 mg/L | 30 s 1 min | 3% BSA | Suspension | 10 ^{-5.5} /0.1 mL | >3 log ₁₀ >4 log ₁₀ |
| (Chan et al., 2020) | SARS-CoV-2 | Bleach | 10% | 1 & 5 min | X | Suspension | 10 ^{6.5} TCID ₅₀ /mL | 1 min :0.83 to 3.25 log ₁₀ 5 min : 0.92 to 3.75 log ₁₀ 3 log ₁₀ |
| (Brown et al., 2021) | SARS-CoV-2 | FAC in pool water | 1.5 mg l ⁻¹ (ppm) FAC | 30 s | X | Suspension | 1 × 10 ⁴ TCID ₅₀ ml ⁻¹ | 3 log ₁₀ |
| (Lee et al., 2023) | SARS-CoV-2 | PAA NaOCl | 173 ppm 2000 ppm | 10 min 30 min | X 5% | Suspension | X | 6.8-56.25% 4 log ₁₀ |
| (Chin et al., 2020) | SARS-CoV-2 | NaOCl | 75 ppm 150 ppm | 5, 10, 30 min | X | Suspension | 7.8 log ₁₀ TCID ₅₀ /ml | >4.8 log ₁₀ |
| (Hatanaka et al., 2021) | SARS-CoV-2 | NaOCl | 80 ppm | 10 s | 1% FBS | Suspension | X | >4 log ₁₀ |
| (Ijaz et al., 2021) | SARS-CoV-2 | NaOCl | 1400 3200 ppm | 0.5 min 5 min | 3g/L BSA 3mg/L erythrocyte | Suspension | 1 × 10 ⁶ PFU | >5.1 log >5.1 log |

Table 2: List of manuscripts using PAA and NaOCl on surfaces.

| Paper | Virus Tested | Disinfectant | Concentration | pH and hard water | Contact time | Soil load | Surface | Viral Inocula Titer | Log Reduction |
|-------------------------|--------------|---|---|------------------------------|--------------|-----------|----------------------------|--|--|
| (Meyers et al., 2021) | HCoV-229E | Ethanol NaOCl | 60-80% 0.525% | X | 15 s | 5% BSA | Ceramic and porcelain tile | X | >4 log ₁₀ |
| (Sattar et al., 1989) | HCoV-229E | NaOCl Ethanol | 5000 ppm 70% | 11.0/Tap water | 1 min | yes | Stainless steel | 10 ⁴ -10 ⁶ PFU | >3 log ₁₀ |
| (Hulkower et al., 2011) | MHV, TGEV | NaOCl | 600 ppm | 380-400 ppm | 1 min | 600 mg/L | Glass | X | MHV: 0.62 log ₁₀ TGEV: 0.35 log ₁₀ |
| (Jung et al., 2023) | SARS-CoV-2 | PAA | 40-4000 ppm | X | 1 & 5 min | X | Lettuce, chicken, salmon | 10 ⁶ log TCID ₅₀ /mL | Lettuce: 160 ppm 1 min: 4 log 80 ppm 5 min: 3 log Meat: 2000 ppm 1 min: 2 log 1000 ppm 5 min: 2 log |
| (Choi et al., 2022) | HCoV-229E | PAA | 200 ppm | 300 ppm | 5 min | 5% | SS and Polypropylene | 6.4-6.8 log ₁₀ TCID ₅₀ /mL | 3.9 log ₁₀ |
| (Wu et al., 2022) | SARS-CoV-2 | PAA (ethanol) NaOCl (ethylene glycol) QAC (ethanol) | 2000 ppm 2000 & 1000 ppm 3000 ppm | X | 5 min | 5% | Cloth carrier | 10 ^{5.50} /0.1 mL | 4 log ₁₀ |
| (Sloan et al., 2022) | SARS-CoV-2 | NaOCl | 5000 ppm | 0.04 % w/v CaCO ₃ | 1 & 5 min | 5% | Stainless Steel | 8.5 logs/ml | >3.45 and 6 log ₁₀ |

Table 3: List of manuscripts using various disinfectants against human coronaviruses.

| Paper | Virus Tested | Disinfectant | Concentration | pH and hard water | Contact time | Soil load | Suspension/ Surfaces | Viral Inocula Titer | Log Reduction |
|-----------------------------|--------------|---|-----------------------|-------------------|--------------|-----------|----------------------|--|------------------------------|
| (Nomura et al., 2021) | SARS-CoV-2 | Ethanol | 36.2% | X | 3 min | 0.03% BSA | Suspension | 1x 10 ⁷ | >4 log ₁₀ |
| (Takeda et al., 2020) | SARS-CoV-2 | AEW (EW) | 74 ppm FAC | pH 2.4 | 1 min | 10% | Suspension | 5.75-7.25 log ₁₀ TCID ₅₀ | >3 log ₁₀ |
| (Suzuki et al., 2021) | HCoV-OC43 | SAIW (EW) | 0.17%KOH | pH 12.5 | 30 s | 0.2% FBS | Suspension | 2.5 x 10 ⁵ PFU | >4.5 log ₁₀ |
| (Huang et al., 2022) | SARS-CoV-2 | QACS: MicroChem Plus FWD | 0.56% | X | 15s | X | Suspension | X | >4 log ₁₀ |
| (Schinköthe et al., 2021) | SARS-CoV-2 | PAA & H ₂ O ₂ | 0.01/0.03 % | DI water | X | X | Dry fogging | 3.37 × 10 ⁵ | >4 log ₁₀ |
| (Urushidani et al., 2022) | SARS-CoV-2 | HOC1 H ₂ O ₂ | 8700 ppm 56400 ppm | X | X | X | Dry fogging | 1.2 × 10 ⁵ TCID ₅₀ | >1.5 and 2 log ₁₀ |
| (Omidbakhsh & Sattar, 2006) | HCoV-229E | ACCEL TB (H ₂ O ₂) | 0.50% | 3.0 | 1 min | 5% | Surface | 4.5 log ₁₀ | >4log ₁₀ |

CHAPTER 3

MATERIALS AND METHODS

Introduction

The objectives of this study were to investigate the efficacy of NaOCl and PAA at inactivating infectious SARS-CoV-2 using the American Society for Testing and Materials (ASTM) standard in suspension and surface methods. The two disinfectants were tested at several concentrations and contact times. Two surfaces, with different texture, stainless steel (SS) and high-density polyethylene (HDPE) were used.

Materials and Methods

Virus propagation and cell culture

The US reference strain SARS-CoV-2 USA-WA 1/2020 (BEI resources NR-52281) was propagated in African green monkey kidney cells (Vero-E6 ATCC CRL-1586) as described previously (Esseili et al., 2022). Handling of SARS-CoV-2 was done under strict BSL3 biosafety protocols at the Center for Food Safety BSL3 laboratory. Briefly, one or two-day-old 90% confluent cells were used to prepare virus stocks using a multiplicity of infection of 0.01. Infection media consisted of DMEM supplemented with 2% fetal bovine serum and 1% antibiotic-antimycotic cocktail. Harvesting the virus was done at 72 h post-infection. Infected cells were collected from the flasks and centrifuged at low speed (450×g for 5 min at 4 °C) to pellet the cell debris, while supernatants containing the virus, were ultra-filtered through an

Amicon 100 K Ultra-15 (Millipore) immediately after harvest to concentrate the viruses 10 times. An aliquot of the virus was immediately titrated as described below. The original viral titer generated was $\sim 7 \log_{10}$ TCID₅₀/ml while the ultrafiltered virus titer was $\sim 8 \log_{10}$ TCID₅₀/ml. The ultrafiltered viruses were first diluted 10x in sterile water before being mixed with or without soil load for in suspension assays or directly diluted in soil load before being used for surface assays.

Testing disinfectants against SARS-CoV-2 following ASTM protocol E1052 in suspension assay:

Sodium hypochlorite solutions were made by diluting commercial bleach in sterile water, using unopened commercial bleach for every experiment. Peracetic acid solutions were made by diluting concentrated peracetic acid (Peroxychem LLC, USA) in sterile water. Disinfectant concentrations tested for in suspension assay were 5, 50 and 200 ppm. ASTM protocol E1052 was followed for the in suspension virucidal assay. Viruses were diluted 1:10 in sterile water before being used in the experiment or added to soil load. Soil load with virus in volumes of 500 μ l is composed of a tripartite mixture of 35 μ l tryptone (Sigma-Aldrich), 25 μ l bovine serum albumin (BSA) (HyClone), 100 μ l bovine mucin (Sigma), and finally 340 μ l virus. Tryptone and BSA solutions were prepared by dissolving 0.5 g each in separate 10 mL phosphate buffer solution (PBS). While bovine mucin solution was prepared by dissolving 0.04 g in 10 mL PBS. All three solutions were sterilized by membrane filtration using 0.22 μ m nitrocellulose filter (VWR). Virus suspension and disinfectants were combined at a ratio 1:10 (v/v) and held for one minute at room temperature before being neutralized with an equal volume from 0.025 M sodium thiosulfate for NaOCl (Sigma) (Cromeans et al., 2014) or Dey/Engley neutralizing broth (Remel) for PAA (Manuel et al., 2017). A control negative (without virus added) and control

positive (virus only) were included in each experiment. All experiments were performed at room temperature (~25 °C).

Testing disinfectants against SARS-CoV-2 following ASTM protocol E2197-17 for surface assay

Surfaces used were stainless steel coupons (type 304, no. 4 finish, Washington Specialty Metal, Athens, GA, USA) and high-density polyethylene surfaces (Kitchen cutting boards, Walmart, USA). The HDPE surfaces were cut into smaller coupons approximately 0.8 in diameter to fit inside 50 ml falcon tubes. Coupons were a kind gift from Dr. Yen-Con Hung with the Center for Food Safety, University of Georgia.

ASTM protocol E1053 was followed for virucidal assay on surfaces. Stainless steel and HDPE surfaces carriers were placed in wells of 6-well plates and inoculated drop wise with 10 µl of virus suspension prepared in soil load as described above. The coupons were allowed to dry at room temperature for 45 min inside the biosafety cabinet. Disinfectants at 200, 400, 600 and 1000 ppm were prepared in 300 ppm hard water. The AOAC hard water solutions guideline which is used to make a range of 100-500 ppm hard water measured in mg/L of CaCO₃ (EPA, 2019). Two solutions were used to make the hard water. Solution 1 is a 0.98 M solution composed of anhydrous MgCl₂ and CaCl₂ (Sigma) dissolved in boiled deionized water. Solution 2 was composed of 0.66 M NaHCO₃ (Sigma). Both solutions were sterilized by membrane filtration using a 0.22 µm nitrocellulose filter (Millipore). Per 1 liter of water needed, 1 ml of solution 1 was added per 100 ppm desired and 4 ml of solution 2 per 1 liter of water final volume. The addition of hard water changed the pH of the final NaOCl solution, so this was adjusted back to pH 6-6.5 using 1 M HCl. While for PAA, hard water did not change the pH which ranged between 4.3-4.5. Disinfectants were added to virus-inoculated coupon at a ratio of

10:1 (v/v). Initially the contact time was fixed at one minute. However, experiments were repeated with two disinfectants concentrations (200 and 1000 ppm) and an increased contact time of 5 or 10 minutes. Disinfectants were neutralized at a ratio of 1:2 (v/v). Coupons were aseptically transferred to a 50 ml Eppendorf tubes containing 990 µl of cell culture infection media (DMEM supplemented with 2% fetal bovine serum and 1% antibiotic-antimycotic cocktail) and vortexed on medium speed for 1 minute. Eluates were transferred to a 1.5 ml tubes and tested immediately using TCID₅₀ assay, as described below. A control negative (without virus added) and control positive (virus only) were included in each experiment. All experiments were performed at room temperature (~25 °C).

Initially, surfaces were prepared by rinsing with sterile deionized water, spraying with 70% ethanol before autoclaving. After using these surfaces, they were sterilized by spraying with 10% Micro-Chem Plus (National Chemical Laboratories) and autoclaved at 121 °C for 120 min to inactivate SARS-CoV-2. After being autoclaved, coupons were sprayed with 70% ethanol and rinsed twice with deionized sterile water to remove Micro-Chem residue before being autoclaved a second time and re-used.

Virus Infectivity quantification by TCID₅₀ assay

Quantification of virus titers is based on the 50% tissue culture infectious dose (TCID₅₀) assay. Briefly, ten-fold serially diluted samples in infection media are added to four wells of 1-2 days old Vero-E6 cells in a 96-well tissue culture plate. After 4-5 days incubation period, the cells are observed for cytopathic effect (CPE). The titer is determined from the dilution where 50% of the wells show CPE and is calculated based on the Reed & Muench method (Reed & Muench, 1938). Negative and positive controls were included in every experiment as described

above. With Each TCID₅₀ assay, cytotoxicity controls were included for the disinfectants, neutralizer, disinfectant and neutralizer combined. In addition, the verification of neutralization (disinfectant and neutralizers were combined, held for one minute, then virus was added) and effect of neutralizers on virus (only virus and neutralizers combined) were included. Neither the neutralizers nor the disinfectant combined with neutralizers had any significant effect on SARS-CoV-2 titers. However, PAA and NaOCl had sometimes a cytotoxic effect on Vero-E6 cells at the undiluted concentrations. Therefore, the detection limit in the TCID₅₀ assay was set at 1.8 log₁₀ TCID₅₀/ml for this treatment when cytotoxicity was observed.

Statistical analyses

All experiments were performed independently three times with at least three technical replicates. The entire data set was transformed to log₁₀. Means and standard errors were calculated from all technical replicates. The log₁₀ reductions were calculated by subtracting virus titers of all technical replicates for a specific treatment from the average virus titer of control no treatment. GraphPad Prism version 5 (Graph Pad Software, USA) was used for all statistical analyses. Data were expressed as mean ± standard error. Two-way analysis of variance (ANOVA) followed by Bonferroni post-test was used to compare means. Factors analyzed include presence/absence of soil load, different concentrations of disinfectants, surface type and contact time. Differences in means were considered significant when *P* value was less than 0.05 and were denoted in figures by either different letters or asterisks. Specifically for surface assays, comparing disinfectant concentrations within a contact time and surface type: means with different letters differed significantly. Comparing the different corresponding disinfectant concentrations between surfaces: significant differences were denoted with asterisks (*).

Comparing corresponding disinfectant concentrations between two contact times: significant differences were denoted with number sign (#).

CHAPTER 4

RESULTS

Effect of NaOCl and PAA on SARS-CoV-2 in Suspension at One Minute Contact Time

When NaOCl at 5 and 50 ppm were incubated with SARS-CoV-2 for one minute, the virus infectivity showed an average of 2.2 and 5.1-log reduction, respectively (Figure 1). However, the presence of soil load significantly impacted the effect of NaOCl on SARS-CoV-2. Specifically, NaOCl at 5 and 50 ppm in the presence of a soil load, showed 0 and 1 log reduction in SARS-CoV-2 infectivity, respectively (Figure 1). Furthermore, in the presence of soil load, increasing the concentration of NaOCl from 5 to 200 ppm, significantly increased the inactivation of the virus to a 5.2 log. However, at 200 ppm NaOCl, soil load did not significantly affect NaOCl virucidal activity as compared to no soil load (Figure 1).

Peracetic acid significantly inactivated SARS-CoV-2 infectivity by 3.1 log at 50 ppm; however, again the presence of soil load significantly reduced this inactivation to 1.3 log (Figure 2). In the presence of a soil load, a 200 ppm of PAA showed 3.7 log reduction in SARS-CoV-2 infectivity (Figure 2). Like the NaOCl results, at 200 ppm PAA, soil load did not significantly affect PAA virucidal activity as compared to no soil load (Figure2).

In comparing disinfectants to each other, it was found that NaOCl performed significantly better at all tested concentrations in the absence of a soil load . However, specifically at the 200 ppm NaOCl showed a significantly higher reduction in SARS-CoV-2 infectivity as compared to 200 ppm PAA both in presence and absence of a soil load.

Effect of NaOCl and PAA on SARS-CoV-2-Contaminated Surfaces at One Minute Contact Time

NaOCl at 200, 400 and 600 ppm exerted less than 2 log reduction in SARS-CoV-2 infectivity on both SS and HDPE (Figure 3). Increasing the concentration to 1000 ppm increased the log reduction in SARS-CoV-2 infectivity to ~1.7 and 2.3 on SS and HDPE, respectively; however, the increase was not significantly different from the other tested concentrations for NaOCl on either surface (Figure 3).

In contrast, PAA at 200 ppm showed 2 and 2.3 log reduction in SARS-CoV-2 infectivity on SS and HDPE, respectively (Figure 4). Increasing the concentration to 400 and 600 ppm increased the log reduction further to at least 3 log but the increase was not significantly different from the 200 ppm concentration (Figure 4). PAA at 1000 ppm showed 3.5 and 2.9 log reduction in SARS-CoV-2 infectivity on SS and HDPE, respectively; however, again the increase was not significantly different from the 200 ppm PAA on either surface (Figure 4).

In comparing disinfectants to each other, it was found that PAA performed significantly better at the 600 and 1000 ppm concentrations on SS in comparison to NaOCl. However, on HDPE, only PAA at 600 ppm showed significantly higher reduction in SARS-CoV-2 infectivity as compared to 600 ppm NaOCl.

Effect of NaOCl on SARS-CoV-2-Contaminated Surfaces at Five- and Ten-Minutes Contact Times

Given that increasing the disinfectant concentrations from 200 to 1000 ppm with a one-minute contact time did not show significant increase in log reduction of SARS-CoV-2 (shown above), the contact time between the disinfectants and SARS-CoV-2-contaminated surfaces was increased to 5 and 10 min.

On SS, NaOCl at 200 ppm and 5 min contact time, a 1.2 log reduction in SARS-CoV-2 infectivity was observed. The latter did not change significantly by further extending the contact time to 10 minutes (1.5 log reduction) (Figure 5). At 1000 ppm, NaOCl showed 3 log reduction in SARS-CoV-2 at 5 min contact time, which again did not change significantly (3.5 log) by further extending the contact time to 10 minutes (Figure 5). On stainless steel, a 1000 ppm NaOCl and 10-minute contact time are required to inactivate SARS-CoV-2 by $> 3.5 \pm 0.3$ log.

On HDPE, NaOCl at 200 ppm and 5 min contact time, exerted ~ 2 log reduction in SARS-CoV-2 infectivity which again did not change significantly (2.3 log) by further extending the contact time to 10 minutes (Figure 6). At 1000 ppm, NaOCl showed 3 log reduction in SARS-CoV-2 at 5 min contact time, which again did not change significantly (3.3 log) by further extending the contact time to 10 minutes (Figure 5). Therefore, on HDPE, a 1000 ppm NaOCl and 10-minute contact time are required to inactivate SARS-CoV-2 by $> 3.4 \pm 0.4$ logs.

In comparing surfaces to each other's, NaOCl action on SARS-CoV-2 was not significantly different between SS and HDPE at either 200 ppm or 1000 ppm.

Effect of PAA on SARS-CoV-2-Contaminated Surfaces at Five- and Ten-Minutes Contact Times

On SS, PAA at 200 ppm and 5 min contact time, a 2.4 log reduction in SARS-CoV-2 infectivity was found which was significantly increased to 3.8 log by further extending the contact time to 10 minutes (Figure 7). At 1000 ppm, PAA demonstrated a 3.9 log reduction in SARS-CoV-2 at the 5 min contact time, which again was significantly enhanced to 5 log by further extending the contact time to 10 minutes (Figure 7). On SS either a 1000 ppm PAA and 5-minute contact time (3.9 ± 0.3 log) or a 200 ppm and 10-minute contact time (3.8 ± 0.4 log) can be implemented to inactivate SARS-CoV-2 by at least 3 logs.

On HDPE, PAA at 200 ppm and 5 min contact time, exerted 2.1 log reduction in SARS-CoV-2 infectivity which was significantly enhanced to 3.3 log by further extending the contact time to 10 minutes (Figure 8). At 1000 ppm, PAA showed 2.5 log reduction in SARS-CoV-2 at 5 min contact time, which did not change significantly (2.7 log) by further extending the contact time to 10 minutes (Figure 8). It is recommended that PAA should be used at 200 ppm for a 10-minute contact time (3.3 ± 0.2 log) on HDPE to inactivate more than 3 logs of SARS-CoV-2.

In comparing surfaces to each other's within a specific contact time, PAA action on SARS-CoV-2 was not significantly different between SS and HDPE at 200 ppm and 5 min or 10 min. However, PAA at 1000 ppm showed significantly higher inactivation of the virus on SS (5 log) as compared to HDPE for both 5 and 10 min contact times.

In comparing disinfectants to each other within a specific surface type, it was found that PAA at both 5 and 10 min contact times, performed significantly better at 200 and 1000 ppm concentrations on SS in comparison to NaOCl. However, on HDPE, both 200 and 1000 ppm PAA at 5 and 10 min contact times, showed similar log reductions for SARS-CoV-2 infectivity as compared to NaOCl.

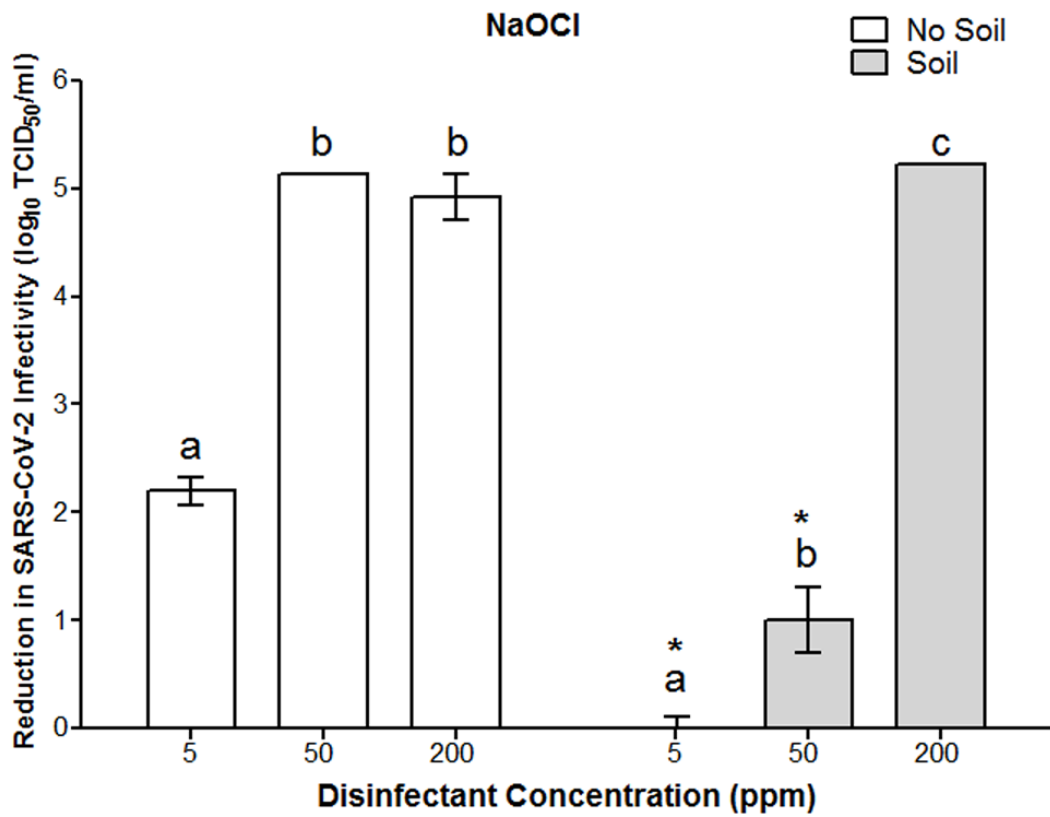


Figure 1: Effect of NaOCl at 5, 50 and 200 ppm on the reduction of SARS-CoV-2 infectivity in solution (log₁₀ TCID₅₀/ml) at one min contact time in the presence and absence of a soil load.

The ASTM E1052 standard method for testing the disinfectants against SARS-CoV-2 in suspension was followed. Data are reported as means ± standard error. Comparing within soil or no soil load: means with different letters differ significantly. Comparing corresponding concentrations between soil and no soil load: significant differences are denoted with asterisks.

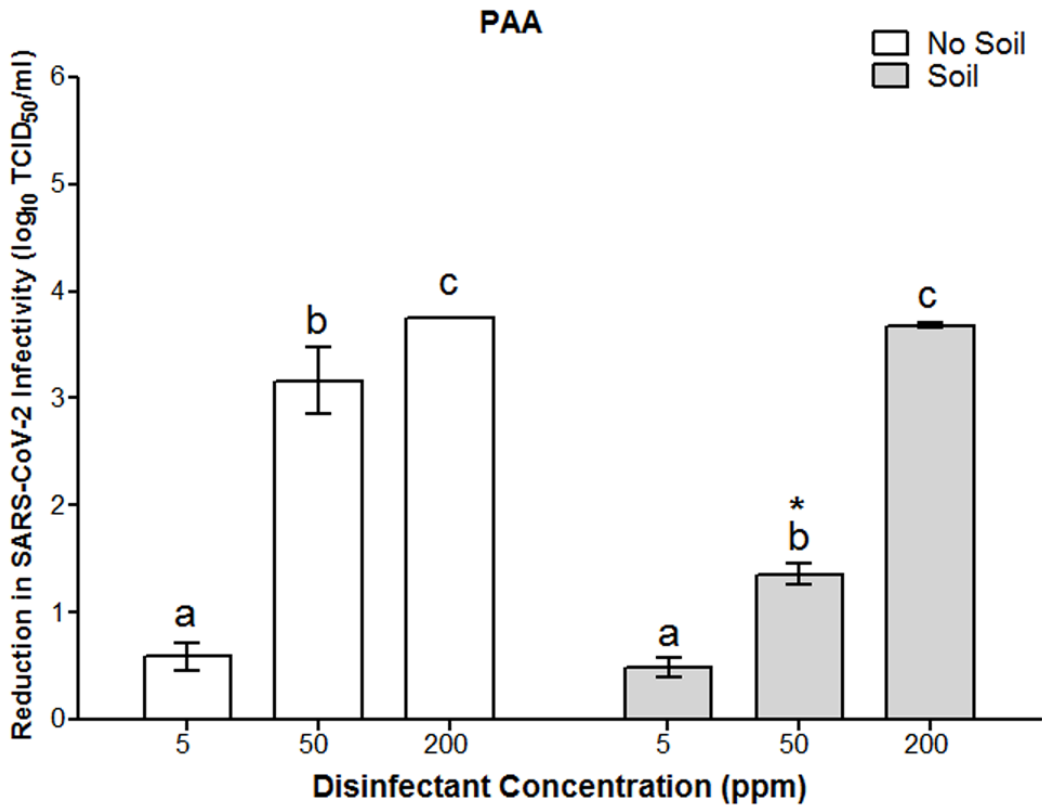


Figure 2: Effect of PAA at 5, 50 and 200 ppm on the reduction of SARS-CoV-2 infectivity in solution (log₁₀ TCID₅₀/ml) at one min contact time in the presence and absence of a soil load. The ASTM E1052 standard method for testing the disinfectants against SARS-CoV-2 in suspension was followed. Data are reported as means ± standard error. Comparing within soil or no soil load: means with different letters differ significantly. Comparing corresponding concentrations between soil and no soil load: significant differences are denoted with asterisks.

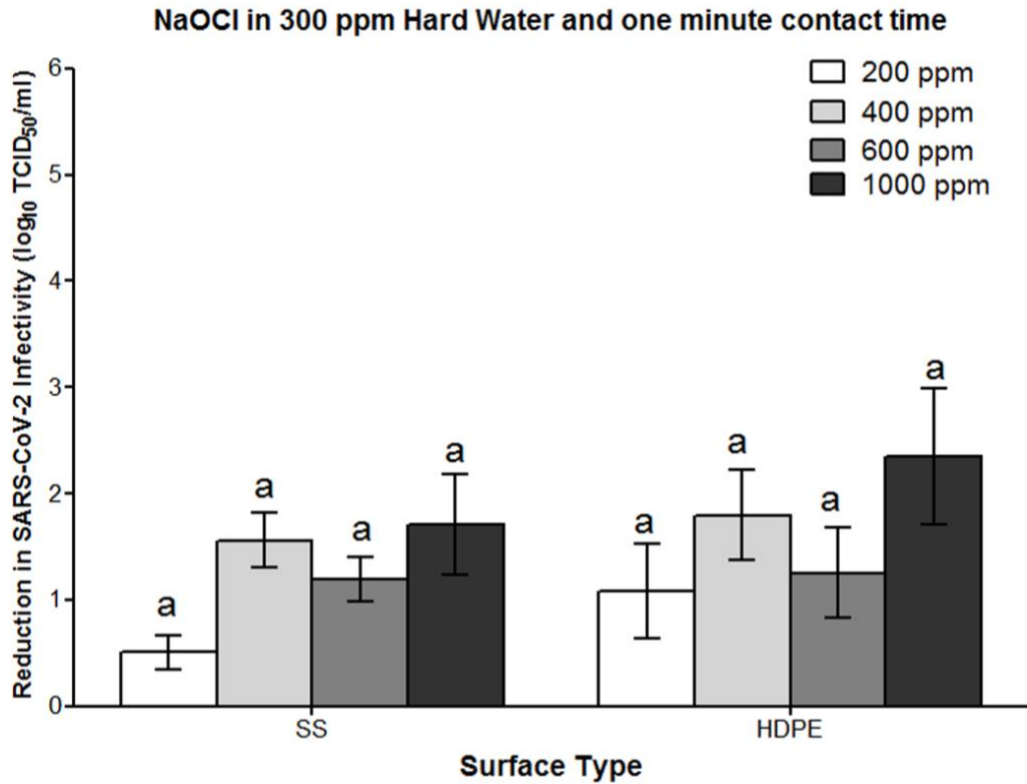


Figure 3: Effect of NaOCl on the reduction of SARS-CoV-2 infectivity (\log_{10} TCID₅₀/ml) at one minute contact time using 200, 400, 600 and 1000 ppm of the disinfectants on SS and HDPE surfaces. The ASTM E2197 standard method for testing disinfectants against SARS-CoV-2 on carrier disks was followed. Comparing within a surface type: means with different letters differ significantly. Comparing corresponding disinfectant concentration between surface types: significant differences are denoted with asterisks.

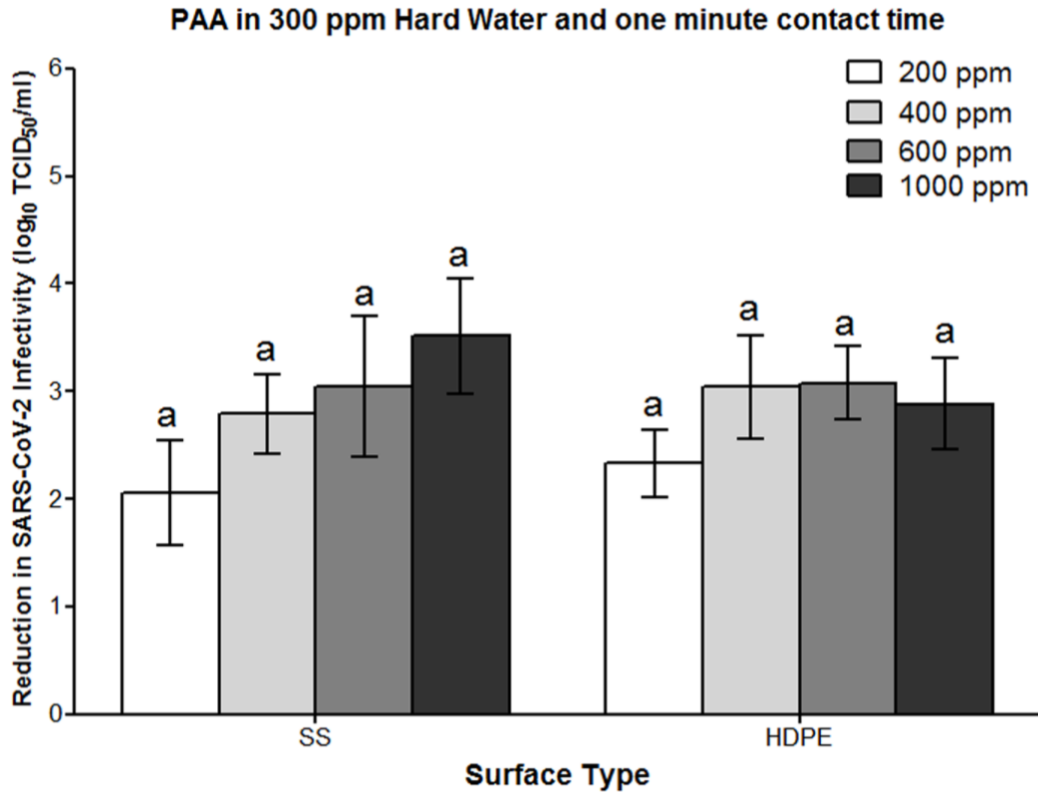


Figure 4: Effect of PAA on the reduction of SARS-CoV-2 infectivity (\log_{10} TCID₅₀/ml) at one minute contact time using 200, 400, 600 and 1000 ppm of the disinfectants on SS and HDPE surfaces. The ASTM E2197 standard method for testing disinfectants against SARS-CoV-2 on carrier disks was followed. Comparing within a surface type: means with different letters differ significantly. Comparing corresponding disinfectant concentration between surface types: significant differences are denoted with asterisks.

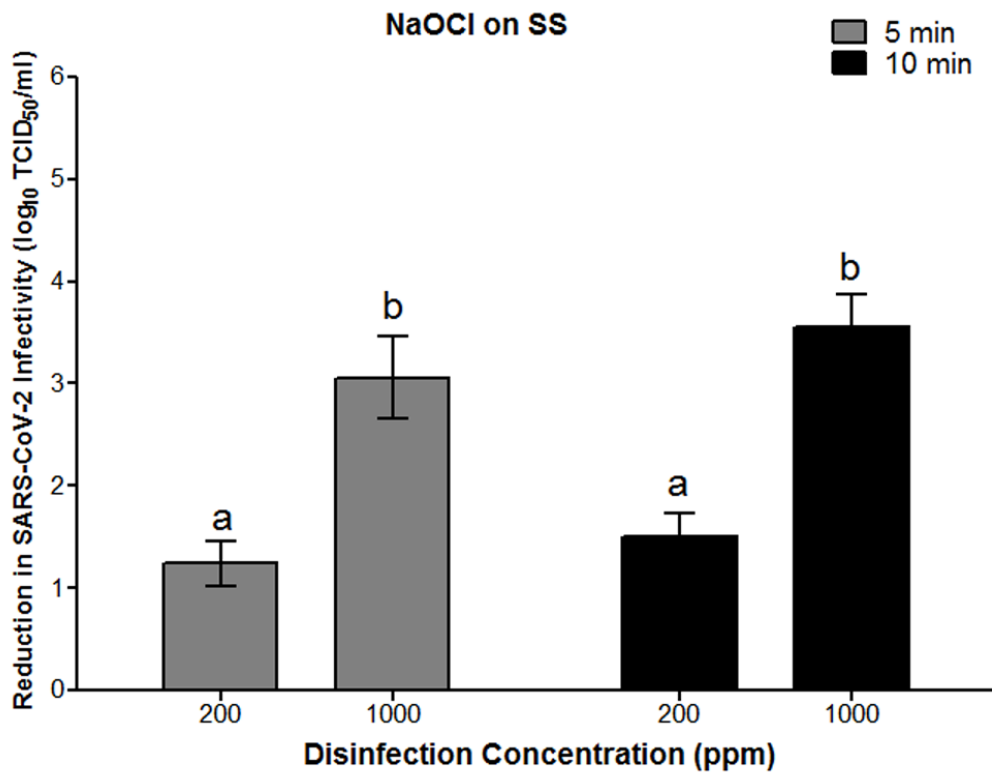


Figure 5: Effect of NaOCl on the reduction of SARS-CoV-2 infectivity (log₁₀ TCID₅₀/ml) at five and ten minutes contact times using 200 and 1000 ppm of the disinfectants on SS surface. The ASTM E2197 standard method for testing disinfectants against SARS-CoV-2 on carrier disks was followed. Comparing within a contact time: means with different letters differ significantly. Comparing corresponding disinfectant concentration between two contact times: significant differences are denoted with #.

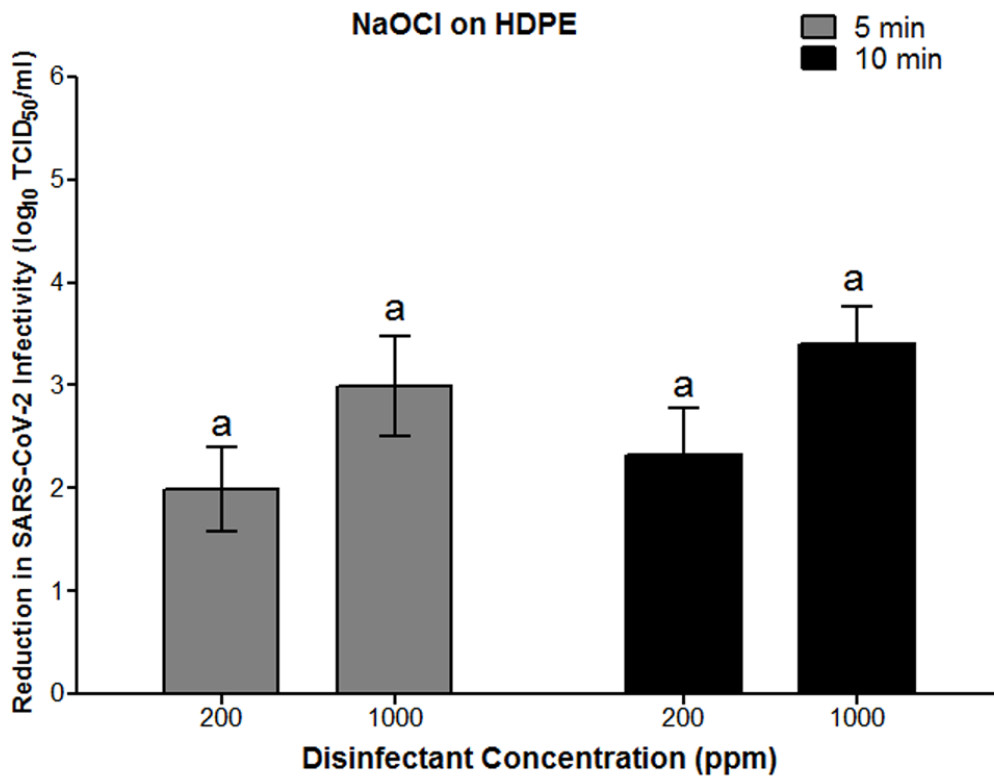


Figure 6: Effect of NaOCl on the reduction of SARS-CoV-2 infectivity (log₁₀ TCID₅₀/ml) at five and ten minutes contact times using 200 and 1000 ppm of the disinfectants on HDPE surface. The ASTM E2197 standard method for testing disinfectants against SARS-CoV-2 on carrier disks was followed. Comparing within a contact time: means with different letters differ significantly. Comparing corresponding disinfectant concentration between two contact times: significant differences are denoted with #.

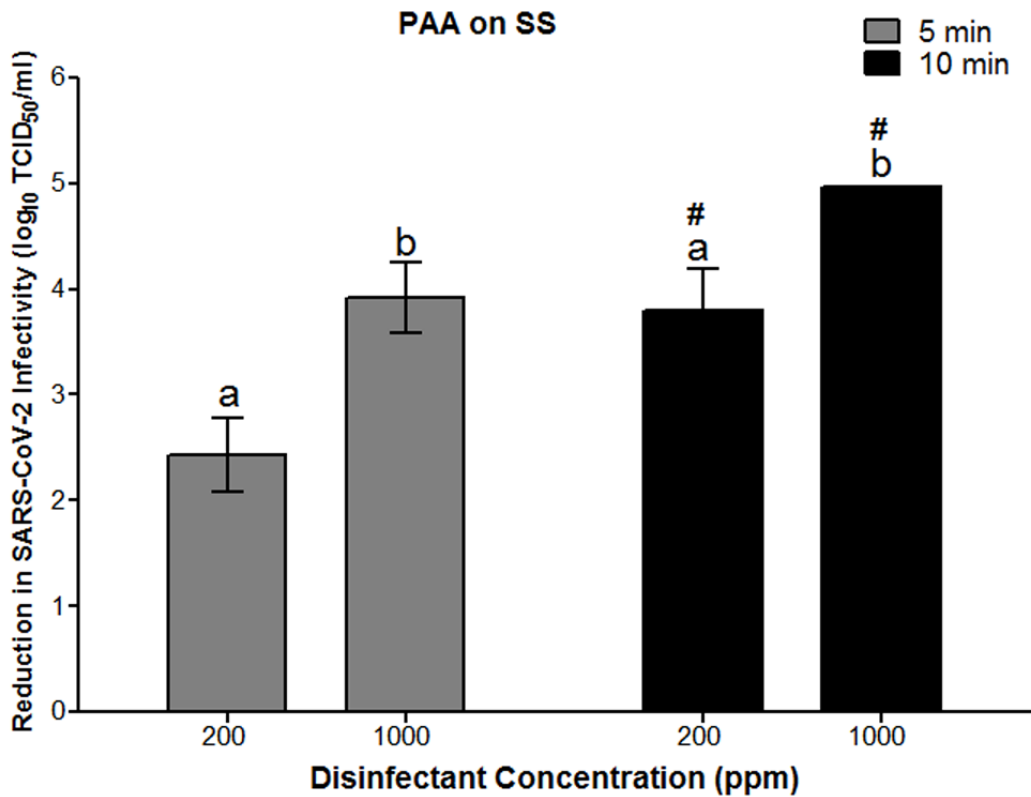


Figure 7: Effect of PAA on SARS-CoV-2 infectivity at five and ten minutes contact time using 200 and 1000 ppm of the disinfectants on stainless steel surface. Comparing within a contact time: means with different letters differ significantly. The ASTM E2197 standard method for testing disinfectants against SARS-CoV-2 on carrier disks was followed. Comparing corresponding disinfectant concentration between two contact times: significant differences are denoted with #.

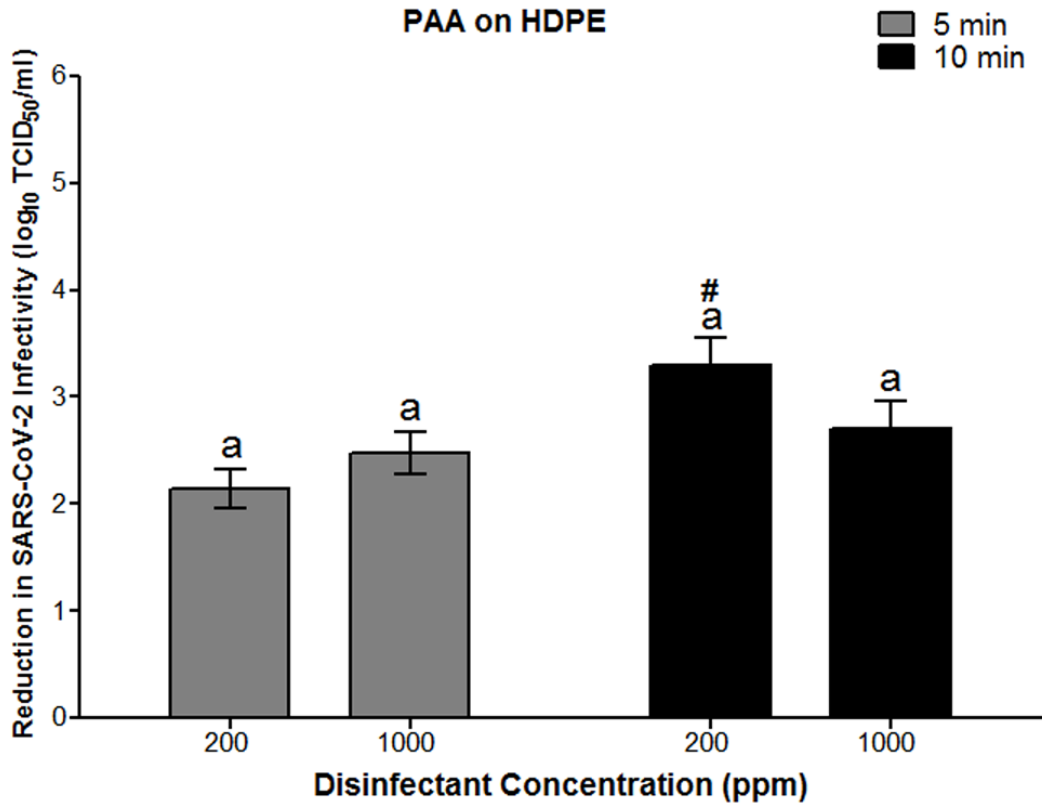


Figure 8: Effect of PAA on SARS-CoV-2 infectivity at five and ten minutes contact time using 200 and 1000 ppm of the disinfectants on HDPE surface. Comparing within a contact time: means with different letters differ significantly. The ASTM E2197 standard method for testing disinfectants against SARS-CoV-2 on carrier disks was followed. Comparing corresponding disinfectant concentration between two contact times: significant differences are denoted with #.

CHAPTER 5

DISCUSSION

In this study, two common surface disinfectants, NaOCl and PAA, were investigated to determine their action against SARS-CoV-2 on contaminated SS and HDPE surfaces. Inactivation studies against SARS-CoV-2 were performed both in suspension and on surfaces following standard ASTM protocols. It was observed that the inclusion of soil load and testing on surfaces did reduce disinfectant virucidal activity. When testing PAA and NaOCl as disinfectants against SARS-CoV-2, adding soil load had an impact on disinfectant efficacy when disinfectants were used at lower concentrations. As can be seen in the results (Fig. 1 and 2), log reduction was significantly reduced upon the introduction of soil load in suspension. PAA and NaOCl previously showing 3 log₁₀ reduction of SARS-CoV-2 at 50 ppm concentration was reduced to 1 log after the introduction of soil load. NaOCl efficacy is generally known to be negatively affected by soil load but in this case, both disinfectants were susceptible at 50 ppm concentration (Lewis & Arens, 1995). However, significance in log reduction between soil load and non-soil load was not observed when both disinfectants were used at 200 ppm. Similar observations were noticed in studies by (Ijaz et al., 2021) and (Hulkower et al., 2011). This phenomenon is likely due to a chemical reaction that may occur between the organic material and disinfectant, a scenario that would require high oxidant demand resulting in consumption of active disinfectant by proteins in the soil matrix (Hulkower et al., 2011). This in turn reduces the concentration of disinfectant molecules available to react with and inactivate the virus particles (Ijaz et al., 2021). Soil load can also act as a barrier, allowing the virus particle to embed in the

organic matrix thus protecting it from penetration of the disinfectant (Hulkower et al., 2011; Lewis & Arens, 1995). The inclusion of organic material is important when determining virucidal efficacy, as SARS-CoV-2 is often shed in respiratory droplets containing organic material (Escudero-Abarca et al., 2022; Hulkower et al., 2011). Increasing the disinfectant concentration may minimize the effects of organic material by providing more readily available disinfectant (Lewis & Arens, 1995). A pre-cleaning step to remove organic material from the surfaces may also help to increase disinfectant efficacy against SARS-CoV-2 (CDC, 2016).

For NaOCl tested in suspension, it was found that only 200 ppm NaOCl in the presence of a soil load was required needed to induce a $>3 \log_{10}$ inactivation of SARS-CoV-2 in one minute. When comparing this to other suspension assay studies (summarized in Table 1) our results differed in some aspects. One study found that 1000 ppm NaOCl was needed to inactivate SARS-CoV-2 in 0.5 min with a 3% bovine albumin soil load and standard hard water concentrations while held in suspension (Xiling et al., 2021). While this study had a shorter contact time, they incorporated a higher NaOCl concentration and a lower soil load concentration. Another study used a 10000 ppm sodium hypochlorite solution (10%) with a 1-minute contact time while held in suspension to achieve a $3.25 \log_{10}$ reduction in SARS-CoV-2 titer. No mention of soil load was found in their study (Chan et al., 2020). In comparison, our results showed a lower NaOCl concentration was required to induce a $>3 \log_{10}$ reduction of viral titer even in the presence of soil load. A study using 80 ppm NaOCl with a 10 second contact time and 1% final FBS concentration while held in suspension was able to achieve a $>4 \log$ reduction in SARS-CoV-2 titer (Hatanaka et al., 2021). When compared to our study, the authors used a lower NaOCl concentration and contact time to achieve $>4 \log$ reduction. In addition, they used a lower soil load concentration composed of only FBS. As NaOCl is affected by soil load,

this could have been a reason a shorter contact time and lower disinfectant concentration was needed. Additionally, in our study, a concentration between 50 and 200 ppm in the presence of soil load was not tested. Therefore, testing a concentration higher than 50 ppm but less than 200 ppm in the presence of soil load may have also induced a >3 log reduction in viral titer.

A more recent study found that 2000 ppm NaOCl diluted in sterile water and incubated for 30 min with SARS-CoV-2 in the presence of 5% FBS induced >4 log reduction in virus titer (Lee et al., 2023). Surprisingly, this contact time was much longer than any other studies observed with one of the highest NaOCl concentrations tested to achieve similar log reductions. A study using 1.5-3 ppm free chlorine from chlorinated pool water at pH 7.4 with a 30 seconds contact time found a >3 log₁₀ reduction of SARS-CoV-2. However, this was also completed in the absence of soil load and hard water. The authors acknowledged that the addition of soil load would likely make an impact on virucidal action (Brown et al., 2021). Two more studies found high inactivation (4.8 to 5.1 log) of SARS-CoV-2 but using two conflicting contact times and concentrations for NaOCl (Chin et al., 2020; Ijaz et al., 2021). Ijaz et al used 0.5 min and 1400 ppm NaOCl while virus mixed in soil load while Chin et al used 75 -150 ppm for 5 min without mixing the virus in soil load. Therefore, the previous in suspension studies using NaOCl against SARS-CoV-2 are not consistent with each other's and none used a standardized method. In our study, a 200 ppm disinfectant concentration for NaOCl achieved > 3 log₁₀ inactivation of SARS-CoV-2 while including a 5% soil load as recommended by ASTM standard E1052.

As application of disinfectants in healthcare and food industry settings will be used on surfaces, disinfectant surface assays were performed. As mentioned, hard water is known to hinder many disinfectants efficacy and should be included as recommended by ASTM protocols. Hard water is generally a combination of calcium and magnesium compounds in water and

measured as ppm CaCO_3 . It is believed that the divalent compounds in the water can react with the disinfectant molecules to form insoluble precipitates, leaving less available disinfectant molecules to interact with the microorganism (CDC, 2016). In our study, 1000 ppm NaOCl was needed at 10-minute contact time to consistently induce a $> 3 \log_{10}$ inactivation of SARS-CoV-2 on SS and HDPE surfaces when using 300 ppm hard water and 5% soil load. Previous studies performed with NaOCl and surfaces either relied on human and animal coronaviruses or was done on food or cloth (summarized in Table 2). For examples, a study found that 1000 ppm NaOCl concentration was needed to induce a 4 \log_{10} reduction in SARS-CoV-2 titer on cloth carriers when used at cold chain temperatures (-20°C) and 5 minute contact time (Wu et al., 2022). While our study also showed that a 5 min could sometimes result in 3 log reduction, but due to variation between experiments a longer contact time (10 min) is required achieve $>3 \log$. Also, in our study the disinfectant was used at room temperature and on stainless steel and HDPE carriers.

The only study that used NaOCl on SS utilized a 0.5% (5000 ppm) NaOCl for 1- and 5-min contact times to show a 3.45 and 6 log reduction SARS-CoV-2 titer, respectively (Sloan et al., 2022). The disinfectant was diluted in 0.04% (w/v) sterile hard water and the assay was completed in the presence of 5% soil load. When compared to our study, a 5 and 10-minute contact time was needed to induce 3.0 and 3.5 log reduction of virus titer respectively. However, our study used a much lower concentration of NaOCl (1000 ppm) and showed that a ten min is needed to consistently achieve $> 3 \log$ reduction in virus titer. A lower concentration was used in our study to minimize toxicity and exposure risks to humans.

Other studies that could be found testing NaOCl on surfaces used human or animal coronaviruses viruses. One study found using 600 ppm NaOCl for 1 minute used in the presence of 380 ppm hard water and soil load could induce $< 1 \log_{10}$ reduction for animal coronaviruses (Hulkower et al., 2011). Finally a study testing 5000 ppm NaOCl for 1 minute contact time on contaminated stainless steel found a $>3 \log_{10}$ reduction in HCoV-229E (Sattar et al., 1989).

Minimal information in the scientific literature is available on the use of PAA as a surface disinfectant against SARS-CoV-2 or other coronaviruses (summarized in Table 1 &2). As PAA decomposes into safe by-products when compared to NaOCl, this disinfectant was of particular interest to be included in our study (Envirotech, 2016; Gil et al., 2019). A study testing the inhibition of the SARS-CoV-2 spike protein using PAA found that an 18 ppm PAA concentration with a 30 minutes contact time, reduced SARS-CoV-2 RNA by 80% (Yamamoto et al., 2022) PAA could block receptor binding protein to ACE-2, therefore inhibiting spike protein mediated infections. However, this was not a standardized disinfectant assay, as the authors were investigating the mechanism of PAA interaction with SARS-CoV-2 pseudovirus i.e., using synthetic recombinant virus that carries the spike protein of SARS-Cov-2 and not the actual pathogen (Yamamoto et al., 2022). However, the authors did state that PAA shows potential to be potent against this virus and that further studies are needed to validate PAA virucidal efficacy against SARS-CoV-2. A second study using the suspension test found that 173 ppm PAA held for 10 minutes without soil load was able to induce a 6.8-56.28% reduction in SARS-CoV-2 (Lee et al., 2023). In contrast, our study tested 200 ppm PAA for 1 min with the presence of soil load and achieved a 3.7 log reduction in SARS-CoV-2 titer.

On food, one study tested 40 to 160 ppm PAA on lettuce and 1000 to 4000 ppm on chicken and salmon for both 1 and 5 min against SARS-CoV-2. They found that 40, 80, 160 ppm for 1 minute on lettuce induced 2.04, 2.46, and 4 log reduction in virus titer (Jung et al., 2023). When the 80 ppm concentration was held for 5 minutes on lettuce, it achieved a 3 log reduction in virus titer. A higher concentration (1000, 2000, 4000 ppm) was tested on the meat products. However, due to their limit of detection, a maximum 2 log reduction of SARS-CoV-2 was observed starting at 2000 ppm for 1 minute and 1000 ppm at 5 minutes (Jung et al., 2023). Therefore, this study differed largely when compared to ours as the carriers used were not similar.

Dry fogging contaminated surfaces with PAA is showing that it can inactivate SARS-CoV-2 in one hour (Cutts et al., 2021). This suggested it could be effective as a surface disinfectant, but more research was needed. As mentioned previously, dry fogging is also not a practice recommended by the CDC or WHO due to health risks. Our research found that PAA must be used at a concentration of at least 200 ppm for 10-minutes when being used on contaminated SS or HDPE surfaces (although a 5 min can be used on SS as reported in Table 6, but to cover diverse surfaces a 10 min is required). This result included the use of 5% soil load and 300 ppm hard water. In contrast, a 4 log₁₀ reduction of SARS-CoV-2 titer was found on cloth food packaging materials when using 2000 ppm PAA for 5 minutes at cold chain temperatures (Wu et al., 2022). A higher concentration of PAA was used (2000 ppm) therefore potentially shortening the contact time. However, the methodology between the two studies differed when comparing the temperature and carriers used. A study using HCoV-229E in soil load found that 200 ppm PAA diluted in hard water when used on stainless steel and polypropylene for 5 min reduced the virus titer by 3.8 log (Choi et al., 2022). The 200 ppm PAA

on stainless steel performed most similarly to our study, but it was used against a surrogate virus instead of SARS-CoV-2. Additional carrier tests were also performed for 5 min contact times on lettuce, chicken, and salmon with 80 ppm PAA on lettuce inducing a 1.68 log reduction and 1500 ppm inducing a maximum of 2.03 log reduction on both chicken and salmon (Choi et al., 2022).

On other enteric non-enveloped viruses such as adenovirus, murine norovirus, and poliovirus, PAA has been demonstrated as effective at concentrations ranging between 400 to 1500 ppm starting at 5 minutes with efficacy decreasing by 1-2 log when used on glass carriers (Becker et al., 2017). Therefore, there was a knowledge gap regarding the use of this disinfectant against SARS-CoV-2 in both suspension and surface assays and our research is the first report indicating that PAA can be used as an alternative to NaOCl at lower concentrations (200 ppm for 10 min).

When comparing NaOCl and PAA disinfectant solutions between the in suspension and surface assays, there was reduced disinfectant efficacy. NaOCl at 200 ppm and a one-minute contact time had a 5-log reduction while in suspension (Fig. 1), but when tested on surfaces, had less than <1 log reduction (Fig. 2). For PAA at 200 ppm and a one-minute contact time, a 4-log reduction was observed while in suspension (Fig. 1) but was reduced to 2-log reduction in viral titer when tested on surfaces (Fig. 2). In this case, there was increased resistance to the disinfectants by SARS-CoV-2 when dried on surfaces. Other studies also noted there were reduced efficacy of disinfectants used against viruses when tested on surfaces (Becker et al., 2017; Hulkower et al., 2011; Ijaz et al., 2021; Kindermann et al., 2020). This increased resistance of SARS-CoV-2 to disinfectants may be due a combination of the virus embedding in the

organic material, thus protecting it from contact with the disinfectant, as well as the virus particle adsorbing to the surface (Hulkower et al., 2011). Both scenarios would make it more difficult to inactivate on surfaces than when suspended in a liquid matrix. However, the reduced efficacy of disinfectants on surfaces was observed to be negligible when contact time or disinfectant concentration increased. While suspension tests may be valuable in conducting preliminary research on disinfectants and its susceptibility to soil load, it may overestimate the virucidal capability of the disinfectant. Testing disinfectant efficacy on surfaces in the presence of soil load may be of more importance as it represents real world scenarios of use and gives a conservative estimate of the true virucidal activity of a disinfectant.

When comparing disinfectants to each other's, NaOCl performed significantly better in suspension assay. A study testing 200 ppm NaOCl against HAV with a 1-minute contact time also found this to be true, with NaOCl inducing a >4 log reduction in HAV titer compared to a <1 log reduction by PAA. When both disinfectants were tested on stainless steel carriers, 1000 ppm NaOCl at 10 minute contact time had 1.78 log reduction in HAV titer while 2500 ppm PAA at 10 minutes induced only 1.25 log₁₀ reduction (Song et al., 2022). In contrast, in our study PAA performed significantly better in surface assay especially for SS, but it was equivalent to NaOCl on HDPE surfaces. This is consistent with the results that surface type had no effect on NaOCl virucidal activity against SARS-CoV-2 whereas it did for PAA. The SS coupons used had a completely smooth surface while the HDPE coupons were textured. This texture could have entrapped virus particles and shielded them from the disinfectants as adhesion mechanisms between virus and fomites can vary due to changes in physiochemical properties and electrostatic charges, causing differences in adsorption to various materials (Owen et al., 2022). Therefore, the surface texture and physiochemical properties between the fomites and virus may explain

why PAA and NaOCl showed similar viral inactivation on HDPE surfaces. Nevertheless, our results show that PAA is either better or equivalent to NaOCl for diverse surface types.

The purpose of the disinfectant will change concentration guidelines. NaOCl concentration for the use on food contact surfaces must be kept between 50-100 ppm while PAA can be used up to 138 ppm (FDA, 2010; Lynch, 2021). When used for produce wash water, 25 ppm NaOCl is often the recommended use found on disinfectant labels while 80 ppm PAA is the allowable concentration limit set by the FDA (CES; FDA, 2010). As organic material presence can decrease disinfectant efficacy, adjustments to concentrations are often made in clinical and health care settings. For NaOCl, concentrations between 500-1000 ppm are most used but up to 10,000 ppm is allowed in situations of high organic material contamination (CDC, 2008; Lin et al., 2020). PAA is known to be effective at inactivating pathogens on surfaces between 12-2250 ppm (CDC, 2016). Overall, our data showed that both PAA and NaOCl virucidal activity against SARS-CoV-2 varied greatly depending on soil load at low concentrations as well as when comparing in suspension assay to surface assay. In general, a one-minute contact time was not enough to inactivate more than 3 log of SARS-CoV-2 on contaminated SS or HDPE. If NaOCl is used to disinfect SARS-CoV-2 on contaminated SS or HDPE, it should be used at 1000 ppm for 10 min. If PAA is used to disinfect SARS-CoV-2 on contaminated stainless steel or HDPE, it can be used as low as 200 ppm for 10 min. In conclusion, PAA is either better or equivalent to NaOCl for diverse surface types and can be used at lower concentrations than NaOCl for similar contact times.

CHAPTER 6

CONCLUSION

For the in-suspension assay PAA and NaOCl were effective ($> 3 \log_{10}$ reduction in infectious virus) at 50 ppm in the absence of soil load. However, in the presence of soil load, a 200 ppm was required for $> 3 \log$ reduction in virus infectivity. For the carrier assay, PAA and NaOCl at 200 ppm and with a one-minute contact time, were not effective against SARS-CoV-2 on either SS or HDPE surfaces. It was found that NaOCl must be used at a concentration of 1000 ppm for 10 minutes to inactivate SARS-CoV-2 on SS or HDPE surfaces. PAA must be used at 200 ppm for 10 minutes to inactivate SARS-CoV-2 on SS and HDPE surfaces. This is first report indicating that PAA is effective against SARS-CoV-2 on surfaces and can be used as safer-and more environmentally friendly alternative to NaOCl, at a lower concentration (200 ppm for 10 min).

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