Technical Report for Chlorine Dioxide Gas-Based N95 Decontamination and Reuse

Much of the available literature on decontamination of N95 FFRs reviewed in this document is a result of recent efforts to relieve the shortage of N95 FFRs during the SARS-CoV-2 outbreak. Because of this, much of the research cited in this document is not yet peer reviewed. **For clarity, wherever non-peer-reviewed results are cited in this report, the citation is preceded by a *.**

Executive Summary

Gaseous chlorine dioxide (ClO_2) is a relatively low-cost gaseous compound capable of eradicating a wide range of microorganisms, including viruses and bacterial spores. The gas can be used to decontaminate medical or laboratory instruments, biosafety cabinets, sensitive equipment, and whole rooms. ClO_2 is a gas over the range of ambient temperatures for most decontamination settings, and will readily diffuse to fill the available space and decontaminate hidden and obscured surfaces. Pure ClO_2 is a mild oxidizing agent that produces much less chlorination of organic molecules than chlorine (Cl_2) or household bleach (sodium hypochlorite). However, the purity of the gas is highly dependent on the processes used to generate, use, and remove it.

There is no peer-reviewed scientific literature to date on the efficacy of ClO_2 gas for disinfection of N95 filtering facepiece respirators (FFRs), nor on the efficacy of gaseous ClO_2 for inactivation of SARS-CoV-2. However, given the proven ability of ClO_2 gas to sterilize even hard-to-kill and difficult-to-reach microorganisms in multiple studies and limited unpublished data on inactivation of bacterial spores in nested N95 FFRs, we conclude that validated commercially available systems are likely to prove effective for decontamination of N95 FFRs contaminated with SARS-CoV-2 and more difficult-to-eradicate pathogens. Preliminary non-peer-reviewed studies suggest that FFR filtration efficiency will be largely preserved even after up to 20 high-dose treatment cycles. The impact of ClO_2 gas treatment on FFR fit seems to vary for different respirator models and particular decontamination cycle parameters, and likely depends on the compatibility of the specific FFR model's material composition (particularly of the straps) with the treatment process.

Importantly, ClO_2 gas is metastable and must be generated on-site for decontamination purposes. ClO_2 gas is a toxic respiratory irritant, and any ClO_2 gas decontamination approach must include occupational safety monitoring of gas levels around each worker performing the decontamination, as well as a process to sufficiently aerate or otherwise remove residual chlorine dioxide from treated FFRs. **Adequate aeration of N95 FFRs after ClO_2 gas treatment, possibly combined with additional removal measures, is also essential for the safety of the individuals wearing the treated FFRs. However, the process and duration required for adequate removal of chlorine dioxide from treated FFRs has not yet been reported in peer-reviewed publications and further experiments are required to establish reliable protocols for ClO_2 removal from treated FFRs.** In one preliminary experiment, one hour of aeration has been found to be sufficient to reduce the concentration of residual chlorine dioxide from a pre-aeration concentration of 80ppmv below the 15-minute short-term exposure

limit of 0.3 ppmv in inhaled breath. We also note that re-use of any N95 FFR may impact FFR fit, and we stress that a user seal check should be performed after every re-donning.

The literature suggests that chlorine dioxide gas at the established sterilization doses and humidity levels will almost certainly inactivate the SARS-CoV-2 virus, as well as more resilient microorganisms on contaminated respirators, and will have negligible effect on the filtration efficiency of N95 respirators (although strap elasticity may be affected).

Further studies on the effect of ClO_2 on N95 FFR fit are needed. Specific data on SARS-CoV-2 or other microorganism inactivation on N95 FFRs would also be valuable. Any decontamination approach should be accompanied by an industrial hygiene workflow involving user training and sterile processing as well as compliance with regulatory guidelines, including those published by the Food & Drug Administration (FDA) and Occupational Safety & Health Administration (OSHA) for applications in the United States.

1. Overview

The novel coronavirus (SARS-CoV-2) that causes coronavirus disease 2019 (COVID-19) has led to a global shortage of N95 Filtering Facepiece Respirators (N95 FFRs, also referred to as 'N95 masks'). While new N95 FFRs should be used with every change in activity, in this emergency, it may be necessary to decontaminate FFRs. In this document, we review the available evidence relevant to the use of chlorine dioxide gas to decontaminate N95 FFRs with the goal of increasing the useful lifetime of N95 FFRs worn by healthcare providers during the COVID-19 pandemic. Effective decontamination requires inactivation of the SARS-CoV-2 virus (and ideally other pathogens) and maintenance of both the fit and filtration efficiency of the N95 FFR while minimizing the risk of cross-contamination.

Chlorine dioxide (ClO_2) is an established sterilant and decontaminant widely used in many industrial and research settings worldwide, including veterinary, medical and laboratory equipment sterilization (Luftman et al., 2006; Mitchell et al., 2019), food processing (*Chlorine Dioxide, 21 C.F.R. § 173.300*, n.d.), building decontamination (Girouard & Czarneski, 2016; Takahashi et al., 2014), and water disinfection (Aieta & Berg, 1986; Kaczur & Cawlfield, 2000; Miller et al., 1978).

Chlorine dioxide gas was developed for use as a medical device sterilant in the 1980s (Rosenblatt et al., 1985), although to date this application has not received FDA clearance. ClO_2 gas is a reddish- to yellow-ish green water-soluble true gas with a boiling point of 11°C (Kaczur & Cawlfield, 2000). The gas is difficult to transport and store safely because it is unstable and explosive above 95,000 ppmv at room temperature (a concentration greatly exceeding that used in typical decontamination processes), and it therefore must be generated on-site for decontamination purposes (Jin et al., 2009; Kaczur & Cawlfield, 2000). Importantly, ClO_2 inactivates microorganisms through oxidation, not chlorination (Kaczur & Cawlfield, 2000). Unlike chlorine, *pure* ClO_2 has not been found to produce carcinogenic trihalomethanes or chloramines in reaction with organic compounds or ammonia, respectively (Miller et al., 1978). However, chlorine can be produced from photochemical decomposition of ClO_2 and/or as a byproduct of ClO_2 gas generation, depending on the approach used (Kaczur & Cawlfield, 2000).

Its highly effective decontamination capability, relatively easy-to-scale implementation, relatively low cost, and high availability make ClO_2 gas a promising method for emergency decontamination or sterilization of N95 FFRs for reuse, including in low-resource settings. However, as mentioned above, high-quality, peer-reviewed data is lacking regarding the impact of ClO_2 gas treatment on N95 FFRs, and there is no data to date on the efficacy of ClO_2 gas for inactivation of SARS-CoV-2 specifically. Furthermore, there is not yet peer-reviewed data on the time required to off-gas an N95 prior to reuse after ClO_2 treatment. Any implementation of a ClO_2-based N95 FFR decontamination process will also need to take into account the toxicity of ClO_2 gas, which has low OSHA and NIOSH exposure limits (0.1 ppmv for 8-hour time-weighted average exposure and 0.3 ppmv time-weighted average short-term exposure over any 15-minute period) (CDC, 2018).

Table 1. Advantages and disadvantages of ClO_2 gas for decontamination

	Advantages	Disadvantages
Accessibility and use	Low-cost and highly effective. Widely used for sterilization of laboratory and veterinary equipment, food processing and water disinfection. **[1. Overview]** A true gas-phase process that tolerates temperature fluctuations. **[1. Overview]** Easily and accurately monitored. **[1. Overview] [7. Strategies]** It can inactivate a wide range of pathogens including viruses. **[4. SARS-CoV-2 and Other Pathogen Inactivation]**	Purity of the gas is highly dependent on the processes used to generate it. **[3. Mode of Action]** ClO_2 gas must be generated on site prior to use. **[1. Overview]**
Chemical properties	True gas at room temperature, readily diffuses rapidly throughout large spaces and permeates small spaces. **[1. Overview] [7. Strategies]**	Storage and transportation not possible because of instability and explosivity in concentrated form. **[1. Overview]**
Safety	Pure ClO_2 has not been found to produce carcinogenic compounds when exposed to organic compounds. **[8. Primary Risks, Occupational Safety, and Unknowns]**	Chlorine can be produced from photochemical and thermal decomposition. **[3. Mode of Action]** Highly toxic gas; process requires aeration or other forms of chlorine dioxide removal from treated FFRs, with limited data available regarding the necessary duration of removal treatment. The long-time (8 hour) permissible exposure limit (PEL) of chlorine dioxide in the air of human workplace is 0.1 ppmv or 0.3 mg/m^3. The 15-min short-term exposure limit (STEL) is 0.3 ppmv. **[8. Primary Risks, Occupational Safety, and Unknowns]**

N95 Decontamination	Sterilization expected at a total ClO_2 gas dose of at least 720 ppmv-hrs delivered over at least two hours at 60% or higher relative humidity. **[2. State of Federal Guidance] [4. SARS-CoV-2 and Other Pathogen Inactivation]** Preliminary evidence suggests minimal impact on fit of most FFR models; FFRs retain high filtration efficiency after ≤20 high-dose cycles. **[5. Integrity of N95 Filtering Facepiece Respirators]**	Treatment may damage fit of particular FFR models, perhaps through strap degradation. **[5. Integrity of N95 Filtering Facepiece Respirators]** Chlorine dioxide remains absorbed in the FFRs for at least one hour after decontamination. **[8. Primary Risks, Occupational Safety, and Unknowns]**

2. State of Federal Guidance

In this unprecedented COVID-19 pandemic, due to a limited supply of N95 FFRs, the Centers for Disease Control and Prevention (CDC) have provided guidance that healthcare workers can practice extended use or limited reuse of N95 FFRs (CDCa, 2020). In addition, the CDC has provided guidance to hospitals on methods for decontaminating N95 FFRs during a crisis (CDCb, 2020)

The Occupational Safety and Health Administration (OSHA) states that cosmetics or other barriers should not be present during respirator use (OSHA, 2015). Emergency use authorizations (EUAs) that the FDA has granted for N95 FFR decontamination during the COVID-19 pandemic also stipulate that cosmetics not be present on respirators sent for decontamination (FDA, 2020b).

After decontamination, the CDC recommends that a 'user seal check' is performed when the respirator is donned to ensure adequate seal (CDCb, 2020). A user seal check after every decontamination cycle is especially important because there is evidence that the fit factor of N95 respirators decreases with numerous don/doffs (Bergman et al., 2012). Per FDA guidelines for N95 FFR decontamination EUAs, bioburden reduction requires ≥ 3 $\log_{10}$ reduction (corresponding to a 99.9% reduction) in viral activity. Based on this guideline, we describe a process as sufficiently "decontaminating" or "inactivating" only when it leads to a ≥ 3 $\log_{10}$ reduction in viral activity. Note that our definition of decontamination in this report, unless otherwise specified, only considers virucidal activity and does *not* consider mycobactericidal or sporicidal activity, for which the FDA has other guidelines (FDA, 2020a). N95 FFR decontamination processes for SARS-CoV-2 may not result in sterilization (≥ 6 $\log_{10}$ reduction of all microorganisms) of the N95 FFR. The level of decontamination achieved with chlorine dioxide gas will vary with the dose used (concentration and duration of treatment), but ClO_2 is recognized by the FDA as a validated sterilant that can achieve a sterility assurance level of 6 $\log_{10}$ (10^{-6}) (FDA, 2019). In this document, we refer to processes with a total ClO_2 gas dose of at least 720 ppmv-hrs delivered over at least two hours at 60% or higher relative humidity as "sterilization" (Girouard & Czarneski, 2016).

Any new methods for decontamination should be verified through organizations' internal processes, which may include FDA clearance, prior to implementation. Currently, we are not aware of any approved FDA EUA for chlorine dioxide gas treatments of N95 FFRs, and

the CDC recommendations similarly do not address the potential use of ClO_2 gas (CDCb, 2020). Please refer to current CDC guidelines that are updated regularly, as well as N95DECON's Full Legal Disclaimer.

3. Mode of Action

Chlorine dioxide (ClO_2) is a chemical sterilant that has been used for decontamination of rooms, isolators, biosafety cabinets, medical equipment, and materials that are intolerant to heat. Chemically, chlorine dioxide gas is an oxidant that leads to microbial inactivation (see evidence summarized in Section 4 and Table 2 below). ClO_2 gas disrupts cell metabolism by absorbing into a humidified microorganism and reacting with key molecules by electron transfer and radical-radical bond formation. Unlike chlorine gas, it is not predominantly a chlorinating agent. Because ClO_2 is a fairly mild oxidant such that the first step of oxidation of most organic molecules is unfavorable, the rates of oxidation vary widely (Neta et al., 1988). The rates of oxidation of the vital amino acids cysteine, tryptophan, and tyrosine are however quite fast (Napolitano et al., 2005; Stewart et al., 2008). These reactions may alter the viral capsid proteins and result in virus inactivation. Studies have also shown that ClO_2 impairs viral ribonucleic acid (RNA) presumably by reaction with guanine (G) or GG sequences (Aieta & Berg, 1986; Alvarez & O'Brien, 1982; Napolitano et al., 2006).

Because it does not possess long-term stability as either a gas or compressed liquid, ClO_2 is generated immediately prior to use. Four examples of processes used to generate ClO_2 gas are shown below:

1. Passing chlorine gas through solid sodium chlorite (Kaczur & Cawlfield, 2000; Luftman et al., 2006, 2008; Rastogi et al., 2010):

$$Cl_2(g) + 2NaClO_2(s) \rightarrow 2ClO_2(g) + 2NaCl(s)$$

2. Mixing a chlorite solution with an acid (Kaczur & Cawlfield, 2000):

$$5NaClO_2(aq) + 4HCl(aq) \rightarrow 5NaCl(aq) + 4ClO_2(g) + 2H_2O(l)$$

$$4NaClO_2(aq) + 2HCl(aq) \rightarrow 2ClO_2(g) + NaClO_3(aq) + 3NaCl(aq) + H_2O(l)$$

3. Mixing a sodium chlorite solution with acid and an oxidant (Kaczur & Cawlfield, 2000; Rastogi et al., 2010):

$$2NaClO_2(aq) + 2HCl(aq) + NaOCl(aq) \rightarrow 2ClO_2(g) + 3NaCl(aq) + H_2O(l)$$

4. Photogeneration of chlorine dioxide from solid $NaClO_2$ (Jain et al., 2017; Wellinghoff et al., 2007):

$$TiO_2(s)/NaClO_2(s) + \text{Light (400-800nm)} \rightarrow ClO_2(g) + \text{reduced solid products}$$

$$NaClO_2(s) + UV\ (320\text{-}380nm) + Humidity \rightarrow ClO_2(g) + various\ co\text{-}products$$

Under typical conditions of use, ClO_2 is a gas and does not condense. While pure ClO_2 gas decontaminates through oxidation rather than chlorination, it is important to note that chlorine can be formed and potentially released in the ClO_2 decontamination process with some of the generation chemistries (see above) or with photochemical decomposition or at elevated temperatures (Kaczur & Cawlfield, 2000). Therefore, ideally the entire decontamination process should be performed in the absence of light in the 300-470 nm range (UV-A and blue light) (Arango et al., 2014); (Vaida & Simon, 1995), and at temperatures below 35°C.

Effective sterilization using ClO_2 gas at ambient temperatures requires a humid environment (Jeng & Woodworth, 1990) and sufficient exposure of the object to be decontaminated to the gas. ClO_2 gas dose is measured as the product of the concentration of ClO_2 in air and the duration of exposure. Individual, unwrapped objects can be present within the decontamination room or enclosure. In typical commercial systems (see Strategies section), the decontamination process involves a conditioning phase to achieve greater than 60% relative humidity; a gas charge phase to rapidly bring the target environment to the desired concentration of ClO_2 gas; a dwell phase in which the concentration of ClO_2 gas may be held constant; and an aeration phase during which the ClO_2 is removed from the environment, e.g. by purging with fresh air or recycling the air through an activated carbon filter to absorb the ClO_2 from the air. ClO_2 concentrations in air can be monitored with a UV spectrophotometer around 360 nm (Kaczur & Cawlfield, 2000), and very low concentrations (from 0.01 to several tens of ppmv) can be monitored with electrochemical sensors, such as those designed to monitor occupational exposures.

4. SARS-CoV-2 and Other Pathogen Inactivation

Chlorine dioxide is a sterilizing agent recognized by the FDA (FDA, 2019). ClO_2 is expected to uniformly decontaminate all surfaces and penetrates materials including polymeric packaging materials (Netramai et al., 2009). Furthermore, it has been shown to destroy other viruses, spores, and other pathogens, several of which are known to be more resistant to decontamination than SARS-CoV-2 (e.g., bacterial spores). Data on the antiviral and antimicrobial activity of ClO_2 is found in Table 1, highlighting 8 examples, also detailed below. Importantly, the efficacy of ClO_2 gas at inactivating microorganisms depends upon the dose of the gas, which is a combination of its concentration and the duration of exposure.

To date, there is no specific published data on ClO_2 inactivation of SARS-CoV-2 or any other microorganism in N95 FFRs. However, it is likely that the findings from studies of ClO_2 decontamination of surfaces and other materials are applicable to decontamination of N95 FFRs by ClO_2 gas, assuming viral particles on the N95 FFR come into contact with ClO_2 gas. It is also likely that the small ClO_2 gas molecules readily diffuse through N95 FFR components. As mentioned above, ClO_2 gas has been shown to penetrate several polymeric packaging materials (Netramai et al., 2009). Unpublished data from a commercial vendor of a ClO_2 gas decontamination system (ClorDiSys) also demonstrated ≥6 $\log_{10}$ inactivation on a

Geobacillus stearothermophilus spore strip within a Tyvek wrapper placed between 2 nested N95 masks after treatment with a total ClO_2 gas dose of 720-730 ppmv-hrs at 65% RH *(P. Lorcheim, personal communication, May 11, 2020). Another commercial vendor (Decon-O-Logic, Belgium) also achieved ≥6 $\log_{10}$ kill after 720 ppmv-hrs of ClO_2 gas treatment on a *Geobacillus stearothermophilus* spore strip buried in the middle of a linen bag filled with 50 European FFP2 respirators *(P. Lorcheim, personal communication, May 11, 2020).

(Rastogi et al., 2010) studied the efficacy of ClO_2 gas generated using the Sabre and ClorDiSys commercial systems (see Strategies) for inactivation of *Bacillus anthracis* spores on six building interior surfaces (carpet, acoustic ceiling tile, unpainted cinder block, painted I-beam steel, painted wallboard, and unpainted pinewood). The study involved RH of 75%, ClO_2 gas concentrations ranging from 500-3000 ppmv and exposure times ranging between 0.5-12 h to achieve 6 $\log_{10}$ reduction. The time necessary to achieve 6 $\log_{10}$ reduction was dependent on the material type, with wood and cinder block coupons requiring longer exposure time.

(Doona et al., 2015) treated two different strains of *B. atrophaeus* characterized by their D_{EtO} values (the times to reduce surviving spores by 90% using ethylene oxide) with a single cycle of ClO_2 gas. The D_{EtO} values of *B. atrophaeus* were determined prior to the ClO_2 gas treatment during another study. 6 $\log_{10}$ reduction was achieved with ClO_2 gas concentrations of 500 ppmv for *B. atrophaeus* with D_{EtO} of 3.1, and 1000 ppmv for that with D_{EtO} of 5.0, varied RH between 40-90% and a fixed 4-h exposure time. The authors found that, at a given dose, increasing RH increased the level of inactivation, and RH levels of ≥80% were optimal for ClO_2 gas decontamination.

(Lowe et al., 2013) spotted strains of *Acinetobacter baumannii*, *Escherichia coli*, *Enterococcus faecalis*, *Mycobacterium smegmatis*, and *Staphylococcus aureus* at 10 sites throughout a hospital room and exposed them to 6 cycles of ClorDiSys-generated ClO_2 gas. The study maintained a ClO_2 gas concentration of 351 ppmv to 385 ppmv for <3 h and RH of 50% and 60%. Duplicate pairs of the samples of each organism were placed at each site. With the interior doors open, complete inactivation was achieved for all samples (specifically reductions of between 7 and 10 $\log_{10}$). With the door to the interior bathroom closed, one site in the bathroom achieved only 6 $\log_{10}$ reduction.

(Jeng & Woodworth, 1990) examined the concentration dependence of ClO_2 gas inactivation of *Bacillus subtilis* subsp. *niger* ATCC 9372 BIs. The study was conducted at 20 - 40% RH and room temperature. The authors showed that exposure times of 30, 60, and 120 minutes were necessary to achieve 6 $\log_{10}$ inactivation for ClO_2 gas concentrations of 30, 15, and 7 mg/L (10866, 5433, and 2535 ppmv) respectively.

(Wilson et al., 2005) tested the efficacy of ClO_2 gas for inactivating sick building syndrome-related fungi. The fungi were exposed to either 500 or 1000 ppmv of ClO_2 gas for 24 hours. Treatment at both levels were successful in completely inhibiting the culturability of and inactivating all organisms except for *C. globosum* colonies which were inactivated an average of 89%.

(Han et al., 2004) studied the efficacy of ClO_2 gas in reducing *E. coli O157:H7* and *L. mono-cytogenes* on strawberries using batch and continuous flow ClO_2 gas systems. In the continuous study, the strawberries were exposed to 0.6, 1.8 or 3.0 mg/L (217, 651 or 1087 ppmv) of ClO_2 gas in 90 to 95% RH for 10 min. Log_{10} reductions of both pathogens increased

with increased ClO_2 gas concentrations, and >5 log_{10} reduction was achieved for both with 3.0 mg/L (1087 ppmv) ClO_2 gas concentration in the continuous study.

One other study found much higher ClO_2 concentrations were required to sterilize some surfaces (Han et al., 2003) This result is inconsistent with other published literature, and it is difficult to determine the actual ClO_2 gas doses used (only the initial ClO_2 concentration was reported, and the concentration was allowed to gradually decrease throughout the decontamination cycle without monitoring or additional gas generation).

5. Integrity of N95 Filtering Facepiece Respirators

To date, there is very limited data on the effect of chlorine dioxide gas treatment on N95 FFRs. Several companies and independent research groups have begun to examine the effect of ClO_2 treatment on N95 FFRs in response to the COVID-19 pandemic in ongoing experiments, only one of which has been published in a peer-reviewed journal to date. However, *the single peer-reviewed study lacks critical methodological details regarding the sterilization processes used, and the results are therefore not included in this report (Cai & Floyd, 2020)*. The existing (mostly non-peer-reviewed) data on the impact of ClO_2 gas treatment on N95 FFR filtration efficiency and structural integrity are highlighted in Table 3 and detailed below.

Importantly, repeated donning of an N95 FFR will gradually reduce the fit, even in the absence of any decontamination process. For some N95 models, one study found that fit deteriorated to below acceptable scores after 5 donnings; for other models, this deterioration only occurred after 15 or more donnings (Bergman et al., 2012).

Much of the currently available data comes from a NIOSH National Personal Protective Technology Laboratory (NPPTL) COVID-19 response effort, “Assessment of filter penetration performance and fit for decontaminated N95 respirators” (NPPTL, 2020d). *These NPPTL assessments do not fully meet NIOSH requirements for STP-0059 (the process used for formal N95 FFR approval) and are NOT equivalent to the NIOSH N95 respirator approval process. Critically, NPPTL has no means of validating the decontamination processes (including method, parameters, and number of cycles) used on the N95 FFRs they test.* The NPPTL COVID-19 response tests consist of particulate filter efficiency testing, static fit testing on a mannequin headform, and tensile testing of N95 FFR straps. The results of these NPPTL tests are detailed below.

One such set of results comes from NPPTL tests on three N95 FFR models treated with the ClorDiSys Minidox-M system, with doses of 720 ppmv-hrs (360 ppmv x 2 hrs) of ClO_2 gas per cycle at 65% RH. The 3M V-flex 1805 model (N=14 treated FFRs) was exposed to a single such cycle resulting in 99.5% filtration efficiency and a passing manikin fit factor, with ≤10% increase in strap tensile strength *(NPPTL, 2020c). The 3M 1860 N95 FFR model was exposed to four cycles (N=15 treated FFRs) with a resulting filtration efficiency of at least 98.82% and a passing manikin fit factor, although there was visible discoloration of the nosepiece foam and a ≥15% increase in the strap tensile strength *(NPPTL, 2020c). The VWR 89201 model (N=15 treated FFRs) was also exposed to four cycles with a resulting filtration efficiency of ≥97.91%; however, **all 5 of the 5 treated VWR 89201 FFRs used for mannequin headform testing**

failed to achieve adequate fit (each of two untreated controls passed fit testing), and there was a <10% decrease in strap tensile strength *(NPPTL, 2020c).

Another set of NPPTL results comes from tests on two N95 FFR models treated with the Sabre DiKlor®-G system, with doses of ≥800 ppmv-hrs (8 hours) of ClO_2 gas per cycle at ≥21°C and ≥75% RH *(NPPTL, 2020a). The 3M 1860 model (N=15 treated FFRs) was exposed to six such cycles, resulting in ≥99.13% filtration efficiency and a passing mannequin fit factor, with a <15% increase in strap tensile strength *(NPPTL, 2020a). The 3M 8210 (N=15 treated FFRs) was also exposed to six cycles, resulting in filtration efficiency of at least 98.73% and a passing mannequin fit factor, with ≤15% increase in strap tensile strength *(NPPTL, 2020b).

A third set of NPPTL results comes from tests on a single N95 FFR model, the 3M 8000, exposed to 1000-2000 ppmv-hrs of ClO_2 gas per cycle for either five, ten, or twenty total cycles (N=15 treated FFRs per condition) *(NPPTL, 2020e). All 3M 8000 FFRs exposed to five or ten such cycles passed the mannequin fit factor tests, with some visible discoloration of the straps and <10% or <15% reduction in strap tensile strength, respectively; filtration efficiency was not tested in these FFRs *(NPPTL, 2020e). Filtration testing was performed on the 3M 8000 FFRs exposed to 20 cycles, with resulting filtration efficiency of at least 98.84% *(NPPTL, 2020e). One of the FFRs treated with 20 cycles *and* one of the control FFRs submitted for this condition failed mannequin fit testing; the treated FFRs in this condition also showed visible discoloration of the straps and <10% reduction in strap tensile strength *(NPPTL, 2020e)

Finally, at least one additional small-scale study, which to date remains unpublished, exposed the 3M 8210 Plus model (N=3 treated FFRs) to a single high-dose sterilization cycle at approximately 4900 ppmv-hrs. The resulting filtration efficiency was at least 97.73% *(C. Chidsey, personal communication, April 24, 2020). FFR fit and strap integrity were not assessed, although the only visible change to the treated FFRs was discoloration of the nosepiece foam.

6. Data Summary Tables

Table 2. Impact of ClO_2 decontamination methods on bacterial spores and viruses

Author	Media	ClO_2 gas concentration (ppmv)	RH (%)	Phase Times (h)	Strain(s)	Effectiveness (log_{10} reduction)
A	Biological indicators on building interior surfaces	500	75	≥3	*Bacillus anthracis* spores	≥4 (except wood)
A	Biological indicators on building interior surfaces	3000	75	≥5	*Bacillus anthracis* spores	≥6 (except wood)
B	Tyvek & no packaging (N=20)	500 or 1000	40 - 90	4	*Bacillus atrophaeus* spores	≥6 for both concentrations
C	Biological indicators throughout hospital rooms (N=10)	351 - 385	50 & 60	<3	*Acinetobacter baumannii* *Escherichia coli* *Enterococcus faecalis* *Mycobacterium smegmatis* *Staphylococcus aureus*	≥6 for all concentrations

D	Biological indicators	2535	20 - 40	2	*Bacillus subtilis subsp. niger* spores ATCC9372	≥6
D	Biological indicators	5433	20 - 40	1	*Bacillus subtilis subsp. niger* spores ATCC9372	≥6
D	Biological indicators	10866	20 - 40	0.5	*Bacillus subtilis subsp. niger* spores ATCC9372	≥6
E	Fungal colonies on filter paper (N = 15)	500 or 1000	*Not specified*	24	*Stachybotrys chartarum* *Penicillium chrysogenum* *Cladosporium cladosporioides* *Chaetomium globosum*	≥4 (except *C. globosum colonies* 1 $\log_{10}$)
F	Biological indicators on strawberries	217, 651 or 1087	90-95	10 min	*Escherichia coli O157:H7* *Listeria monocytogenes*	≥3 (217 ppmv) ≥4 (651 ppmv) ≥5 (1087 ppmv)

A: (Rastogi et al., 2010), B: (Doona et al., 2015), C: (Lowe et al., 2013), D: (Jeng & Woodworth, 1990), E: (Wilson et al., 2005), F: (Han et al., 2004)

Table 3. Impact of ClO_2 decontamination methods on N95 FFR filter efficiency by NIOSH method

Author	**N95 FFRs** *(surgical models in italics)*	**Dose**	**# cycles**	**Filtration efficiency**	**FFR integrity**
*G	*3M 1860* (N=10 total)	720 ppmv-hrs (ClorDiSys)	4	≥98.82%	Passing mannequin fit factor. Visible discoloration of nosepiece foam. ≥15% increase in strap tensile strength.
*G	*3M V-flex 1805* (N=10 total)	720 ppmv-hrs (ClorDiSys)	1	≥99.5%	Passing mannequin fit factor. <10% increase in strap tensile strength.
*G	VWR 89201 (N=10 total)	720 ppmv-hrs (ClorDiSys)	4	≥97.91%)	Significant reduction in fit factor (**5 of 5 tested treated FFRs failed fit testing**). <10% decrease in strap tensile strength.
*H	3M 8210 (N=10 total)	≥800 ppmv-hrs (DiKlor-G®)	6	≥98.73%	Passing fit factor. Visible discoloration of nosepiece foam. ≥15% increase in strap tensile strength.
*I	*3M 1860* (N=10 total)	≥800 ppmv-hrs (DiKlor-G®)	6	≥99.13%	Passing fit factor. Visible discoloration of nosepiece foam. <15% increase in strap tensile strength.
*J	3M 8000 (N=5 total)	1000-2000 ppmv-hrs	5	Not assessed	Passing fit factor. Visible discoloration of straps. <10% decrease in strap tensile strength.
*J	3M 8000 (N=5 total)	1000-2000 ppmv-hrs	10	Not assessed	Passing fit factor. Visible discoloration of straps. <15% decrease in strap tensile

					strength.
*J	3M 8000 (N=10 total)	1000-2000 ppmv-hrs	20	≥98.84%	One treated and one control N95 FFR failed fit test. Visible discoloration of straps. <10% decrease in strap tensile strength.
*K	3M 8210 Plus (N=3 total)	~4900 ppmv-hrs	1	≥97.73%	Not tested. Visible discoloration of nosepiece foam.

G: *(NPPTL, 2020c), H: *(NPPTL, 2020b), I: *(NPPTL, 2020a), J: *(NPPTL, 2020e), K: *(C. Chidsey, personal communication, April 24, 2020)

7. Strategies

As noted above (see Mode of Action), the basic requirements for effective ClO_2 gas decontamination or sterilization of N95 FFRs are 1) sufficient N95 FFR exposure to the gas and 2) sufficient humidity. Based on the available literature, a total ClO_2 gas dose of at least 720 ppmv-hrs delivered over at least two hours at 60% or higher relative humidity is likely to achieve 6 $\log_{10}$ inactivation of most microorganisms. Additional process controls to minimize chlorine production, prevent gas leakage, and ensure sufficient ClO_2 clearance from treated materials are important to ensure the safety of the worker(s) performing the decontamination as well as the end users of the treated N95 FFRs.

Decontamination can take place in a variety of settings, from very small to very large scale. Preexisting chambers sealed off from their surroundings, such as biological safety cabinets or glove boxes, work well (Girouard & Czarneski, 2016; Luftman et al., 2008). ClO_2 gas can also be used to decontaminate much larger spaces (from rooms or laboratories to entire facilities), although this approach may require additional engineering controls to seal off the decontamination area, as well as increased airflow and/or longer cycle duration to ensure diffusion of the gas throughout the entire space (Girouard & Czarneski, 2016; Lowe et al., 2013; Luftman et al., 2006; Takahashi et al., 2014). ClO_2 gas diffuses readily, including under closed internal doors and into closed cabinets (Lowe et al., 2013). A relative advantage of ClO_2 gas as a decontaminant is its compatibility with laboratory and medical equipment, including electronics, simplifying decontamination of entire rooms or buildings. ClO_2 gas treatment has been shown to have no effect on personal computers or household electronic devices (Doona et al., 2015; Girouard & Czarneski, 2016) and has been used to decontaminate sensitive, high-end equipment (B. Sherman et al., 2015). However, ClO_2 gas has been shown to damage some materials, including corrosion of some metals (particularly carbon steel and copper, as well as some alloys of stainless steel and aluminum), fading of inkjet printed paper, and yellowing of photographs (EPA, 2010).

Currently, there are two commercially viable systems, from Sabre (DiKlor®-G) and ClorDiSys, that have EPA registration numbers, have received historic FDA approvals for other other applications, and have made significant progress toward validating their process for sterilization of N95 FFRs, including preliminary fit and filtration efficiency studies through NPPTL (Table 3) and submission of Emergency Use Authorization (EUA) applications to the FDA. **Note that, to date, the FDA has not approved any EUA for a chlorine dioxide**

decontamination approach. These commercial systems each use the same general five-step process to sterilize N95 FFRs with chlorine dioxide gas: 1) preconditioning, 2) conditioning, 3) charging/ClO_2 gas generation, 4) exposure phase, and 5) aeration. Each step is described in more detail below.

In the preconditioning phase, the sterilization space or chamber is brought to the target relative humidity (RH, 60–75%). In the conditioning phase, the raised RH is held, allowing the objects inside the chamber to be hydrated. Note that while ClO_2 gas decontamination has been shown to be more effective at higher RH (Jeng & Woodworth, 1990), high humidity has also been shown to be important for effective sterilization of bacterial spores using other decontamination methods (Spotts Whitney et al., 2003).

In the charging phase, ClO_2 gas is introduced into the sterilization space. The ClorDiSys Minidox-M® process generates ≥99% ClO_2 gas by passing 2% chlorine gas in 98% nitrogen through a cartridge containing a combination of sodium chlorite and other proprietary ingredients (see Eqn 1 in Mode of Action). This gas is then mixed with the air in the sterilization space to a concentration of 360 ppmv (Czarneski & Lorcheim, 2005) (Girouard & Czarneski, 2016) (Eylath et al., 2003). The Sabre DiKlor®-G system generates gaseous chlorine dioxide using 15% HCl, 12.5% NaOCl, and 25% $NaClO_2$ in solution (see Eqn 3 in Mode of Action), with a 95% yield rate (Rastogi et al., 2010) (EPA, 2014). Sabre's DiKlor-G® chlorine dioxide gas phase generation employs two closed loop systems working together to both produce and apply the chlorine dioxide in the target treatment zone. The generator loop must generate the aqueous chlorine dioxide product, flow through an emitter, and return the spent aqueous solution to the generator for recharging. The gas loop sucks air from the target treatment zone, flows through the emitter, and returns the air containing chlorine dioxide gas into the treatment zone. This system only applies gaseous chlorine dioxide to the treatment zone, while the salts and any non-volatile contaminants remain in the aqueous loop of the generator system (Rogers et al., 2006) (EPA, 2014).

These commercial systems continuously monitor the ClO_2 gas concentration spectrophotometrically to ensure the final target dose (the product of concentration and time) is reached in the sterilization space. The ClorDiSys system typically uses a dose of ≥720 ppmv-hrs (Girouard & Czarneski, 2016; NPPTL, 2020c), while the Sabre DiKlor®-G system uses ≥800 ppmv-hrs (NPPTL, 2020a, 2020b).

Finally, in the aeration phase at the completion of the sterilization cycle, the residual ClO_2 gas is removed from the decontamination space by air flow. The ClorDiSys system can use a carbon-based scrubber to remove the ClO_2 from the space (ClorDiSys, 2014) if venting through an exhaust stack to the atmosphere is not appropriate. The Sabre DiKlor®-G system neutralizes residual ClO_2 gas in a treatment zone, by dosing the liquid generation loop with sodium erythorbate (chemically similar to vitamin C). This neutralizes chlorine dioxide gas as the gas loop pulls air from the treatment zone (EPA, 2014). For faster gas removal, the gas emitters can be converted into active gas scrubbers by adding an alkaline sodium sulfite solution (Hua & Reckhow, 2007) based on chemistry in (Horvath & Nagypal, 2006)), or an alkaline erythorbic acid solution to the liquid ClO_2 solution process flow loop. This solution is then circulated through the emitters so that ClO_2 gas is removed from the air drawn through the emitters.

Lower-resource approaches to ClO_2 gas decontamination are also possible with less specialized equipment, with corresponding loss of complete control over and continuous monitoring of cycle parameters. Some of these approaches can achieve sterilization (≥6 $\log_{10}$ kill) at a tiny fraction of the cost of the larger-scale commercial systems (Mitchell et al., 2019). One challenge in these lower-resource processes is preventing ClO_2 gas leakage throughout the entire decontamination cycle, including aeration/scrubbing, in order to maintain occupational exposure levels below safety limits. Another commercial vendor (Aptar) has adopted an alternative approach of dramatically reducing the quantity of ClO_2 gas produced, thereby mitigating the risk of occupational exposure by reducing the peak concentration and allowing the gas to simply diffuse through a plastic ziplock-style bag during a two-hour decontamination cycle; however, this strategy suffers from dramatically reduced decontamination efficacy (AptarGroup, Inc., 2020). This process has *not* been proven to achieve 3-log kill of SARS-CoV-2 or a surrogate virus, and it is highly unlikely to inactivate harder-to-kill pathogens which may also be present on used N95 FFRs, particularly in healthcare settings.

8. Primary Risks, Occupational Safety, and Unknowns

Studies on N95 FFR decontamination by chlorine dioxide, including the effects of treatment on FFR integrity, are limited. There are no peer-reviewed studies to date showing successful ClO_2 decontamination of N95 FFRs contaminated with SARS-CoV-2 or any other pathogens. The single peer-reviewed study on the effects of ClO_2 treatment of N95 FFRs lacks crucial methodological details regarding the sterilization process (Cai & Floyd, 2020).

There are important concerns about occupational safety and toxicity for workers performing ClO_2 decontamination of N95 FFRs. As previously noted, chlorine dioxide gas is explosive at concentrations above 95,000 ppmv in air (these concentrations greatly exceed typical concentrations generated for use in decontamination) (Bretherick, 1990; Deshwal & Lee, 2005; Dobson & Cary, 2002; Jin et al., 2009). ClO_2 is a hazardous and highly irritant gas (Deshwal & Lee, 2005). ClO_2 gas has been shown to be toxic by inhalation in rodents at much higher concentrations than regulatory exposure limits; it also causes eye and respiratory tract irritation in animals and humans (Dobson & Cary, 2002). Proper ventilation in work places is recommended (Deshwal & Lee, 2005). Based on the US Occupational Safety and Health Administration (OSHA) the long-time average (8 hour) permissible exposure limit (PELs) of chlorine dioxide in the air of human workplace is 0.1 ppmv or 0.3 mg/m^3. The short-term average (15 min) exposure limit is 0.3 ppmv (OSHA, 2019). Electrochemical sensors are available to monitor occupational exposures and alert users to concentrations exceeding OSHA limits.

Different methodological approaches such as exposure of various viruses and bacteria in the wet state on glass dishes for many hours to ClO_2 gas at 0.05 ppmv (less than the permissible exposure limit of 0.1 ppmv) have been claimed to reduced viability by factors from 2 $\log_{10}$ to 5 $\log_{10}$; the presence of 1% fetal bovine serum in the wet layers led to reduced deactivation of pathogens (Morino et al., 2011).

Chlorine dioxide is less toxic to humans and animals as compared to microorganisms, an effect that may be related to size selectivity. The time to eradicate an organism with chlorine dioxide is proportional to the square of its size, such that smaller organisms would be eradicated much faster than larger ones (Noszticzius et al., 2013).

Long term exposure to low levels of chlorine dioxide has been studied. (Akamatsu et al., 2012) evaluated chronic exposure of chlorine dioxide at concentrations up to 0.1 ppmv in rats for a period of six months and did not find any toxic effects in the rats. Data on chronic carcinogenicity is lacking, and there is no data on reproductive toxicity or teratogenicity of ClO_2 gas specifically (Dobson & Cary, 2002). However, the CDC has stated that "[c]hlorine dioxide is not mutagenic or carcinogenic in humans" (NIOSH, 2017; *Other Sterilization Methods | Disinfection & Sterilization Guidelines | Guidelines Library | Infection Control | CDC*, 2019).

It is also important to note that the generating method could affect the impurities generated which in turn could have serious impacts on human health. For example, byproducts such as Cl_2 could form trihalomethane when it reacts with organic matter. Trihalomethane is carcinogenic, and other products such as chloroxy anions (ClO_2^- or ClO_3^-) could also negatively affect human health (Couri et al., 1982). (Ma et al., 2017) produced chlorine dioxide gas contaminated with smaller amounts of chlorine gas by electrolysis of aqueous sodium chloride and assessed safety related issues in vitro, and in vivo rabbit ocular irritation, inhalation toxicity and subchronic oral toxicity. Findings of their study indicated no eye irradiation at 50 ppm by mass ClO_2 in aqueous solutions, no mortalities or abnormalities at 20 ppm of ClO_2 and subchronic oral toxicity tests showed no toxicity up to 40 ppm in mice for 90 days.

In a systematic review by (Tofanelli et al., 2020) it was noted that disposable aqueous chlorine dioxide wipes for high-level disinfection in an endoscopy department did not have any safety related issues for patients and staff. (Chang et al., 2018) evaluated the occupational exposure to airborne chlorine dioxide during high-level disinfection of nasal endoscopes in hospital staff and found insignificant occupational health exposure.

Proper removal of the gas from treated N95 FFRs before use is crucial. With time, ClO_2 gas will eventually outgas and break down. Commercial sterilization systems include aeration cycles, often combined with neutralization or scrubbing of the gas (see Strategies section above). Commercial suppliers report no measurable ClO_2 gas in the large volume of the treatment chamber after aeration (P. Lorcheim, personal communication, June 30, 2020). **However, to date there is no published method to measure the time course of residual levels of ClO_2 emitted from treated N95 FFRs during use.**

Preliminary results from ongoing unpublished experiments show that ClO_2 continues to outgas from some types of N95 FFRs for at least one hour. In one experiment, two 3M 8200 respirators were nested around a biological indicator strip holding 10^6 colony forming units (cfu) of *B. atrophaeus* spores and the edges of the respirator were taped tightly together with clear packaging tape. This assembly was sealed in a 6 x 9 inch Tyvek envelope and placed in a 2 L glass jar with metal lid. A dose of approximately 700 ppmv-hrs ClO_2 gas over two hours was generated by mixing 100 µL saturated aqueous sodium chlorite and 100 µL saturated aqueous sodium hydrogen sulfate in an inverted polypropylene bottle cap inside the glass jar. 10 mL aliquots of gas were periodically withdrawn through a luer lock stopcock sealed into the

lid of the jar and then flushed through a fused silica cuvette with a 1-cm pathlength. The optical density at 351 nm was used to determine the concentration of the ClO_2 gas (Wahner et al., 1987). The concentration peaked at about 10 minutes at about 800 ppmv. At the end of the two-hour treatment, the concentration of ClO_2 gas in the jar had dropped to about 80 ppmv. All 10^6 cfu of spores on the biological indicator were subsequently found to have been killed, providing proof that the ClO_2 had permeated completely through the envelope and the full thickness of the respirators. Because no drop in concentration was observed when the ClO_2 gas was generated in the absence of the respirators and the Tyvek envelope, it was concluded that most of the gas was absorbed over many tens of minutes into the nonwoven polypropylene fabrics of the respirators and the envelope. After removal and separation of the respirators and the biological indicator strip, nitrogen gas was passed through one of the respirators at 6 L/min (the average inhalation rate of an adult at rest). The concentration of ClO_2 in the effluent gas as measured by a hand-held electrochemical ClO_2 gas sensor (GasAlert Extreme, BW Technologies by Honeywell) dropped steadily to 0.04 ppmv after 45 minutes. The respirator and sensor were then transferred to a 1-L polyethylene terephthalate bag, which has minimal ClO_2 permeability. The ClO_2 concentration in the bag rose slowly over the next 10 minutes to 0.4 ppmv, illustrating both the slow rate of ClO_2 outgassing and the significant dilution caused by 6 L/min gas flow through the respirator. From these results, one can infer that the ClO_2 outgasses by a factor of ten in 25 to 30 minutes. Thus, for a final treatment concentration of 80 ppmv, aeration should conservatively last at least an hour in a gentle flow of fresh air to confidently bring the ClO_2 concentration inhaled by a user down from 80 ppmv to below the 15-min STEL of 0.3 ppmv when diluted by a resting breathing rate of 6 L/min. In a second, similar experiment but with a larger dose of ClO_2 of about 1400 ppmv-hrs, it took 70 minutes of 6 L/min nitrogen gas flow through one of the respirators before the ClO_2 concentration in the flowing gas dropped below 0.1 ppmv. From these experiments, one can conclude that aeration requires only minimal air flow; much less than 6 L/min should suffice due to the slow rate of ClO_2 outgassing. Two hours of aeration is expected to reliably reduce the initial inhaled concentration below 0.01 ppmv. Additional aeration time will be required for higher ClO_2 concentrations at the end of the treatment cycle. In another experiment, clearance of the gas was found to be dramatically shortened by exposure to direct sunlight, presumably due to photodecomposition of ClO_2 while still absorbed in the polypropylene of the respirator *(C. Chidsey, personal communication, August 3, 2020).

9. Conclusions

To date, there is less federal guidance and less literature available specifically supporting N95 FFR decontamination during the COVID-19 pandemic with ClO_2 gas compared to several other methods (e.g., hydrogen peroxide vapor or UV-C decontamination). The FDA has yet to approve any of the submitted Emergency Use Authorizations (EUAs) for decontamination of N95 FFRs with ClO_2 gas. However, if implemented properly and adequate concentration of ClO_2 gas and humidity is maintained in the sterilization chamber or other space for an adequate duration to achieve a dose of at least 720ppmv-hrs, it is likely that ClO_2 gas will completely inactivate SARS-CoV-2 and harder-to-kill pathogens on N95 FFRs. This inference is

based on results from similar viruses, but has not yet been confirmed directly for SARS-CoV-2 by peer-reviewed studies. **Damage to the strap integrity and fit of some models of N95 FFR has been reported after ClO_2 treatment, and therefore model-specific integrity of the FFRs used in each setting should be verified before implementing a ClO_2 gas decontamination approach**. We once again stress that (i) after each round of decontamination, a user seal check should be performed, and (ii) extended cycles of doffing and re-donning may affect FFR fit. Finally, **adequate aeration of N95 FFRs after ClO_2 gas treatment, possibly combined with additional removal measures, is also essential for the safety of the individuals wearing the treated FFRs.** Further experiments are required to determine adequate processes and aeration duration for ClO_2 removal from treated FFRs prior to implementation. **Further independent data and peer review would be needed to demonstrate the proper engineering controls to preserve FFR integrity, achieve FFR decontamination, and prevent occupational exposure.**

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