

Ozone disinfection for viruses with applications in healthcare environments: a scoping review

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Abstract: The aim of this scoping review was to provide sufficient information about the effectiveness of ozone gas in virus inactivation of surfaces and objects under different environmental conditions. The review was performed according to the list of PRISMA SrC recommendations and the JBI Manual for Evidence Synthesis for Scoping Reviews. The review was registered in Open Science Framework (OSF). EMBASE (Ovid), Lilacs, LIVIVO, MEDLINE (PubMed), SciELO, Scopus and Web of Science were primary sources, and “gray literature” was searched in OpenGray and OpenThesis. A study was included if it reported primary data on the effect of ozone gas application for vehicle-borne and airborne virus inactivation. No language or publication date restriction was applied. The search was conducted on July 1, 2020. A total of 16,120 studies were screened, and after exclusion of noneligible studies, fifteen studies fulfilled all selection criteria. Application of ozone gas varied in terms of concentration, ozone exposure period and the devices used to generate ozone gas. Twelve studies showed positive results for inactivation of different virus types, including bacteriophages, SARS-CoV-2 surrogates and other vehicle-borne viruses. Most of the studies were classified as unclear regarding sponsorship status. Although most of the population has not yet been vaccinated against COVID-19, disinfection of environments, surfaces, and objects is an essential prevention strategy to control the spread of this disease. The results of this Scoping Review demonstrate that ozone gas is promising for viral disinfection of surfaces.

Keywords: Disinfection; Ozone; Virus Inactivation.

Introduction

SARS-CoV-2 spread from China worldwide in less than 4 months, directly impacting the economy, health and lifestyle of affected countries. Transmission can occur via human-to-human contact, contact with infected surfaces (fomites or skin-to-skin), or airborne transmission, which occurs through the mouth, nose, and eyes or through inhalation of small respiratory droplets suspended in the air.^{1,2}

There is currently no approved and effective antiviral treatment against SARS-CoV-2,^{1,3} and ongoing COVID-19 vaccination programs have not reached most of the population; thus, prevention remains the main strategy

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for controlling the spread of the disease. However, considering the difficulty in directly conducting research with SARS-CoV-2, which requires biosafety level 3 (BSL3) or higher, many studies using surrogate viruses have been conducted to develop methods to prevent SARS-CoV-2 infection.⁴ Hence, efforts have focused on disinfection of environments and objects as well as individual protection and preventative measures to avoid disease spread and potential outbreaks.¹ Aerosol, droplet, and fomite transmission are important routes for the spread of many viral diseases.⁵ Among the numerous disinfection and sanitization methods proposed,⁶ devices that generate ozone are commonly applied due to the ability of gaseous ozone to easily penetrate into all areas of a room, furniture and other objects.⁷

Ozone is a gas that forms chemically via an unstable triatomic molecule of oxygen (O₃) that it quickly degrades to its stable state (diatomic oxygen),⁸ leading to formation of secondary oxidants (hydroxyl radicals) with high reactivity and a short reaction time.^{9,10} In brief, the disinfection mechanism is based on the reaction of ozone with organic compounds containing double bonds.¹¹ Virus inactivation occurs as a consequence of envelope protein denaturation, impairing virus adhesion to cells, oxidation of unsaturated fatty acids present in the lipid envelope, and destruction of single-stranded RNA.⁷ However, the effectiveness of ozone as a virucide is related to various factors, including the ozone concentration,^{8,12} exposure time,⁸ and temperature^{8,12,13} and relative humidity of the environment.^{12,13}

In the current pandemic of SARS-CoV-2, identifying a disinfection process that interrupts virus transmission routes, including contaminated surfaces, is of utmost importance. Therefore, the present scoping review aims to provide sufficient information about the effectiveness of ozone gas for inactivation of vehicle-borne and airborne viruses under different environmental conditions.

Methodology

Protocol registration

The scoping review protocol was performed according to Preferred Reporting Items for Systematic

Review and Meta-Analysis Protocols (PRISMA-P)¹⁴ and was registered in Open Science Framework (<https://osf.io/2a3nu/> - DOI 10.17605/OSF.IO/2A3NU). This review is reported following Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines – extension for scoping review¹⁵ and was conducted according to Joanna Briggs Institute Critical Appraisal tools in JBI Manual for Evidence Synthesis for Scoping Reviews¹⁶. The Arksey and O'Malley methodological framework¹⁷ was employed to conduct this scoping review: a) identifying the research question, b) identifying relevant studies, c) study selection, d) data charting process and e) summarizing and reporting results.

Research question and eligibility criteria

Eligibility criteria were based on the following research question: "Is there evidence of the effectiveness of the application of ozone gas in inactivating vehicle borne and airborne viruses with applications to healthcare environments"? The "PCC" (Population, Concept, and Context) mnemonic was used to guide this scoping review, where P denotes vehicle borne and airborne viruses, C ozone disinfection, and C reducing surface transmission.

Inclusion criteria

A study was eligible for inclusion if it reported primary data on the effect of ozone gas application on inactivation of vehicle-borne (blood, fluids and fomites) and airborne viruses. No language or publication date restriction was applied. In vitro studies, clinical trials, and experimental and observational studies (prospective and retrospective) were included.

Exclusion criteria

Exclusion criteria were as follows: a) studies involving virus inactivation in water for sewage treatment; b) studies involving food and agriculture; c) studies using ozone application as a coadjuvant method for virus inactivation or studies in which ozone was a byproduct from a different decontamination system (not gas); d) case reports and case series; e) systematic reviews; f) conference abstracts, letters, and editorials; and g) personal opinions and books and/or book chapters.

Literature search and study selection

The following databases were searched on July 1, 2020: MEDLINE (PubMed), Scopus, EMBASE, Lilacs, LIVIVO, SciELO, and Web of Science. Part of the “gray literature” was searched in OpenGray and OpenThesis. Descriptors were selected using Medical Subject Headings (MeSH), Descriptors in Health Science (DeCS), and Embase Subject Headings

(Emtree). Boolean operators (AND and OR) were used to combine descriptors and improve the search strategy by means of different combinations (Table 1). The search strategy for MEDLINE was adapted for the other databases, respecting their rules of syntax.

The results obtained from the primary databases were initially exported to EndNote Web™ (Clarivate™, Analytics, Philadelphia, USA), excluding duplicates.

Table 1. Strategies for database searches.

Database	Search strategy (July 2020)
Embase (http://www.embase.com)	('ozone' OR 'ozonotherapy' OR 'ozone therapy' OR 'o3' OR 'ozonized') AND ('disinfection' OR 'infection control' OR 'prevention and control' OR 'biological control agents' OR 'disease transmission' OR 'safety management' OR 'personal management' OR 'disinfectant' OR 'decontamination' OR 'ppe' OR 'virus inactivation' OR 'airborne transmission' OR 'healthcare workers') NOT ('food' OR 'agriculture' OR 'drinking water')
LILACS (http://lilacs.bvsalud.org/)	((“Ozone” OR “Ozonotherapy” OR “Ozone Therapy” OR “O3” OR “Ozonized”) AND (“Disinfection” OR “Infection Control” OR “Prevention and Control” OR “Biological Control Agents” OR “Disease Transmission” OR “Safety Management” OR “Personal Management” OR “Disinfectant” OR “Decontamination” OR “PPE” OR “Virus Inactivation” OR “Airborne Transmission” OR “Healthcare Workers”))
LIVIVO (http://livivo.de)	((“Ozone” OR “Ozonotherapy” OR “Ozone Therapy” OR “O3” OR “Ozonized”) AND (“Disinfection” OR “Infection Control” OR “Prevention and Control” OR “Biological Control Agents” OR “Disease Transmission” OR “Safety Management” OR “Personal Management” OR “Disinfectant” OR “Decontamination” OR “PPE” OR “Virus Inactivation” OR “Airborne Transmission” OR “Healthcare Workers”) NOT (“Food” OR “Agriculture” OR “Drinking Water”))
PubMed (http://www.ncbi.nlm.nih.gov/pubmed)	((“Ozone” OR “Ozonotherapy” OR “Ozone Therapy” OR “O3” OR “Ozonized”) AND (“Disinfection” OR “Infection Control” OR “Prevention and Control” OR “Biological Control Agents” OR “Disease Transmission” OR “Safety Management” OR “Personal Management” OR “Disinfectant” OR “Decontamination” OR “PPE” OR “Virus Inactivation” OR “Airborne Transmission” OR “Healthcare Workers”) NOT (“Food” OR “Agriculture” OR “Drinking Water”))
SciELO (http://www.scielo.org/)	((“Ozone” OR “Ozonotherapy” OR “Ozone Therapy” OR “O3” OR “Ozonized”) AND (“Disinfection” OR “Infection Control” OR “Prevention and Control” OR “Biological Control Agents” OR “Disease Transmission” OR “Safety Management” OR “Personal Management” OR “Disinfectant” OR “Decontamination” OR “PPE” OR “Virus Inactivation” OR “Airborne Transmission” OR “Healthcare Workers”))
Scopus (http://www.scopus.com/)	((“Ozone” OR “Ozonotherapy” OR “Ozone Therapy” OR “O3” OR “Ozonized”) AND (“Disinfection” OR “Infection Control” OR “Prevention and Control” OR “Biological Control Agents” OR “Disease Transmission” OR “Safety Management”)) ((“Ozone” OR “Ozonotherapy” OR “Ozone Therapy” OR “O3” OR “Ozonized”) AND (“Personal Management” OR “Disinfectant” OR “Decontamination” OR “PPE” OR “Virus Inactivation” OR “Airborne Transmission” OR “Healthcare Workers”))
Web of Science (http://apps.webofknowledge.com/)	((“Ozone” OR “Ozonotherapy” OR “Ozone Therapy” OR “O3” OR “Ozonized”) AND (“Disinfection” OR “Infection Control” OR “Prevention and Control” OR “Biological Control Agents” OR “Disease Transmission” OR “Safety Management” OR “Personal Management” OR “Disinfectant” OR “Decontamination” OR “PPE” OR “Virus Inactivation” OR “Airborne Transmission” OR “Healthcare Workers”) NOT (“Food” OR “Agriculture” OR “Drinking Water”))
OpenGrey (http://www.opengrey.eu/)	((“Ozone” OR “Ozonotherapy” OR “Ozone Therapy” OR “O3” OR “Ozonized”) AND (“Disinfection” OR “Infection Control” OR “Prevention and Control” OR “Biological Control Agents” OR “Disease Transmission” OR “Safety Management” OR “Personal Management” OR “Disinfectant” OR “Decontamination” OR “PPE” OR “Virus Inactivation” OR “Airborne Transmission” OR “Healthcare Workers”))
OpenThesis (http://www.openthesis.org/)	((“Ozone” OR “Ozonotherapy” OR “Ozone Therapy” OR “O3” OR “Ozonized”) AND (“Disinfection” OR “Infection Control” OR “Prevention and Control” OR “Biological Control Agents” OR “Disease Transmission” OR “Safety Management” OR “Personal Management” OR “Disinfectant” OR “Decontamination”))

The remaining references retrieved from OpenGray and OpenThesis were exported to Microsoft Word™ 2019 (Microsoft™ Ltd., Washington, USA) software, and duplicates were manually removed. The reviewers (MSI and GSL) independently performed a methodical analysis of all study titles, specifically evaluating the study design and excluding those that did not meet the inclusion criteria. When reading abstracts, all eligibility criteria were taken into account. The complete texts were read, and possible disagreements were resolved by a third reviewer (LD). In cases where the title or abstract provided insufficient information to accomplish a proper inclusion or exclusion decision, the full text was read to address any doubts. The references of the eligible studies were evaluated as well as those identified by experts in the field. These strategies were performed to minimize selection and publication bias.

Data charting process and data items

After study selection, the reviewers performed a calibration exercise that consisted of selecting and extracting data from three randomly selected articles. The studies were analyzed independently by two reviewers (MSI and GLS). All of the data were checked by another author. The following information was extracted: meta data (author, date, year of publication), virus type, treatment groups, virus carrier/contact surface, method of assessing virus inactivation, ozonation method, device, ozone concentration (ppm), pH, temperature, relative humidity (RH), exposure time to ozone and key findings.

Sponsorship status evaluation

Information regarding the source of funding of the selected studies was also assessed. These data were extracted because industry sponsorship might be associated with risks of publication, reporting and selection biases.¹⁸ The sponsorship status was classified as follows:¹⁹

- a. Unclear - when it was not possible to affirm the sponsorship status even after an attempt to contact the authors by email;
- b. Nonsponsored - when the authors declared that the study did not receive any type of financial support from companies related to ozone-generating devices;

- c. Sponsored - when the authors reported any financial contribution (financial support, provision of equipment or supplies, discounts, etc.) from companies related to ozone-generating devices.

The main text and acknowledgments were checked to collect this information. In cases of missing information or unclear data, the authors were contacted twice by e-mail at an interval of one week.

Synthesis of results

The results of eligible studies were summarized in a descriptive/narrative manner, including study characteristics, and virus inactivation efficiency of interest relative to the healthcare environment was synthesized. Studies reporting ozone concentrations expressed in mg/L were transformed to part per million (ppm) for analysis.

Results

Selection of evidence sources

During the first phase of study selection, 16,120 results among nine electronic databases, including the gray literature, were obtained. After removing repeated/duplicate results, 9937 articles remained for analysis of titles and abstracts. After detailed analysis, only 59 studies were eligible for full-text analysis. One study was included as an expert suggestion. The references of the potentially eligible studies were evaluated carefully, and one additional article was selected, resulting in 61 studies for full-text assessment. After reading the full text, studies that did not fulfill the inclusion criteria were eliminated. Ultimately, 15 studies were included in the scoping review. Figure 1 illustrates the search process, identification, inclusion and exclusion of eligible studies.

Characteristics of evidence sources

Overview

The studies included were published between 1990 and 2020, and most were performed in North America (46.6%) (Figure 2). These studies analyzed the effectiveness of ozone application using different study designs. Most tested the effectiveness of ozone

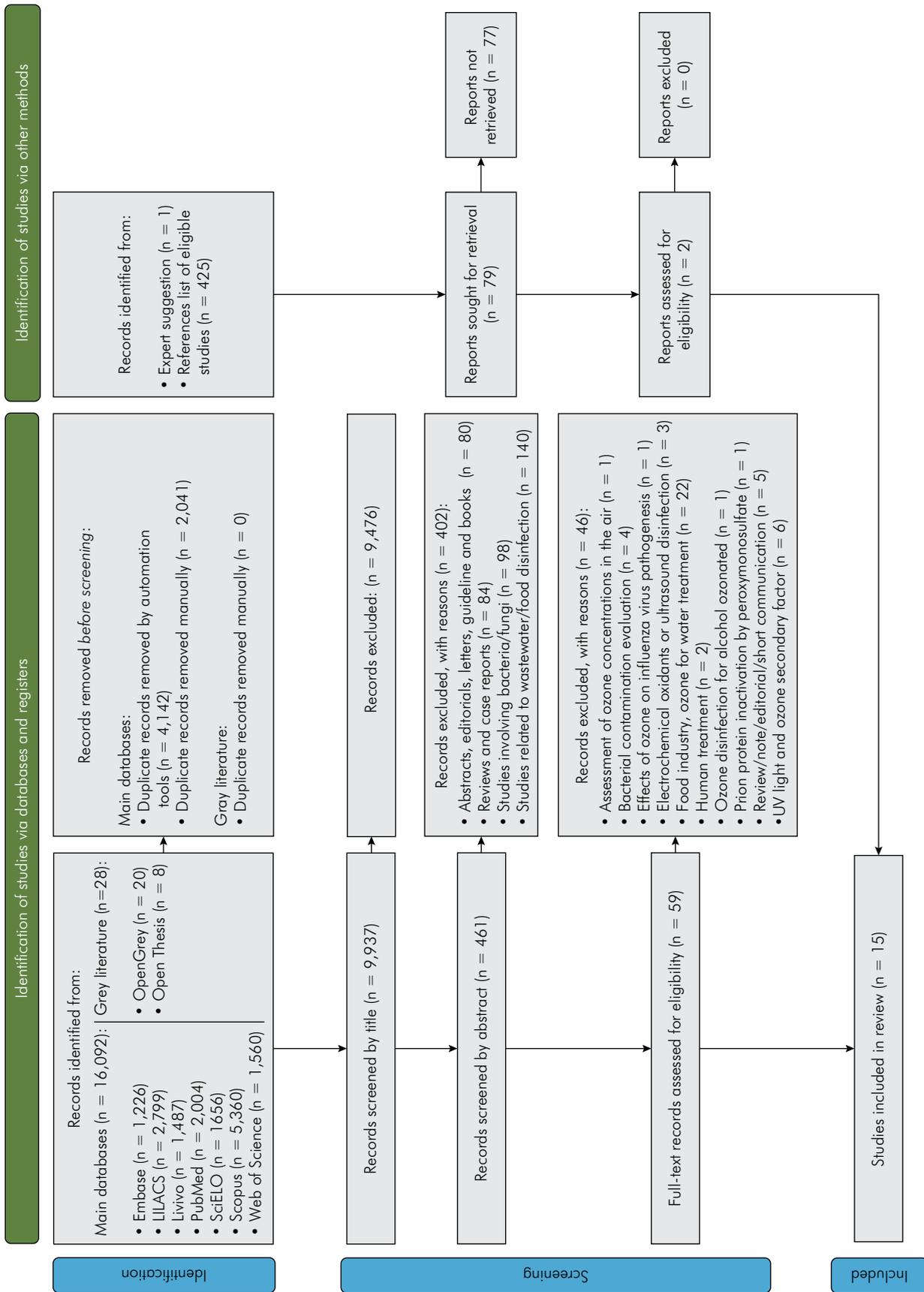


Figure 1. Flowchart of the search process.

gas by adding aliquots of virus stock solutions in Petri dishes with the following sources: ^{7,20-24} hospital textiles (fabrics, cloth, carpet, cotton); ^{7,25,26} N95, PFF2 masks and respirators ^{27,28}; stainless steel disks ^{7,29} or plastics. ^{24,30} Only 3 studies ³¹⁻³³ used aerosol generators to simulate virus spread in the environment. Ozone application was considered efficient in reducing virus infectivity or virus integrity in 12 (80%) of the 15 included articles (Table 2).

Virus type

The effect of ozone application was investigated on several viruses, including hepatitis B, ²⁶ influenza H1N1, ²⁸ H3N2, ⁷ human respiratory syncytial RSV, ²⁸ lentiviral vector, ³³ human coronavirus HCoV-229E, ²⁷ murine norovirus, ^{7,20,31} feline calicivirus, ^{7,20,25,29} phages $\phi 6$ and $\phi X174$, ^{21,31,32} MS2 bacteriophages, ^{21,31,32} phage PR772, ³¹ twist synthetic SARS-CoV-2 RNA, ³⁰ Theiler's murine encephalomyelitis virus (TMEV), Reo type 3 virus (RV) and murine hepatitis virus (MHV), ²⁴ phage T7, ^{21,32} herpes simplex virus, ^{22,25} rhinovirus, ⁷ adenovirus types 3 and 11, ⁷ sindbis virus (SINV) ⁷, yellow fever virus (YFV) ⁷, vesicular stomatitis virus (VSV) ⁷, poliovirus (PV vaccine strain) ⁷, 7 vaccinia virus (VV) ⁷ and enterovirus 71 ²³. Ozone gas was ineffective in hepatitis B ²⁶, lentiviral vector ³³ and enterovirus 71 ²³ inactivation.

All of the studies included in this scoping review published in 2020 and 2021 ^{27,28,30,31,33} focused on viral strains similar to SARS-CoV2. The authors described the following viruses as biosafe substitutes for SARS-CoV-2: lentiviral vector, ³³ HCoV-229E, ²⁷ influenza A virus (IAV; strain A/WSN/33) and human respiratory syncytial virus (RSV; strain A2). ²⁸ Three of these studies ^{28,30,31} reported good results of virus inactivation after ozone exposure for at least 40 minutes.

Ozone application

Standardization of the unit of measurement used to assess the ozone concentration in ppm was obtained using the formula $1 \text{ ppmv} = 0.002 \text{ mcg/ml}$ for conversion, as suggested by Bocci (2011). ³⁴

Different types of equipment were used for ozonation, all of which use oxygen from the air as the source; an exception was Tseng and Li, ^{21,32} in which pure oxygen was the source used to feed

the ozone generator OZIPCS-V/SW (Ozotech Inc., Yreka, USA). The devices produce ozone through electrical discharges or through plasma (Table 3). Five studies evaluated the effect of low ozone concentrations ($< 1.3 \text{ ppm}$), ^{21,22,23,31,32} and two of them ^{31,32} used aerosol generators to simulate virus spread. Considering the results of these studies, 40 minutes of ozone application with high RH (85%) was most effective. Two studies showed that increased exposure time (44 minutes ²¹ to 180 min ²²) was required when using low ozone concentrations for virus inactivation. One study ²³ demonstrated that a low ozone concentration ($< 1.3 \text{ ppm}$) was not effective in reducing virus survival.

Most of the experiments ^{20,21,24,27,28,30,31,32} utilized high ozone concentrations and were carried out in chambers and cabinets to avoid ozone leakage. One study demonstrated that ozone cannons were not efficient for disinfecting the surfaces of ambulances. ³³ The efficacy of ozone gas in an office, a laboratory, a hotel room and a cruise liner cabin was also tested. ^{7,25} Overall, the variation in ozonation protocols among the studies did not allow direct comparison of results.

Relative humidity (RH)

Ten articles reported RH during the tests. ^{7,20-24,28,31,32,33} Among them, eight evaluated the influence of RH on virus degradation and/or virus infectivity. ^{7,21,24,25,28,29,31,32} The results of these experiments controlling RH demonstrated that RH plays a key role in ozone reactivity, and in most studies, the optimum efficacy of ozone treatment was achieved under high RH ($> 50\%$). All of these studies reported a positive effect of elevated RH on the overall efficacy of ozone treatment.

Industry sponsorship status

The sponsorship status is shown in Table 4. None of the studies reported any type of financial support from companies related to ozone-generating devices. In 7 studies, the authors declared no conflicts of interest. Two studies ^{29,33} provided information by e-mail. Unclear information was observed in 8 of the selected studies, and in these cases, it was not possible to affirm sponsorship status even after attempts to contact the authors by email.

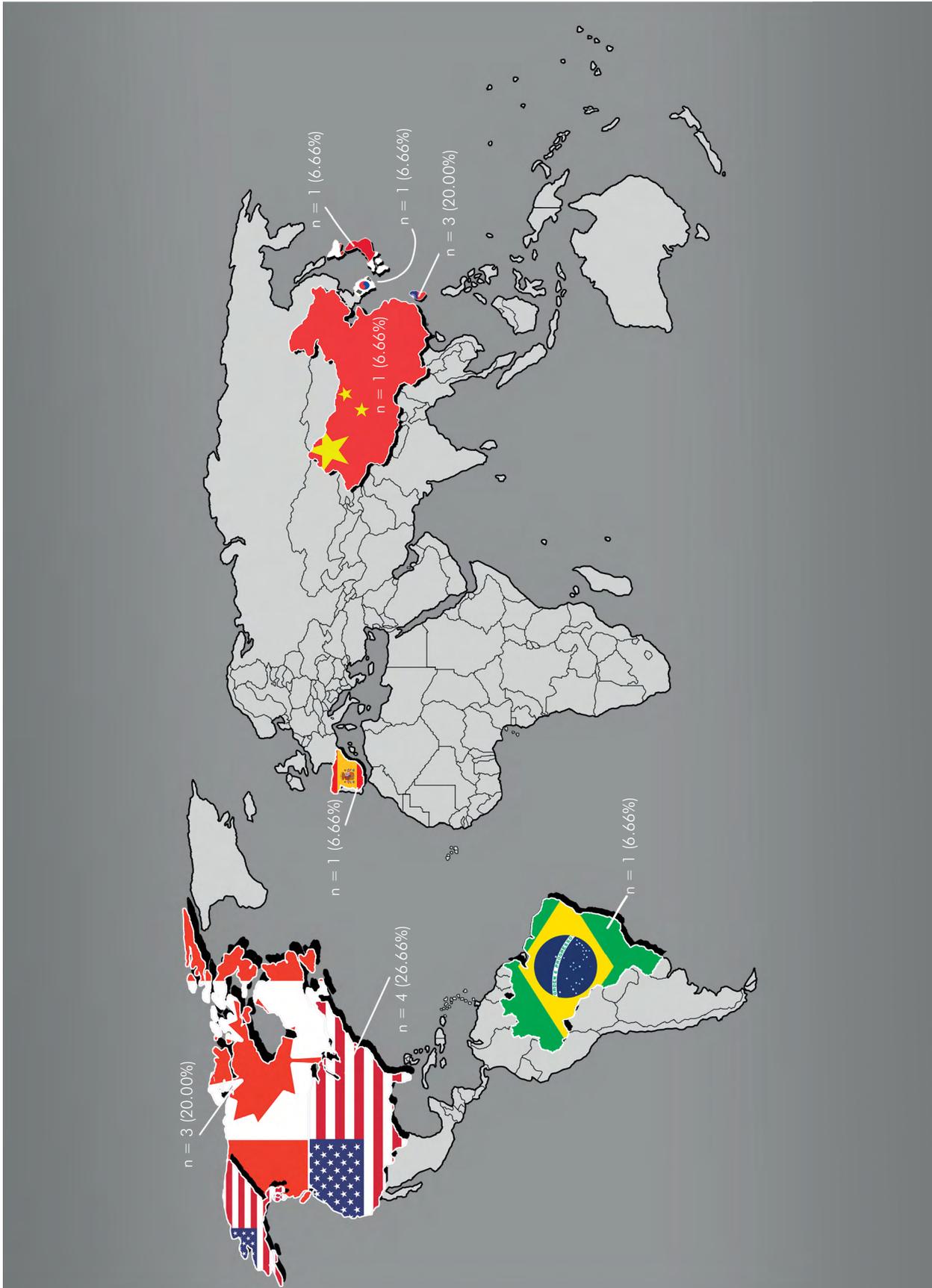


Figure 2. Distribution of works according to the country in which they were developed.

Table 2. Main characteristics of the eligible studies.

Authors (year)	Virus type	Interventions	Virus carrier/contact surface	Method of assessing viral inactivation	Main results
Sato, Wananabe, Miyata, 1990 ²⁴	TMEV	Ozone	Dry phase: Lyophilized virus samples in glass vials were exposed to ozone	Plaque assay	Effective: More than 100 ppm ozone and 80% of RH was strongly virucidal
	HVJ	Control (no treatment)	Liquid phase: A portion of a hundredfold virus sample in 35-mm dishes		
Tseng, Li, 2006 ³²	RV	Ozone exposed Ozone unexposed	Virus stock solutions were diluted in sterile, deionized water for nebulization; 3% gelatin plates were used to collect virus-containing aerosols before and after ozone treatment.	Plaque assay	Effective: The survival fraction of all four viruses decreased exponentially with increasing ozone dose
	MHV				
	MS2				
	φ6				
Hudson et al., 2007 ²⁵	Norovirus and its animal surrogate feline calicivirus	Ozone exposed	Virus samples were dried on sterile plastic or other surfaces (fabrics and carpet, cotton tips, plastic)	Plaque assays	Effective: Substantial inactivation of FCV and NV samples was achieved, with a comparable reduction in RT-PCR values, indicating that infectivity of both viruses would be similarly affected if it were possible to assay for NV infectivity.
		Ozone unexposed		RT-qPCR assay	
Lin et al., 2007 ²³	Enterovirus 71	Ozone Control (no treatment)	Virus stock in glass dishes	Plaque assay	Ineffective: No statistically significant differences in cell viability were noted among the control group and 0.5 or 1 ppm ozone exposed infected cells (Fig. 3B). The 1.5 or 2 ppm-exposed cells had 45–40% viability.
Tseng, Li, 2008 ²¹	MS2 φ6 φX174 T7	Ozone exposed Ozone unexposed	A diluted culture of virus stock solution was spread on the surface of gelatin-based medium.	Plaque assay	Effective: The survival fraction of all four viruses decreased exponentially with increasing ozone dose
Hudson et al., 2009 ⁷	Influenza	Ozone exposed	Aliquots of virus, diluted when necessary in PBS, were spotted onto glass slides, stainless steel circular disks, and pieces of fabric and cotton.	Plaque assays	Effective: All viruses tested, showed similar kinetics of virus inactivation on three hard surfaces, plastic, glass and stainless steel. The combination of ozone gas plus high RH consistently yielded substantial inactivation.
	HSV	Ozone unexposed			
	Rhinovirus				
	Adenovirus				
	Mouse coronavirus				
	Sindbis virus				
	Yellow fever virus				
	Vesicular stomatitis virus				
Poliovirus					
Cannon, Kotwal, Wang, 2013 ²⁰	Murine norovirus (MNV-1)	Ozone	FCV or MNV-1 stocks were spread uniformly onto glass Petri dishes using a cell scraper. One uninoculated Petri dish was also included in each experimental replicate and served as a negative control.	Plaque assays	Effective: exposure of two norovirus surrogates to 20 ppm atmospheric ozone for 18 min and 80% of RH significantly reduced virus infectivity on smooth glass surfaces.
	Feline calicivirus (FCV)				

Continue

Continuation

Authors (year)	Virus type	Interventions	Virus carrier/contact surface	Method of assessing viral inactivation	Main results
Petry et al., 2014 ²²	Herpes Simplex Virus 1 (HSV-1) Bovine Herpes Virus 1 (BoHV-1)	Ozone Control (no treatment)	Aliquots of HSV-1 and BoHV-1 propagated in MDBK cells were added to 35-mm Petri dishes.	Plaque assay	Effective: Ozone promoted a significant reduction of more than 90% of viral replication for both viruses tested after 3 h of exposure.
Guo et al., 2015 ²⁶	Hepatitis B	Formaldehyde oxidization fumigation Ozone	Serum was collected from HBV-infected people with a HBV DNA copy number of 10 ⁷ copies/ml. The serum was diluted 10-fold with sterile distilled water and added to cloth. Negative control groups were composed of sterile distilled water samples.	RT-qPCR	Ineffective: Application of ozone to disinfect HBV-contaminated hospital linen was ineffective.
Nayak et al., 2018 ²⁹	FCV	Ozone Control (no treatment)	Gas-phase: Sterile stainless steel discs were placed in wells of a 24-well microtiter plates. The surface of each disc was spiked with 15 µl of FCV. Liquid-phase: Aliquots of FCV were added to 96-well plates.	Plaque assay	Effective: Gas-phase FCV inactivation: Complete inactivation was achieved within 3 min of treatment for the humidified biosamples at 1 cm distance from the discharge; 1 cm is similar to 40 cm. Liquid-phase FCV inactivation was also effective: Significant reduction in FCV titer was achieved by treating sterile water for 5 min at 1 cm in dry air.
Cía et al., 2020 (preprint) ³³	Lentiviral vector	UV-C light Ozone	Open Petri dishes containing pSIN-GFP lentivector stock in DMEM and containing dried bacterial cultures inside an ambulance.	Fluorescence microscopy Flow cytometry Plaque assays	Ineffective: Ozone treatments currently applied in emergency vehicles in this study did not significantly affect virus or bacteria viability.
Blanchard et al., 2020 (preprint) ²⁸	Influenza A Human respiratory syncytial	Ozone 70% ethanol	Virus solutions in growth medium were deposited by pipette onto pieces of each candidate material: cloth face masks, Tyvek (spun high-density polyethylene) fabric used in disposable gowns and PAPR (powered air purifying respirator) hoods, and N95 respirators. Uninoculated samples of each material served as a negative control.	RT-qPCR Plaque assay NanoLuc	Effective: Ozone treatment at 20 ppm or greater, 70% or greater RH at room temperature, and for at least 40 minutes should reliably inactivate enveloped viruses on a variety of materials used for medical PPE.
Dubuis et al., 2020 ³¹	φ6 φX174 PR772 MS2 MNV-1	Ozone	The virus buffer was placed in an aerosol generator and nebulized for 10 minutes.	Plaque assays qPCR	Effective: 40 minutes and 55% of RH of exposure was required for φX174 and MS2 inactivation. 10 minutes and 85% RH for φX174, PR772 and MS2. φ6 and MNV-1 viruses showed inactivation levels of at least two orders of magnitude after 40 minutes.

Continue

Continuation

Authors (year)	Virus type	Interventions	Virus carrier/contact surface	Method of assessing viral inactivation	Main results
Lee et al., 2021 ²⁷	Human coronavirus HCoV-229E	Ozone	HCoV-229E culture was added to face masks.	Plaque assays RT-qPCR	Effective: When face masks experimentally contaminated with a human coronavirus (HCoV-229E) as a surrogate were exposed to ozone gas (approximately 120 ppm) produced by the plasma generator for either 1 or 5 min, the virus lost infectivity.
Westover et al., 2020 (preprint) ³⁰	Twist Synthetic SARS-CoV-2 RNA Control 2	Ozone Control (no treatment)	Aliquots of Twist Synthetic SARS-CoV-2 RNA were added to open tubes in the Sani Sport Supreme.	qPCR r	Effective: Synthetic SARS-CoV-2 RNA was shown to undergo significant degradation for 1 hr under ozone treatment. Ozone-treated samples exhibited 65.13%, 25.82%, 11.24%, 12.46% and 6.16% of RNA remaining for 30 mins, 1 hr, 2 hr, 3 hr, and 4 hrs. RNase-treated samples showed complete degradation.

Discussion

Our search identified articles with sufficient evidence for a survey of the effectiveness of ozone gas for inactivating some vehicle-borne viruses. This scoping review provides useful insight for the design, implementation, and effectiveness of ozone gas applications for virus inactivation on surfaces and objects under different conditions, including health services.

The outbreak of SARS-CoV-2 has reinforced the need to develop methods for disinfection, especially those applicable to health care environments. Several methods have been proposed, such as aerosolized hydrogen peroxide, H₂O₂ vapor, ultraviolet C light, pulsed xenon, and gaseous ozone.³⁵ Among these, the efficient penetrability of a gas allows the decontamination of inaccessible locations and disinfection of much more than just surfaces, such as crevices, fixtures, and the undersides of furniture. In addition, air treatment should be considered to reduce the infectivity of airborne diseases.⁷ Thus, several devices that generate ozone for this purpose have been developed in recent months.

To narrow the search to microorganisms (or their surrogates) commonly transmitted in health care

environments, vehicle-borne and airborne viruses were considered in this scoping review. Thus, studies involving sewage treatment, decontamination in the food and agriculture fields were excluded. Disinfection with ozone gas was effective against many types of viruses with or without an envelope, and decreased virus infectivity was reported in most of the studies (80%). Gaseous ozone was efficient in reducing the infectivity of several common viruses acquired in hospital settings, such as norovirus,^{7,20,31} influenza,^{7,28} and RSV.²⁸

Such effectiveness has also been recently demonstrated for SARS-COV2 biosafe surrogate viruses. As handling of SARS-CoV-2 requires biosafety level (BSL) 3 facilities,³⁶ surrounding viruses are commonly used to facilitate research. Twist synthetic SARS-CoV-2 RNA,³⁰ HCoV-229E,²⁷ influenza A virus (IAV; strain A/WSN/33) and human respiratory syncytial virus (RSV; strain A2)²⁸ are considered biosafe substitutes for SARS-COV-2 due to their similarity in form, structure and function. Although the analyzed studies employed surrogates for SARS-CoV-2, some^{21,24,32} published before the pandemic showed the efficiency of ozone gas application against viruses that are actually considered surrogates for SARS-CoV-2, such as MHV (mouse hepatitis virus)

Table 3. Ozone application parameters.

Authors (year)	Ozonation method	Ozone concentration (ppm)	Temperature (°C)	Humidity (%)	Time evaluated (min)
Sato, Watanabe, Miyata, 1990 ²⁴	Ozone Generator (Elios Ozonizer, Shinryo. Reinetsu. Co. Tokyo. Japan)	HJV samples: 200 TMEV samples: 100 and 200	22–25	50–90	30 mi
Tseng; Li, 2006 ³²	Ozone generator (OZ1PCS-V/SW, Ozotech Inc., Yreka, CA)	0.1–10	25–28	55–85	13.8 sec 18.4 sec
Hudson et al., 2007 ²⁵	Multiple corona discharge units (Viroforce 1000; Viroforce Systems, Kelowna, BC, Canada)	20–25	23	70 in excess	20
Lin et al., 2007 ²³	Ozone Generator (Tenco, XV1043CA, Taiwan)	0.5, 1, 1.5; 2	25		120 5 10 15 20 40 60 90 120
Tseng; Li, 2008 ²¹	Ozone generator (OZ1PCS-V/SW, Ozotech Inc., Yreka, CA)	0.6; 0.9; 1.2	25–28	55–85	20 40 60 90 120
Hudson et al., 2009 ⁷	Ozone generators (Treated Air Systems) for the initial field trials Multiple corona discharge units (Viroforce 1000; Viroforce Systems, Kelowna, BC, Canada)	25	20	40 > 90	10 (RH tests > 90%) 60
Cannon, Kotwal, Wang, 2013 ²⁰	Ozone generator (ZONOSanitech, Alpharetta, GA).	20	Room temperature	80	18 60
Petry et al., 2014 ²²	Ozone generator (Brizzamar, Ronda Alta, RS, Brazil)	0.02– 0.05	26.2– 29.2	30–37	120 180 15
Guo et al., 2015 ²⁶	Computer-controlled bed unit ozone sterilizer (Kz-x-dL1, Guangdong Kangzhen Medical Equipment Co. Ltd., Guang-dong province, China)	150*			30 60 80
Nayak et al., 2018 ²⁹	Dielectric barrier discharge	20			5 20
Cía et al., 2020 ³³	Ozone generator (Industrial Global Supply S.L.)	Higher than 10		37–48	10 20
Blanchard et al., 2020 ²⁸	Global Ozone Decon-Zone 4201A Cabinet// Global Ozone OT-100 Trailer//Zono SC 1 Cabinet// VirtuCLEAN 2.0 Waterless CPAP Cleaning Pouch	Approximately 20	Room temperature	40 50–70 80	160 320
Lee et al., 2020 ²⁷	Dielectric barrier discharge	Approximately 120	25	--	1 5
Dubuis et al., 2020 ³¹	Ozone generator (model EMO3-VTTL, EMO3, Quebec City, CANADA)	Phages: 1.13 ± 0.26 MNV-1: 0.23 ± 0.03		20% 55% 85%	10 40 70 30 60
Westover et al., 2020 ³⁰	Ozone generator (Sani Sport Supreme)	20			120 180 240

*Conversion 1 ppm = 0.002 mcg/ml

Table 4. Sponsorship status of the studies.

Authors (year)	Sponsorship status		
	Sponsored	Nonsponsored	Unclear
Sato, Wananabe, Miyata, 1990 ²⁴			x
Tseng, Li, 2006 ³²			x
Hudson et al., 2007 ²⁵			x
Lin et al., 2007 ²³			x
Tseng, Li, 2008 ²¹			x
Hudson et al., 2009 ⁷			x
Cannon, Kotwal, Wang, 2013 ²⁰			x
Petry et al., 2014 ²²			x
Guo et al., 2015 ²⁶		x	
Nayak et al., 2018 ²⁹		x	
Cía et al., 2020 ³³		x	
Blanchard et al., 2020 ²⁸		x	
Dubuis et al., 2020 ³¹		x	
Lee et al., 2021 ²⁷		x	
Westover et al., 2020 ³⁰		x	

and Phi6.⁴ Dubuis et al.³¹ demonstrated the effect of ozone as a disinfectant against multiple phages with different features to represent a broad range of eukaryotic viruses and their resistance when airborne and when exposed to disinfecting agents.

Nevertheless, three studies^{23,26,33} showed evidence that gaseous ozone was ineffective against specific types of viruses. For example, Cía et al.³³ found that gaseous ozone treatments applied for emergency vehicles do not significantly affect virus viability; in this study, the effect of an ozone cannon device (10 ppm) against HIV-1-derived lentivectors inside an emergency vehicle was investigated. Guo et al.²⁶ demonstrated that the application of ozone (140 ppm) to disinfect HBV-contaminated hospital linen was also ineffective, even when the disinfection time was prolonged to 80 min. Lin et al.²³ observed approximately 85% viability for cells exposed to 1.5 and 2 ppm ozone, and increasing the ozone concentration to 2.5 and 3 ppm achieved 40 and 50% cell mortality with 1 and 2 h exposure, respectively. Interestingly, the study involving the emergency vehicle³³ used low RH (37%–48%), but the other studies^{23,26} did not mention the RH of the experiment. The literature is clear regarding the role RH plays in the disinfection process using ozone.³⁷ Blanchard et al.²⁸ suggested

that humidity may act through two mechanisms: (a) water may promote the generation of highly reactive hydroxyl radicals from ozone, and (b) greater humidity may facilitate ozone interacting with surface-bound species, such as by diffusion, to reach viruses. Therefore, the evidence of this scoping review found optimum efficacy of ozone treatment under high RH (> 50%).

It is not appropriate to determine the effectiveness of a specific disinfection method by relying only on the resistance of the virus being analyzed. Indeed, each virus has different tolerances for various protocols.³¹ The ozone generation mechanism may also affect results. Ozone is obtained by transforming oxygen via electrical discharges, and there are three different systems exist for generating ozone gas: ultraviolet, plasma or corona systems. The ozone gas produced by ultraviolet light is obtained at low concentrations and acts as a secondary product complementary to the UV effect. The cold plasma system is very commonly used to purify water and air in public environments, and the corona discharge system, which is mainly used in health care settings, produces ozone more effectively and at a greater concentration. The mechanism of artificial ozone production occurs through electrochemical discharge known as the corona effect. Another advantage of this method is that it can be applied in water.³⁸

The type of material or virus carrier applied varied among the studies, which did not allow for direct comparisons. The experimental setup and methodology used most commonly were virus stock spread on glass dishes,^{7,20,22,23,24,33} stainless steel disks,²⁹ or gelatin medium²¹ and allowed to dry before ozone exposure. In general, disinfection of hospital equipment was the main focus after the SARS-COV2 outbreak. Disinfection of face masks,^{27,28} Tyvek (spun high-density polyethylene) fabric used in disposable gowns and PAPR (powered air-purifying respirator) hoods²⁸, N95 respirators,²⁸ and samples of fabrics and carpet²⁵ has been demonstrated. No substantial loss of filtration performance was found for ozone-treated masks^{27,28}. Thus, in times of shortages, ozone treatment has proven to be an effective method for disinfecting personal protective equipment (PPE) for safe reuse.

Viral quantification can be performed by the standard plaque assay method³⁹ or viral titration for quantitative assay of the infectivity of virus recovered in monolayer cells of selected bacterial strains as hosts^{23,40,41,42}. RT-PCR is another quantification method that measures a defined sequence of the viral genome; as one would expect this method be more resistant to the damaging effects of ozone gas than infectivity, it probably underestimates the effectiveness of antiviral agents. Thus, it would be worthwhile to use two methods to analyze infectivity and viral degradation. In fact, the importance of these two methods of quantification was clear in the study of Lee et al.²⁷ In this experiment, no viable HCoV-22E was recovered from face masks, and the virus was shown to lose infectivity in a human cell line (MRC-5) when exposed for a short period of time (1 min) to ozone gas produced by a DBD plasma generator. However, the short exposure may not fully degrade the viral RNA because no significant difference in the amount of amplifiable RNAs between treated and untreated masks was observed. These results suggest that loss of infectivity could be due to damage to the viral envelope or envelope proteins, resulting in failure of the virus to attach to host cells.

The concentration of ozone required for efficacy also depends on the exposure time. The ozone concentration in the majority of the studies included in this review was several magnitudes higher than the exposure limits defined for Occupational Safety and Health regulations. For this reason, most of them were performed in a proper chamber or cabinet. The major disadvantage of the use of ozone is its potential toxicity at high concentrations; thus, precautions must be taken to avoid such exposure. In general, limits for workers are <0.1 ppm or 0.2 mg/m³ on average over 8 h, <0.3 ppm if the exposure time is only 15 min; PPE is needed if the concentration is >0.3 ppm. Challenges can be overcome by ensuring that the area is emptied of staff, closed off, and sealed.³⁷ Ozone gas decays quickly to oxygen, with a half-life of approximately 20 min. Another plausible option is the use of a catalytic converter (scrubber) near closed doors and inside them to accelerate ozone degradation and avoid leakage. Ozone concentrations below 0.1 ppm may be feasible for treating air inside unoccupied hospital rooms.³¹ More studies using

lower concentrations but longer exposure periods to treat air in an unsealed unoccupied room should be performed to avoid worker and patient toxicity.

It should also be noted that most studies used ozone generators with industrial and domestic applications that obtain ozone from ambient air. Such equipment produces ozone but can also release other compounds derived from gases present in the atmosphere. Similar to ozone, the concentration of these contaminants in air varies according to temperature and air humidity.^{43,44} It is important to note that there is a possibility that such devices produce ozone concentrations higher or lower than those indicated by the manufacturer, which can also affect results. Thus, such rates and concentrations should be measured.⁴⁵ Devices that use sterile oxygen for ozone generation, on the other hand, will not only provide byproducts free of such gases and compounds but will also have effective flow regulation.⁴³ Future studies should be carried out to detect compounds generated by domestic and industrial generators, as well as the exact concentrations of the generated ozone in both systems. Comparative studies between generators that use ambient air and medicinal oxygen should also be performed.

Another limitation was possible sponsorship bias present among the selected studies. Most of the studies^{7,20-25,32} were classified as unclear with regard to sponsorship status. Authors did not state whether they received any type of funding or provision of devices from companies related to ozone generators, and they did not mention no conflict of interest. Sponsorship from companies, mainly in studies evaluating specific devices or products, might lead to bias. Risks of publication, reporting and selection biases as well as publication of selected results have been associated with industry sponsorship.^{18,46} Therefore, the results of this study should be interpreted with caution, and future studies should be performed to assess the presence of sponsorship bias among studies evaluating the effectiveness of ozone generators as disinfecting agents.

Conclusion

Our findings suggest that ozone should be considered as an effective method to decrease the infectivity of several viruses commonly acquired

inside hospitals and other healthcare environments. This scoping review provides direction for a future systematic review to investigate not only the effectiveness but also a better protocol for the use of ozone as a disinfectant or sterilization method for environments and surfaces, thus contributing to disease prevention in both emergency situations and pandemics, as well as in daily routines.

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