



Nota tecnica:

Uso sicuro ed efficace dell'ozono per la sanitizzazione di aria e superfici in combinazione con il COVID-19

Il presente documento estrapola i dati quantitativi per l'attività virucida dell'ozono sulla base delle informazioni disponibili della letteratura scientifica per casi d'uso di misurazioni in sicurezza sviluppate sotto lo stimolo della situazione generatasi dall'attuale emergenza sanitaria.

L'ozono è un potente ossidante che reagisce con molecole organiche e quindi ha una funzione battericida, virucida e fungicida. Allo stesso tempo, si tratta di una sostanza tossica, che ha effetti negativi sulla salute e la sicurezza.

Il suo uso è proposto per la disinfezione dei luoghi di lavoro e dei luoghi pubblici nei quali è necessario provvedere alla sanitizzazione di aria e superfici con particolare riferimento ad attrezzature di lavoro e dispositivi di protezione personali per il riutilizzo (ivi comprese maschere monouso), con particolare riferimento all'epidemia da COVID-19.

L'ozono può essere generato in situ mediante generatori di ozono compatti, che utilizzano l'aria presente in ambiente "essicata" come precursore. Dovrebbe essere iniettato nel locale da disinfettare fino al raggiungimento della concentrazione di ozono desiderata;

dopo il tempo necessario per la disinfezione, le sue concentrazioni devono essere ridotte ai livelli richiesti per la sicurezza dei lavoratori. L'uso ottimale dell'ozono è per la disinfezione dell'aria e della superficie senza presenza, utilizzando una concentrazione che è effettiva per la distruzione di virus, ma non abbastanza alto per deteriorare i materiali.

Le concentrazioni utilizzate per i cicli di trattamento delle apparecchiature OZY sono le seguenti:

- VIRUS: concentrazione di 4,2 PPM per un tempo di 20 minuti - *ciclo completo della durata di circa 40/60 minuti*
- BATTERI: concentrazione di 2,2 PPM per un tempo di 40 minuti - *ciclo completo della durata di circa 60/80 minuti*
- FUNGHI: concentrazione di 2 PPM per un tempo di 40 minuti - *ciclo completo della durata di circa 60/80 minuti*
- INSETTI: concentrazione di 5 PPM per un tempo di 60 minuti - *ciclo completo della durata di circa 60 minuti*

Technical Note

Safe and Effective Use of Ozone as Air and Surface Disinfectant in the Conjunction of Covid-19

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Abstract: The present paper extrapolates quantitative data for ozone virucidal activity on the basis of the available scientific literature data for a safe and effective use of ozone in the appropriate cases and to explore the safety measures developed under the stimulus of the current emergency situation. Ozone is a powerful oxidant reacting with organic molecules, and therefore has bactericidal, virucidal, and fungicidal actions. At the same time, it is a toxic substance, having adverse effects on health and safety. Its use is being proposed for the disinfection of workplaces' and public places' atmosphere, and for disposable masks and personal protective equipment disinfection for reuse, with particular reference to the COVID-19 pandemic outbreak. Ozone can be generated in situ by means of small, compact ozone generators, using dried ambient air as a precursor. It should be injected into the room that is to be disinfected until the desired ozone concentration is reached; after the time needed for the disinfection, its concentrations must be reduced to the levels required for the workers' safety. The optimal use of ozone is for air and surface disinfection without human presence, using a concentration that is effective for the destruction of viruses, but not high enough to deteriorate materials.

Keywords: SARS-CoV-2; ozone generators; sanification; disinfection; virucidal efficacy; occupational exposure; personal protective equipment

1. Introduction

The Report ISS Covid-19 n. 25/2020 [1] issued on May 2020 by the Italian Institute of Health (ISS), reports recommendations on the sanification of non-healthcare settings during the COVID-19 emergency. In this document, the term "sanification" is intended as the process of cleaning and/or disinfecting and maintaining good air quality, taking into account scientific evidence of COVID-19 virus persistence on different surfaces, and the efficacy of cleaning and disinfection products for indoor environments. Ozone is evaluated, among other disinfectants, on the basis of the available literature and of the statements of internationally recognized organizations. European Chemical Agency (ECHA), Centers for Disease Control and Prevention (CDC), Food and Drug Administration (FDA), United States—Environmental Protection Agency (US-EPA) and the International Ozone Association (www.iao-pag.org) confirm the effectiveness of ozone for the inactivation of many viruses, although without being aware of specific research on SARS-CoV-2.

Ozone, an allotropic form of oxygen, is an inorganic gas (CAS n. 10028-15-6) constituted by three oxygen atoms (O_3) arranged in a bent structure, where the distance among oxygen atoms is 1.26 Å. It easily decomposes into oxygen (O_2) and one single, very reactive oxygen atom. Ozone is present in nature and its concentration in the atmosphere is approximately 0.04 parts per million (ppm: 1 ppm \sim 2 mg/m³). It is also produced by ultraviolet irradiation of oxygen or other precursors such as volatile organic compounds and nitrogen oxides present in the atmosphere. About 90% of the atmospheric ozone is located in the stratosphere, and therefore called stratospheric ozone. The solubility of ozone in water is 49.0 mL/100 mL (at 0 °C), tenfold than oxygen, thus causing an immediate reaction with any biomolecule in biological fluids. Its density (2.14 kg/m³) is higher than that of air, letting it concentrate close to the ground in indoor environments.

Ozone is well known for its protective role in the earth's ecological environment. Being a powerful oxidant, it is able to react with organic molecules containing double or triple bonds. This is the origin of its bactericidal, virucidal, and fungicidal actions, which are used in water treatment, odor control and medicinal applications [2].

The inhibitory and lethal effects of ozone on pathogenic microorganisms have been observed since the 19th century, and the most cited explanation of these effects is based on the disruption of their envelopes through peroxidation of phospholipids and interaction with proteins [3]

The ozone virucidal effect has been also studied by Roy et al. in 1981 [4], showing that after 30 s of exposure, 99% of the viruses were inactivated: their envelope proteins had been damaged, causing possible failure of attachment to normal cells, and their single-stranded RNA was broken.

Viruses are small particles built of macromolecules; unlike bacteria, they need a host cell to multiply, and being unable to repair oxidative damage, they are more susceptible to oxidative action than prokaryotic or eukaryotic organisms [5].

Ozone interacts with viruses in which a lipoprotein envelope is present, by spreading through the protein coat into the nucleic acid core, damaging their DNA or RNA [6–8]. Most studies on ozone's virucidal effects have focused on ozone's ability to break apart lipid molecules with multiple bonds. In fact, if the envelope of the virus is fragmented, its DNA or RNA core cannot survive. The enveloped viruses are usually more sensitive to physical–chemical challenges than naked ones.

Naked viruses only have a nucleocapsid, which is a protein capsid that covers the nucleic acid core (DNA or RNA). Even if ozone's effect on unsaturated lipids is one of its best-documented biochemical actions, it can interact with proteins, carbohydrates, and nucleic acids [7]. Due to its high reactivity, the toxic effects of ozone reaction products should also be considered.

Manufacturers and sellers of ozone devices use a variety of terms that suggest that ozone is a "healthy" kind of oxygen, but ozone is a toxic gas with very different properties from oxygen, and concentrations that are safe to breathe are unlikely to be effective in controlling indoor air pollution [8].

A comprehensive evaluation of ozone human health effects was recently performed by the US EPA, as part of the "Integrated Science Assessment for Ozone and Related Photochemical Oxidants" [9]. According to this report, recent studies support and expand upon the strong body of evidence that short-term ozone exposure causes respiratory effects. The strongest evidence comes from controlled human exposure studies demonstrating ozone-induced decreases in lung function and inflammation in healthy, exercising adults at concentrations as low as 60 ppb after 6.6 h of exposure. In addition, epidemiologic studies continue to provide strong evidence that ozone is associated with respiratory effects, including asthma and chronic obstructive pulmonary disease exacerbations. The available evidence was inadequate to determine whether there was a causal relationship between exposure to ambient ozone and cancer.

Currently, in Italy, ozone can be used exclusively as a sanitizer. It is presently being reviewed by the European Environmental Agency and/or Switzerland for use as a biocide, in disinfection, food and animal's feeds, drinking water, and as a preservative for liquid systems, under the Biocidal Products Regulation (BPR) of ECHA.

The objective of this study was to conduct a literature review on the ozone virucidal efficacy in order to individuate the optimal conditions (concentration, contact time, microclimate) for its possible use as disinfectant for indoor environments and Personal Protective Equipment (PPE), with particular reference to SARS-CoV-2. In addition to this, some critical aspects that currently prevent the use of this gas as a disinfectant, including safety risks and the lack of Indicative Occupational Exposure Limit Values (IOELVs) at European Union level, were further explored.

2. Methods

2.1. Literature Search

A literature review, started on June and ended November 2020, was carried out using PubMed, Scopus and Google Scholar databases. A first search was conducted to identify publications dealing with the ozone virucidal activity under different experimental conditions, as gas concentration, contact time and microclimatic parameters. Articles were identified using the following keyword combinations: (“ozone” OR “ozone generator” OR “gaseous ozone”) AND (“disinfectant” OR “disinfection” OR “sanitation” OR “efficacy” OR “treatment” OR “activity”) AND (“coronavirus” OR “COVID-19” OR “SARS-CoV-2” OR “SARS-CoV-1” OR “viruses”).

In the same databases, another search was performed in order to find articles regarding the use of ozone as a disinfectant for the safe reuse of PPE and the possible damage caused to materials. For this purpose, the following search string was used: (“PPE” OR “Personal Protective Equipment” OR “N95 Mask” OR “KN95 Mask” OR “Filtering Face Piece” OR “FFP” OR “gowns” OR “gloves” OR “glasses” OR “shields” OR “goggles”) OR (“rubber” OR “polyethylene” OR “polypropylene” OR “cotton fabric”).

Publications titles and abstracts were reviewed and only those that met the criteria described above were eligible for the inclusion. Documents on the use of ozone in aqueous solution or those about ozone therapy for patients were not taken in consideration. Only articles written in English were included.

2.2. Metrics for Airborne Microorganisms Inactivation

By examining the scientific literature about ozone virucidal efficiency, the ozone concentration values that were able to inactivate viral microorganisms at normal ambient temperature and relative humidity, in a certain exposure time, were extracted.

Normalized infectious ratios (NIRs) were calculated by dividing the mean sample plaque-forming unit (PFU)/mL by the mean control PFU/ml and the mean control genomes/ml by mean sample genomes/ml. Results were multiplied together. NIRs were calculated both for ozone and for the reference (air) conditions.

Lastly, relative infectious ratios (RIRs) were obtained by dividing each ozone-NIR with the corresponding median air-NIR.

$$\text{RIR} = \text{NIR O}_3 / \text{median [NIR air]} \quad (1)$$

The RIRs represent the effect of ozone only, since data were corrected for the effect of relative humidity (RH) and aerosol aging without ozone [10].

3. Results

3.1. Ozone Virucidal Efficacy

A total of 109 documents were retrieved in databases using all the above-mentioned keywords and search strings, but only 17 papers were eligible for inclusion. The review of the existing scientific literature demonstrates that ozone is an extremely rapid and potent virucidal; however, exact gaseous

ozone dosages are limited to only a few studies, as virucidal action of ozone happens in seconds or fractions of seconds and this makes the measurement of viral inactivation technically challenging [3].

The data available in the literature do not cover all virus types. In some studies, the virucidal activity of ozone was tested on pathogenic viruses of particular clinical interest such as Poliovirus, Norovirus, Herpes Simplex Virus and Influenza virus [4–6,10]; in others, instead, the bacteriophages were used in place of airborne human pathogenic viruses [8].

Hudson [5] developed a prototype ozone generator (Viroforce 1000) that contains eight corona discharge units, and that subsequently removes ozone by a built-in catalytic converter. In this study, the maximum anti-viral efficacy was obtained at a concentration of 25 ppm of ozone for 15 min, followed by a short period of >90% relative humidity. All the 12 viruses tested were inactivated by at least three orders of magnitude, both in laboratory and in simulated field trials, on different hard and porous surfaces, and in the presence of biological fluids. Viral activity was tested through cellular culture.

Inactivation of herpes simplex virus (HSV) by ozone was studied by Petry et al. [7], generating ozone by a commercial air purifier (Brizzamar, Ronda Alta, RS, Brazil). The ozone concentration in the environment was monitored through the EcoSensor Model OS-4 (Ozone Switch TM, Newark, CA, USA). With an ozone exposure of 1–3 h at concentrations between 0.02 and 0.05 ppm, a 68–90% reduction in viral activity tested through cellular culture was obtained.

Dennis [11] tested five commercial ozone generators used to reach the target ozone concentration in a box for DPI regeneration. The antiviral efficacy was guaranteed at 10–20 ppm for 10 min, based on the results of other papers.

Tseng and Chihshan [12] studied the inactivation of four different viruses, representative of ssDNA, ssRNA, dsDNA, and enveloped dsRNA categories, using an ozone generator with pure oxygen at 3 L/min (OZ1PCS-V/SW, Ozotech Inc., Yreka, CA, USA). Airborne Ozone concentrations were measured by an ozone analyzer (model 401, Advanced Pollution Instruments, San Diego, CA, USA), with a detection limit of 1.0 ppb. Ozone levels of 0.6–1.2 ppm for 20–112 min time guaranteed 90% and 99% of inactivation, respectively. For all the tested viruses, the ozone concentration required for the same inactivation level was lower at 85% RH than at 55%.

Zhang et al. [13] demonstrated that ozone in aqueous solution is able to inactivate SARS-CoV-1, the etiologic agent responsible for the Severe Acute Respiratory Syndrome (SARS) spread in 2003, which has a very similar structure to the SARS-CoV-2 virus, causing the current COVID-19 pandemic. Afterwards, Hudson et al. [5] tested the virucidal action of ozone on murine coronavirus (MCV), a species of coronavirus which infects mice, commonly used as a surrogate for SARS-CoV-1.

As SARS-CoV-2 is an enveloped virus, particularly sensitive to oxidant action, the scientific evidence suggested that ozone would effectively inactivate this new coronavirus too.

Moreover, some studies [8,12,14] showed that the important factor for inactivation of viruses and other microorganisms is the total ozone dose, which can be calculated as the product of exposure time and concentration. In fact, low concentrations of ozone for longer exposure times produce the same results as shorter exposures at high concentrations.

Dubuis ME et al. [10] tested the efficacy of ozone and RH combinations for the inactivation of different airborne viruses. Four phages (ϕ X174, PR772, MS2 and ϕ 6) and one eukaryotic virus (murine norovirus MNV-1) were exposed to low ozone concentrations (1.23 ppm for phages and 0.23 ppm for MNV-1) at different levels of RH, for time intervals ranging from 10 to 70 min. A two orders of magnitude inactivation was achieved for ϕ X174, MS2 and MNV-1 with an ozone exposure of 40 min at 85% RH. For PR772 and ϕ 6, exposure to the reference condition at 20% RH for 10 min gave the same results. In according to previous studies [15], these results suggest that ozone, even when used at a low concentration, is a powerful disinfectant for airborne viruses and other microorganisms if combined with a high RH level. It is well known that ozone is an oxidizing agent in aqueous solutions, and when in the gas phase, reacting with water, it generates free radicals that increase its disinfection power.

Tseng and Li [8] also observed that the phages inactivation increased when high 85%RH was used and further studies demonstrated that ozone under high RH conditions leads to the formation of more free radicals than in dry air [14,16].

In another study, Blanchard et al. [17] showed that a concentration of 20 ppm of ozone is able to inactivate some enveloped respiratory viruses (influenza A virus and respiratory syncytial virus) that are very similar in form and function to SARS-CoV-2. The authors performed a series of experiments under different relative humidity conditions, demonstrating that, at 50–70% RH, the ozone treatment was highly effective (99.99% reduction in viral infectivity) because of a greater production of highly reactive hydroxyl radicals. Therefore, they conclude that the humidity is an important parameter to evaluate, because, under rather dry environmental conditions, the disinfection procedure could require considerably longer exposure times.

More recently, Yano et al. [18] reported the first results about the inactivation of SARS-CoV-2 using ozone. In this study, the viral load of SARS-CoV-2 (JPN/TY/WK-521 strain) was determined by using the plaque assay on confluent layers of VeroE6/TMPRSS2 cells in culture plates. Cell monolayers were allowed to dry before exposure to ozone at concentrations of 1.0 and 6.0 ppm, for 60 and 55 min, respectively. Viral infection assays were performed at room temperature (25 °C) and 60–80% RH and the results expressed in PFU/mL. At 6.0 ppm of ozone for 55 min, results showed a significant reduction in the number of plaques (1.0×10^3 PFU/mL) compared to control (2.0×10^6 PFU/mL). Therefore, authors suggest that it is plausible to believe that at slightly higher concentrations and for shorter periods, this gas can be used in particularly critical environments (healthcare setting, hospices, etc.) where a rapid and effective disinfection is required. Table 1 shows the results of ozone virucidal efficacy on some pathogenic viruses including SARS-CoV-2.

Table 1. Contact times and ozone concentrations needed for 90% inactivation of different viruses.

Ozone Concentration (ppm)	90% Inactivation Time (min)	Relative Humidity	Viruses	Reference
25	15	>95% after cycle	12 different viruses	[5]
0.05	180	35%	Herpes	[7]
10.33	0.3	55%	4 kind: ssDNA, ssRNA, dsDNA, Enveloped dsRNA	[8]
1.23	70	55%	4 phages	[10]
10	11.36	55%	Different viruses	[11]
0.6	100	55%	4 kind: ssDNA, ssRNA, dsDNA, Enveloped dsRNA	[12]
1.2	14	55%	4 kind: ssDNA, ssRNA, dsDNA, Enveloped dsRNA	[12]
20	40	>70%	Influenza A and respiratory syncytial virus	[17]
1 6	60 55	60–80%	SARS-CoV-2	[18]

3.2. Total Ozone Dose

From the results of these studies, we can extrapolate a relationship between the ozone concentration used and the contact time needed for a viral inactivation >90% (Figure 1).

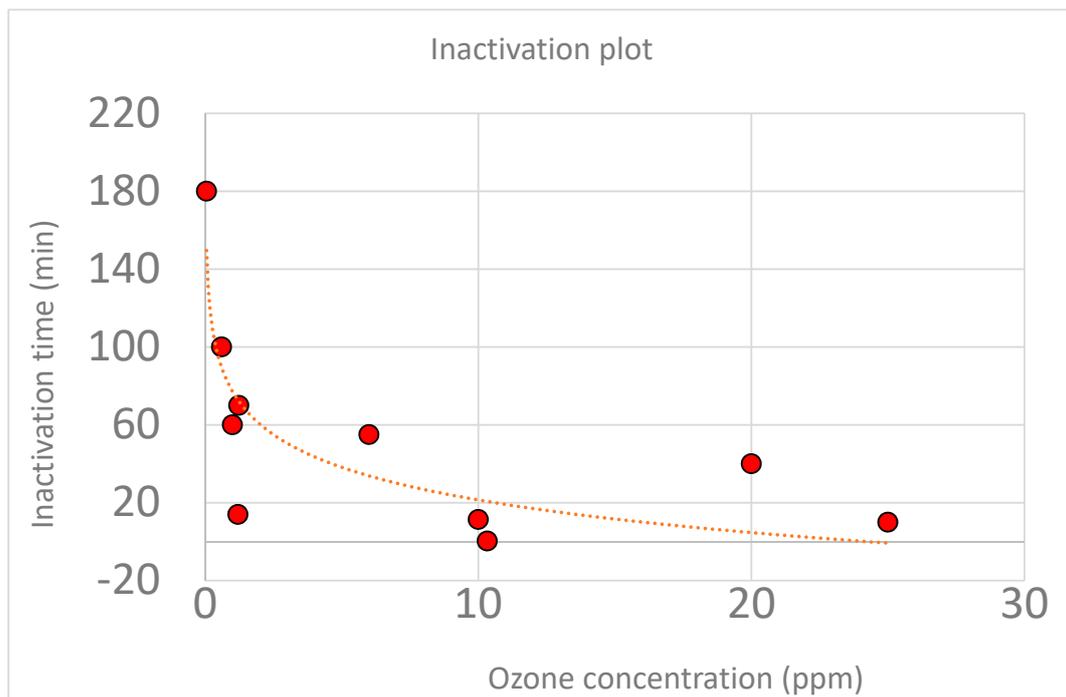


Figure 1. Relationship between ozone concentrations and contact time for viral inactivation.

From these data, we could extrapolate that the time needed in minutes is

$$y = -24.18 \ln(x) + 77.10 \quad (2)$$

where x is the ozone concentration expressed in ppm, with a good correlation ($R^2 = 0.73$, $p = 0.0015$).

The last point of the curve (25 ppm) derives from the experiment of Hudson [5], where contact time was not determined by inactivation but set a priori, and therefore it does not fit into the curve. However, contact times below 1 min are not technically advisable.

The choice of a higher concentration and a shorter time or vice versa should be based on the specific issues of the location to be disinfected.

The measurement of the ozone concentration is based on the spectrophotometric technique of the absorption, by the ozone molecules, of ultraviolet radiation with a wavelength equal to 254 nm. There are also ozone meters with electrochemical detection principle, both fixed and portable, with extremely affordable costs.

3.3. Ozone Production

Due to its high reactivity, ozone cannot be stored, but it is usually generated in situ from air, oxygen or water by means of different energy sources.

The methods normally used to produce ozone are:

- Electrolysis of water (water);
- Photochemical method (air, oxygen);
- Dielectric barrier discharge (air, oxygen).

Electrolysis of water is the preferred technology for ozone water production and, as such, it is not useful for air disinfection.

Ultraviolet irradiation of oxygen and nitrogen oxides is the main source of natural ozone, but it is not very practical when high quantities are needed. The ozone production efficiency increases, decreasing the wavelength, with a peak efficiency at 185 nm [19], well below the 254 nm wavelength used by germicidal lamps.

Therefore, consumer-oriented UV-ozone “air purifiers” produce, if any, an amount of ozone just above the 0.04 ppm olfactory threshold. However, for applications not requiring high concentrations, as for laboratory standards, UV lamps are an option.

When high yields are required, ozone is generated via the dielectric barrier discharge (DBD), also known as the corona effect. The name derives from the shape of the region of glowing gas formed around an electrode when the electric field strength is high enough for gas ionization, without forming an arc. The feeding gas can be pure oxygen or air, and since the ozone yield is reduced by air humidity, a compressor with the purpose of drying air shall be installed at the inlet. This is one of the most effective technologies for ozone production, and therefore most of the commercial generators are based on DBD.

Ozone production is generally affected by several parameters like the nature of the electrode, the reactor geometry and configuration, the power source used, the gas source and flow rate in reactor, and physical variables like pressure, humidity and temperature. Only DBD generators fed with environmental air will be addressed here. The air that feeds the generator must be purified from possible contaminants like particulate matter, VOC, etc., in order to avoid the generation of reaction by-products that are harmful to human health.

Ozone generators specifications usually provide a production of ozone amount expressed in mg/hour.

The volume of the space to be disinfected should be measured and expressed in cubic meters (m³).

The amount of ozone produced in 1 h, divided by the volume of the room, will provide the concentration of ozone that can be reached in 1 h, or in 1 min if it is divided by 60.

For example, a small commercial ozone generator can produce 2000 mg/hour. For a room of 10 × 10 × 3 m (300 m³) the ozone concentration of 6.6 mg/m³ can be reached after 1 h, which can be converted to 3.36 parts per million (ppm) as follows

$$\text{ppm} = \text{mg/m}^3 \times 24.45/48 \quad (3)$$

where 24.45 is the volume of 1 mole of an ideal gas at 1 atm and 25° C and 48 g/mol the molecular weight of ozone.

Some papers use the product of ozone concentrations for the exposure time in order to compare the efficacy of different conditions, so the unit becomes min.ppm or min.mg/m³.

The actual ozone yield strongly depends on environmental parameters such as humidity [20], with decreased efficiency at a higher humidity. When the generator is stopped, the interaction of the ozone molecules with the environment, and even among one another, leads to the recombination of ozone into oxygen [21] within a time depending on air flow, temperature and humidity.

Low-cost generators, designed to be manually started and stopped, do not allow for achieving a reliable concentration and holding it for a definite time, if other technical measures are not set up.

3.4. Disposable Masks and Personal Protective Equipment Disinfection for Reuse

A total of 55 documents were retrieved using the mentioned keywords in bibliographic databases, but only 20 were selected for this study.

In times of emergency, disinfection and sterilization protocols, even for disposable PPE (gloves, glasses, face shields, gowns, filtering facepieces respirators), may be needed. A summary of the possible issues for each piece of equipment is reported here.

It is important to keep in mind that reuse should be regulated by a good practice procedure in order to guarantee:

- That disinfection for reuse should not be applied to PPE that are exhausted for another specific use (for example protection from dusts or fibers);
- That an individual PPE would be reused only by the same individual;
- That the acceptable number of reuses has been determined and has not been reached.

Most non-reusable PPEs are made of polymeric materials, which are, by nature, among the cheapest and easiest to work. Ozone contributes strongly to the aging of rubber through the splitting of alkenic double bonds, according to the ozonolysis mechanism described for the first time by Criegee [22]. Ozone, therefore, has a high affinity with unsaturated polymers. To test the resistance of rubber to ozone exposure there are several technical standards, in which the applied ozone concentrations are in the order of a few ppm [23–26].

Jaffe reports that the rate of oxidation at ordinary temperatures is very low, but, depending on the type of rubber compound, the rate increases significantly with each rise of about 10 °C [27]. The same author also states that the main factors influencing the action of ozone on rubber are the stress degree, the nature of the rubber compound, the ozone concentration, the exposure period, the speed of contact with the surface and the temperature. The simultaneous action of stress and ozone is a necessary condition for the manifestation of visible cracks [28]. It is also known as the combined effect between ozone and other agents, such as UV [29,30] or chemicals [31], causes a greater and faster oxidation than that obtained from ozone alone.

In conclusion, materials such as natural rubber (latex) and nitrile are the least resistant to ozone. Natural rubber or some synthetic rubbers show cracks if they are just 2–3% stretched and simultaneously exposed to an atmosphere containing 0.01–0.02 ppm of ozone [32]. Instead, butyl rubbers, neoprene and polyurethane, exposed to the same ozone concentrations, withstand almost triple the amount of stress compared to natural rubbers. Finally, rubbers such as silicone, polyacrylic, chlorosulfonated polyethylene, and ethylene–propylene copolymer, characterized by saturated chemical structures, are the most resistant [27].

Gloves are among the most common and cheapest protective devices. The disposable gloves used in healthcare settings are normally made of nitrile or latex. Considering that the reuse of disposable gloves is rather difficult, it is not considered particularly useful to further investigate any sterilization procedure, even with agents different from ozone.

Potentially infected droplets of liquid and splashes directed towards the eyes can be stopped by goggles or safety glasses or by a shield covering the face down to the chin. Because of its excellent optical and mechanical properties, these PPE are normally composed of polycarbonate, which offers excellent resistance to ozone. Therefore, the use of ozone sterilization procedures could adapt very well to this type of PPE.

Disposable filtering facepieces respirators (FFR) are designed to reduce the inhalation of particulate contaminants (such as droplets or aerosols). The filtering action is carried out by the non-woven fabric, usually polyethylene and/or polypropylene, of which the FFR is made. These materials are normally resistant to the chemical action of ozone. Regarding the sterilization of FFR, Zhang et al. [13] studied the inactivation of SARS-CoV-1 by using different concentrations of ozone and discovered that this virus can be inactivated using a concentration of ozone equal to 27.73 mg/L for 4 min. A recent study [33] showed that exposure to ozone concentrations of 400 ppm for up to 10 cycles of two hours (at room temperature and RH 75–90%) did not degrade the filtration and fit capabilities of the FFR but caused an unclear residual odor that needs further, more detailed, investigation. In another study [11], authors exposed the non-woven polypropylene material used for the N95 FFR at ozone concentrations of 10 and 20 ppm, for a duration of 10, 20 and 60 min. They observed the absence of microscopically visible damage to the fibers; in addition, they performed tests according to the NIOSH standard and also demonstrated the absence of loss of filtration efficiency.

A critical aspect could relate to the retention straps and therefore to the fitting: in fact, in some FFR models, straps can be made of latex and they could lose tensile strength or even break.

In any case, the fact that ozone is a gaseous virucidal makes it a particularly effective method for reaching shadows and crevices and sterilize porous and fibrous materials better than other methods (for example, UV).

Medical gowns can be made of different materials, on which their reusability depends. The reusable gowns are made of cellulose fabrics (like cotton) while the disposable gowns are generally made

of artificial rubber (propylene or polypropylene). The elective disinfection treatment for cotton gowns includes hot water, soap and disinfectants like chlorine [34]. On the other hand, the use of low ozone concentrations (0.02–0.06 ppm) on cotton fibers can increase fluidity and decrease breaking strength [35] as well as a progressive whitening of dyed fabrics [36,37]. The reactions between ozone and dirty, used clothes can lead to the production of harmful volatile organic compounds like aldehydes and acetone [38].

Ozone does not seem to have particular contraindications for sterilizing propylene or polypropylene gowns (like Tyvek®), and it is able to easily reach the internal parts of the folds of the fabric.

3.5. Ozone Negative Impact on Consumer Goods and Other Materials

Ozone, being a strong oxidizing agent, can damage materials such as rubber, plastics, fabrics, paint and metals. Exposure to airborne ozone can progressively damage both the functional and aesthetic qualities of materials and products, shortening their life. This inconvenience can cause significant economic losses for industries and other activities, because of the increased costs of maintenance or of the replacement of the damaged goods [39].

Limited data are available, but they indicate significant damage to rubber products and surface coatings, while textiles and other polymeric materials seem not to be affected in the range of atmospheric concentrations [40]. Ozone is also able to fade dyes on nylon and acetate, and most of the natural dyes and dye-based pigments used by artists. It also plays a role in the process of the corrosion of metals like copper and aluminum [31].

3.6. Occupational Exposure Limits

The U.S.A. Occupational Safety and Health Administration (OSHA) has set Public Health Air Standards of 0.1 ppm for 8 h or 0.3 ppm for 15 min as the limit of the amount of ozone to which people can be safely exposed.

The Directive 2008/50/EC of the European Parliament and of the Council of 21 May 2008, on ambient air quality and cleaner air for Europe, transposed in Italy as Legislative Decree 155/2010, sets two ozone concentration thresholds: an information threshold of 180 $\mu\text{g}/\text{m}^3$ per hour, and a long-term objective for the protection of human health of 120 $\mu\text{g}/\text{m}^3$ (0.24 ppm), calculated as the daily maximum of the moving average of 8 h.

The olfactory perception threshold of ozone is 0.04 mg/m^3 , equal to ~ 0.02 ppm, concentrations that have no effect on human health.

A guidance on the safe use of ozone provided by manufacturers and importers of ozone generators is available on the ECHA website [41].

For ozone, no Indicative Occupational Exposure Limit Values (IOELVS) have been set at European Union level, but some Member States have established national occupational exposure limit values, both for long- and for short-term exposures.

In the Italian legislation (attachment XXXVIII of Legislative Decree 81/08) there are no limit values for the occupational exposure to ozone.

Limit values in use in several countries can be found on the German IFA-GESTIS database [42]. Alternatively, the values indicated by the American Conference of Governmental Industrial Hygienist (ACGIH) [43] can be taken into account, which also are related to the physical activity carried out by the workers. Two European countries, Ireland and Spain, have adopted the same ACGIH limit values.

Occupational Exposure Limit Values in different European and extra European countries are reported in Table 2 [44]. Values are very consistent and go from a minimum of 0.05 ppm for long-term to a maximum of 0.3 ppm for short-term exposures.

Table 2. Occupational Exposure Limit Values in different European and extra European countries.

Country or Agency	Limit Value—Eight Hours		Limit Value—Short Term	
	ppm	mg/m ³	ppm	mg/m ³
Austria	0.1	0.2	0.2	0.4
Belgium			0.1	0.2
Denmark	0.1	0.2	0.1	0.2
Finland	0.05	0.1	0.2	0.4
France	0.1	0.2	0.2	0.4
Hungary	0.1	0.2	0.1	0.2
Ireland	heavy work 0.05	heavy work 0.1	heavy, moderate and light works < 2 h 0.2	heavy, moderate and light works < 2 h 0.4
	moderate work 0.08	moderate work 0.16		
	light work 0.1	light work 0.2		
Latvia	0.05	0.1		
Poland	0.075	0.15		
Romania	0.05	0.1	0.1	0.2
Spain	heavy work 0.05	heavy work 0.1	heavy, moderate and light works < 2 h 0.2	heavy, moderate and light works < 2 h 0.4
	moderate work 0.08	moderate work 0.16		
	light work 0.1	light work 0.2		
Sweden	0.1	0.2	0.3	0.6
Switzerland	0.1	0.2	0.1	0.2
The Netherlands	0.06	0.12		
ACGIH (American Conference of Governmental Industrial Hygienists)	heavy work 0.05	heavy work 0.1	heavy, moderate and light works < 2 h 0.2	heavy, moderate and light works < 2 h 0.4
	moderate work 0.08	moderate work 0.16		
	light work 0.1	light work 0.2		
USA—NIOSH (National Institute for Occupational Safety and Health)			0.1	0.2
USA—OSHA (Occupational Safety and Health Administration)	0.1	0.2		
United Kingdom			0.2	0.4
Canada—Ontario	0.1	0.2	0.3	0.6
Canada—Québec			0.1	0.2
Japan JSOH (Japan Society for Occupational Health)	0.1	0.2		
New Zealand			0.1	0.2
Republic of China			0.15	0.3
Singapore			0.1	0.2
South Korea	0.08	0.16	0.2	0.4

3.7. Safety Issues

Ozone generators are currently being promoted as effective tools for removing pollution and odours from indoor air, but it must be remembered that ozone is associated not only with adverse health effects but also with safety issues.

Ozone generators are high voltage electric machines, with all the safety implications involved.

Moreover, the corona effect, whichever the frequency of the applied current, produce a broad spectrum of radio frequencies depending on many different parameters [45].

The ECHA safety guidance forbids the entire room where the ozone generator is deployed to pacemaker bearers. The actual extension of the area where the electromagnetic field exceeds the reference levels set for the protection of general public and workers should be addressed in the risk assessment. Depending on the configuration of the generator, this area could range from centimeters to meters; however, a too high radio frequency emission could impair the correct functioning of other devices, including medical equipment.

4. Discussion

Ozone, being a gas, is very effective in reaching all the points in the space and on the surfaces, performing a rapid and extensive disinfection of the environment simply by exploiting its natural air dispersion. The UV-C radiation has effective antimicrobial properties but is well known that there are shady areas where the disinfection could not occur. Liquid disinfectants can be sprinkled as natural air diffusion is limited.

Nevertheless, to date, few studies are available on ozone virucidal activity and these were carried out using very different experimental conditions on some pathogenic viruses or their surrogates. Since standardised methods for the assessment of ozone virucidal efficacy are not available, the heterogeneity of experimental procedures performed makes it difficult to compare the results. In the studies examined, the experiments were carried out using various ozone generators, under different conditions of temperature and relative humidity, on different viruses. By extrapolating data on ozone virucidal activity at room temperature and medium relative humidity between 35 and 55%, we found that an opportune combination of ozone concentration, in the range 1–25 ppm, and contact times between 10 min and 3 h are able to efficiently inactivate (>90%) some pathogenic viruses or their surrogates. A mathematical relationship between ozone concentration and the needed contact time is reported.

In these studies, some authors showed that a parameter that plays an important role is the relative humidity that increases ozone virucidal activity. Among these, very recently, Yano et al. [22] reported the first in vitro inactivation study on SARS-CoV-2 virus by 6.0 ppm ozone at room temperature and 60–80% RH, obtaining, in only 55 min, a 99.9% reduction (which equates to about 3-Log) in the number of the PFU/mL

Overall, findings suggest that ozone used at a low concentration is a powerful disinfectant (up to 99% reduction) when combined with a high RH because, when air humidity is higher, more free radicals will be formed, which are able to kill airborne viruses.

There is no harmonized classification of ozone. According to the classification provided by companies to ECHA, ozone should be classified for acute inhalation toxicity (Acute Tox. 1), skin corrosion (Skin Corr. 1B), serious eye damage (Eye Damage 1) and specific target organ (airways) repeated toxicity (STOT Rep. Exp. 1). As reported by the ECHA Registry of classification and labelling (CLH) Intentions, a proposal to classify ozone also for mutagenicity (Muta. 2) and carcinogenicity (Carc. 2) was submitted by Germany in 2016.

Due to its toxicological properties and its capability to degrade several materials, the optimal use of ozone for the disinfection of air and surfaces is in the absence of humans, using a dose and time of usage sufficient to destroy viruses, but having minimal degradation effects on materials.

If it is not possible to demonstrate that, after the disinfection of a workplace, airborne ozone concentrations guaranteeing the safety of the workers have been reached, it is necessary to determine said concentrations with an appropriate method.

The use of ozone as a disinfectant for the re-use of PPE has been further investigated in the light of COVID-19 pandemic as possible solution to the shortage of personal protective equipment. On the basis of the available information, the use of ozone disinfection procedures could adapt very well to goggles, safety glasses and face shields, propylene or polypropylene gowns, while for disposable filtering facepieces' respirators, further detailed investigations are needed.

The Italian Institute for Public Health (ISS) [44] concludes that this gas must be used in unoccupied and confined environments, and that it is necessary to evaluate the risk of exposure of both operators (who must be trained and equipped with suitable personal protective equipment) and of the staff who will use the disinfected premises.

5. Conclusions

The examination of the literature on ozone virucidal activity showed that ozone, used at a low concentration, is a powerful agent (up to 99% reduction) when combined with a high RH, for the disinfection of air, surfaces and some personal protection devices. The optimal minimal dose and the needed contact time can be calculated in order to sufficiently to destroy viruses, but this combination should be tested for possible degradation effects on materials and goods.

The major drawbacks for the use of ozone as a disinfectant are the risks that it poses to human health and safety: it is able to induce decreases in lung function and inflammation, and is associated with respiratory effects, including asthma and chronic obstructive pulmonary disease exacerbations. Moreover, ozone generators are high-voltage electric machines, with all the safety implications involved. If other, less harmful, disinfectants are not applicable, ozone is a valid option, provided that people/workers are never present during the treatment.

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