PRELIMINARY INDICATIONS FOR THE USE OF OZONE AS AIR AND SURFACE DISINFECTANT IN THE CONJUNCTURE OF COVID-19

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Abstract

The main objective of the present document is to review the available scientific information on ozone virucidal activity in order to extrapolate quantitative data for the use of ozone in the appropriate cases and to explore the safety measures offered by new technologies, developed under the stimulus of the current emergency situation.

Ozone, best known for its protective role in the earth's ecological environment, is a powerful oxidant reacting with organic molecules containing double or triple bonds, and therefore has bactericidal, virucidal, and fungicidal actions. As such, it is used in air care products and biocides, it is being proposed for the disinfection of workplaces and public places atmosphere, and also for disposable masks and personal protective equipment disinfection for reuse (in the face of shortages), with particular reference to productive and social activities being started again following the COVID-19 pandemic outbreak.

Ozone can be generated in-situ by means of small, compact ozone generators, using dried ambient air as precursor. Typical phases of a treatment cycle are: the conditioning phase, in which ozone is injected into the room to be disinfected until the desired ozone concentration is reached; the treatment phase, lasting for the time necessary for the disinfection; the ventilation or ozone conversion phase, which guarantees the elimination of ozone from the room until the concentrations required for the workers' safety are reached.

Due to its toxicological properties and its capability to degrade several materials, the optimal use of ozone is for air and surfaces disinfection without human presence, using an ozone concentration effective for the destruction of viruses, but not high enough to deteriorate materials. The choice of a higher concentration for a shorter time or viceversa should be based on the specific issues of the location or the materials to be disinfected.

Introduction

On May 2020, the Italian Institute of Health (ISS), issued its Report 19 n. 25/2020 [ISS, 2020] addressing interim recommendations on cleaning and disinfection of non-healthcare settings. The document presents an overview concerning "sanification" intended as the process of cleaning and/or disinfecting and maintaining good air quality in non-healthcare settings taking into account scientific evidence of COVID-19 virus persistence on different surfaces and efficacy of cleaning and disinfection products for indoor environments. The document also considers the environmental impact and human health risk associated with the use of the products. In this report, ozone is also evaluated on the basis of the available literature and of the statements of Internationally recognized organizations like ECHA (European Chemical Agency), CDC (Centers for Disease Control and Prevention), FDA (Food and Drug Administration), US-EPA (United

States- Environmental Protection Agency), and the International Ozone Association [www.iao-pag.org] who confirms the effectiveness of ozone for the inactivation of many viruses even if it is not aware of specific research on SARS-CoV-2.

The ISS document concludes that the use of ozone must take place in unoccupied and confined environments, 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 uses the disinfected premises, and lastly, that therefore disinfection by ozone treatment is not suitable for domestic use.

The main objective of the present document is to review the available scientific information on ozone virucidal activity in order to extrapolate quantitative data for the use of ozone in the appropriate cases and to explore the safety measures offered by new technologies, developed under the stimulus of the current emergency situation.

In this document we did not take in consideration the use of ozone in aqueous solution nor the ozone therapy for patients, but only the use of airborne ozone, generated *in situ* for the disinfection purpose.

Chemical and physical properties

Ozone, an allotropic form of oxygen, is an inorganic gas (CAS n. 10028-15-6), whose molecule is constituted by three oxygen atoms (O_3), arranged in a cyclical structure with a distance among oxygen atoms of 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 ppm (1 ppm ~ 2 mg/m3). Its production is also catalyzed in the atmosphere by ultraviolet ray irradiation of oxygen or other precursors such as volatile organic compounds and nitrogen oxides. About 90% of the ozone in the atmosphere exists in the stratosphere (stratospheric ozone).
Ozone, best known for its protective role in the earth's ecological environment, is a powerful oxidant reacting with organic molecules containing double or triple bonds, and therefore has bactericidal, virucidal, and fungicidal actions that have been used in water treatment, odor control, and medicinal applications [Knobler S. et al., 2004].
The solubility in water (at 0°C) of ozone is 49.0 ml/100 ml, tenfold compared with oxygen, what allows its immediate reaction with any soluble compounds and biomolecules present in biological fluids.
It is heavier than air and therefore, inside buildings, it is concentrated close to the ground.

Ozone production

Due to its high reactivity, ozone cannot be stored, but it is usually generated in situ from air, oxygen or water by applying various 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, is not useful for air disinfection.
Ultraviolet irradiation of oxygen and nitrogen oxides is the main source of natural ozone but is not very practical when high quantities are needed. The ozone production efficiency increases with decreasing

wavelength with a peak efficiency at 185 nm [Al shammah et al., 2001], 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 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 but 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 to dry air can be installed at the inlet. (DBD) is one of the most effective technologies in producing ozone and is the basis for most of the commercial ozone generators. Ozone production is generally affected by electrode material, reactor geometry, reactor configuration, pressure, gas flow rate, frequency, humidity, power source, temperature and gas source in reactor [Yulianto E et al., 2019].
This work is mainly addressed to the use of ozone as a simple and fast disinfection method, both in healthcare facilities and other workplaces. Therefore oxygen cylinders or pressurized vessel are not advisable.
In the following, only DBD generators fed with environment air will be addressed. 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 harmful to the 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 expressed in cubic meters (m^3).
The amount of ozone produced in 1 hour, divided by the volume of the room will provide the concentration of ozone that can be reached in 1 hour, or in 1 minute if it is divided by 60.
For example a small commercial ozone generator can produce 2000 mg/hour.
For a room of 10x10x3meters (300 m^3) the ozone concentration of 6.6 mg/ m^3 can be reached after 1 hour, that can be converted to 3.36 parts per million (ppm) as follows:
ppm= mg/ m^3 x 24.45/48
(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/ m^3.
In fact, the actual ozone yield strongly depends on environmental parameters such as humidity [Xuming et al, 2016] with decreasing efficiency higher humidity. When the generator is stopped, the interaction of ozone molecules with the environment and even among one another leads to the recombination of ozone into oxygen [Mc Clurkin et al, 2013] within a time depending on air flow, temperature and humidity.
Low cost generators, designed to be manually started and stopped by a timer, don’t allow to achieve a reliable concentration and hold it for a definite time if other technical measures are not set up.

Literature on ozone virucidal efficacy

Ozone is currently under review for use as a biocide in the European Environmental Agency and/or Switzerland, in application to: disinfection, food and animals feeds, drinking water, preservation for liquid systems, under the Biocidal Products Regulation (BPR) of ECHA.
Although the inhibitory and lethal effects of ozone on pathogenic microorganisms have been observed since the latter part of the 19^{th} century, the mechanisms for these actions have not yet been satisfactorily highlighted. The most often cited explanation for ozone’s bactericidal effects focuses on the disruption of

envelope integrity through peroxidation of phospholipids. There is also evidence for interaction with proteins [Mudd et al., 1969].

Viruses have been studied during their interaction with ozone [Roy et al., 1981]. After 30 seconds of exposure to ozone, 99 percent of the viruses were inactivated and demonstrated damage to their envelope proteins, which could result in failure of attachment to normal cells and breakage of the single-stranded RNA.
Typically, viruses are small, independent particles, built of macromolecules. Unlike bacteria, they multiply only within the host cell. Viruses are unable to repair oxidative damage, and therefore they are more susceptible to oxidative antimicrobial action than prokaryotic (bacteria or fungi) or any eukaryotic organisms [Dennis R. et al., 2020].

Ozone destroys viruses, in which the envelope is present, by spreading through this protein coat into the nucleic acid core, resulting in damage of the viral DNA or RNA [Tseng CC. et al., 2006; Tseng C. et al., 2008; Rojas-Valencia, MN, et al., 2012]. At higher concentrations, ozone destroys the capsid or exterior protein shell by oxidation. Most research efforts on ozone's virucidal effects have centered upon ozone's propensity to break apart lipid molecules at sites of multiple bond configuration. Indeed, once the lipid envelope of the virus is fragmented, its DNA or RNA core cannot survive.

Non-enveloped viruses, called "naked viruses" are constituted of a nucleic acid core (made of DNA or RNA) and a nucleic acid coat, or capsid, made of protein.
The enveloped viruses are usually more sensitive to physico-chemical challenges than naked virions. Although ozone's effects upon unsaturated lipids are some of its best-documented biochemical actions, ozone is known to interact with proteins, carbohydrates, and nucleic acids. [Thailand Medical News, 2020].
A review of the existing scientific literature reveals that the virucidal action of ozone is extremely rapid and potent; however, requisite gaseous ozone dosages are limited to only a few studies.
The virucidal action of ozone happens in seconds or fractions of seconds; therefore, it is technically difficult to measure viral inactivation [Wolf C. et al., 2018].

The scientific literature has not been very rich until now on the antiviral properties of ozone against SARS-COV-2. Data available in literature don’t cover all virus types. In some studies, the virucidal activity of ozone was tested on pathogenic viruses of particular clinic interest such as Poliovirus, Norovirus, Herpes Simplex Virus and Influenza virus [Roy D et al., 1981; Hudson JG et al., 2009; Dubuis ME et al., 2020; Tanaka H. et al., 2009]; in others instead, the bacteriophages were used in place of airborne human pathogenic viruses [Tseng CC. et al., 2006].

Hudson et al. [Hudson et al., 2009] developed a prototype ozone generator (Viroforce 1000). The generator contains 8 corona discharge units, a powerful circulating fan, and a catalytic converter to convert ozone back to oxygen after the treatment. Maximum anti-viral efficacy required an ozone concentration of 25 ppm for 15 min followed by a short period of high humidity (>90% relative humidity). All 12 viruses tested, on different hard and porous surfaces, and in the presence of biological fluids, could be inactivated by at least 3 log10, in the laboratory and in simulated field trials. Viral activity was tested by cellular culture. The ozone was subsequently removed by the built-in catalytic converter.

Inactivation of Herpes Virus by ozone was studied by Greici et al. [Greici et al.2014]. The ozone was generated by a commercial air purifier (Brizzamar, Ronda Alta, RS, Brazil). The ozone generator was kept on for 3 h, and the ozone total concentration in the environmental was monitored through the sensor EcoSensor Model OS-

4 (Ozone Switch TM, Newark, CA, USA). 68 - 90% reduction of viral activity (tested by cellular colture) was obtained with ozone exposure of 1-3 hours at concentrations between 0.02 and 0.05 ppm.

Dennis 2020 [Dennis R. et al., 2020] has 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 per 10 min, based on the results of other papers.

Tseng and Chihshan [Tseng Chun Chieh, and Chihshan Li, 2008] tested 4 different viruses representative of ssDNA, ssRNA, dsDNA, and enveloped dsRNA categories, using an ozone generator (OZ1PCS-V/SW, Ozotech Inc., Yreka, CA) with pure oxygen at 3 L/min. Ozone levels were measured by an ozone analyzer (model 401, Advanced Pollution Instruments, San Diego, CA) with a detection limit of 1.0 ppb. Ozone concentrations of 0.6 - 1.2 ppm for 20 - 112 min time guaranteed 90% and 99% inactivation, respectively. For all tested viruses at the same inactivation, the required ozone concentration at 85% RH was lower than that at 55% RH.

Zhang et al. [Zhang et al., 2004] demonstrated that ozone in aqueous solution is able to inactivate SARS-CoV-1, etiologic agent responsible for Severe Acure Respiratory Syndrome (SARS) in 2003, very similar in structure to the SARS-CoV-2 virus causing the current COVID-19 pandemic. Afterwards Hudson et al. [Hudson J., et al. 2009] 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.

Although no data are available in literature on the efficacy of ozone at inactivating SARS-CoV-2, being this an enveloped virus, thus particularly sensitive to oxidant action, the scientific evidence suggests that ozone will effectively inactivate the new coronavirus too.

Moreover, some studies [Tseng CC et al., 2006; Tseng C et al., 2008; Li CS et al., 2003] showed that the important factor for inactivation of viruses and other microorganisms is the total ozone dose which is calculated as the product of exposure time and concentration. In these studies, low concentrations for longer duration achieve the same results as high concentrations for short duration.

In a recent study, Dubuis ME et al. [Dubuis ME et al., 2020] tested the efficacy of an air treatment using ozone and relative humidity (RH) for the inactivation of airborne viruses. Four phages (ϕX174, PR772, MS2 and ϕ6) 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) and various levels of RH for 10 to 70 minutes. An inactivation of at least two orders of magnitude for ϕX174, MS2 and MNV-1 was achieved with an ozone exposure of 40 minutes at 85% RH. For PR772 and ϕ6, exposure to the reference condition at 20% RH for 10 minutes yielded the same results. In according to previous studies [Sharma M., Hudson JB. Et al. 2008], these results suggest that ozone used at a low concentration is a powerful disinfectant for airborne viruses and other microorganisms when combined with a high RH. In fact, as well known, ozone is an oxidizing agent in aqueous solutions and when it is in the gas phase, reacting with water, generates free radicals that increase its disinfection power.

Tseng and Li [Tseng Chun-Chieh & Li Chih-Shan, 2006] also observed that the inactivation of phages increased when high RH (85%) was used and further studies have demonstrated that the presence of ozone under high RH conditions leads to the formation of more radicals than in dry air [Foarde K.K et al., 1997; Li C.S. and Wang Y.C. 2003].

Metrics for airborne microorganisms inactivation

By examining the scientific literature about ozone virucidal efficiency, the ozone concentration values that are 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 first dividing the mean sample PFU/ml by the mean control PFU/ml. Then, mean control genomes/ml were divided by mean sample genomes/ml. Finally, both results were multiplied together. NIRs were calculated for ozone and the reference (air) conditions.

RIR ¼ NIR O_3=Med½[NIR air]

Lastly, relative infectious ratios (RIRs) were obtained by dividing each ozone-NIR with the corresponding median air-NIR. This step removed the humidity and aerosol aging effects. As a result, RIRs represent solely the ozone effect for each exposure time and RH.

<u>Relative infectious ratios (RIRs)</u>: RIRs represent the effect of ozone only, since data were corrected for the effect of RH and aerosol aging without ozone [Dubuis ME et al., 2020]

From results of the cited literature data, considering a RH not increased (35-55%), reported in Table 1 we can extrapolate a relationship between the ozone concentration used and the contact time needed for a viral inactivation >90% (fig 1.)

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

Ozone concentration (ppm)	**90% Inactivation time (min)**	**Relative Humidity**	**Virus**	**Reference**
25	15	> 95% after cycle	12 different viruses	Hudson 2009
0.05	180	35%	Herpes	Grieci 2014
10	11.36	55%	Different viruses	Dennis 2020
0.6	100	55%	4 kind: ssDNA, ssRNA, dsDNA, Enveloped dsRNA	Tseng 2008
1.2	14	55%	4 kind: ssDNA, ssRNA, dsDNA, Enveloped dsRNA	Tseng 2008
10.33	0.3	55%	4 kind: ssDNA, ssRNA, dsDNA, Enveloped dsRNA	Tseng 2006
1.23	70	55%	4 phages	Dubuis 2020

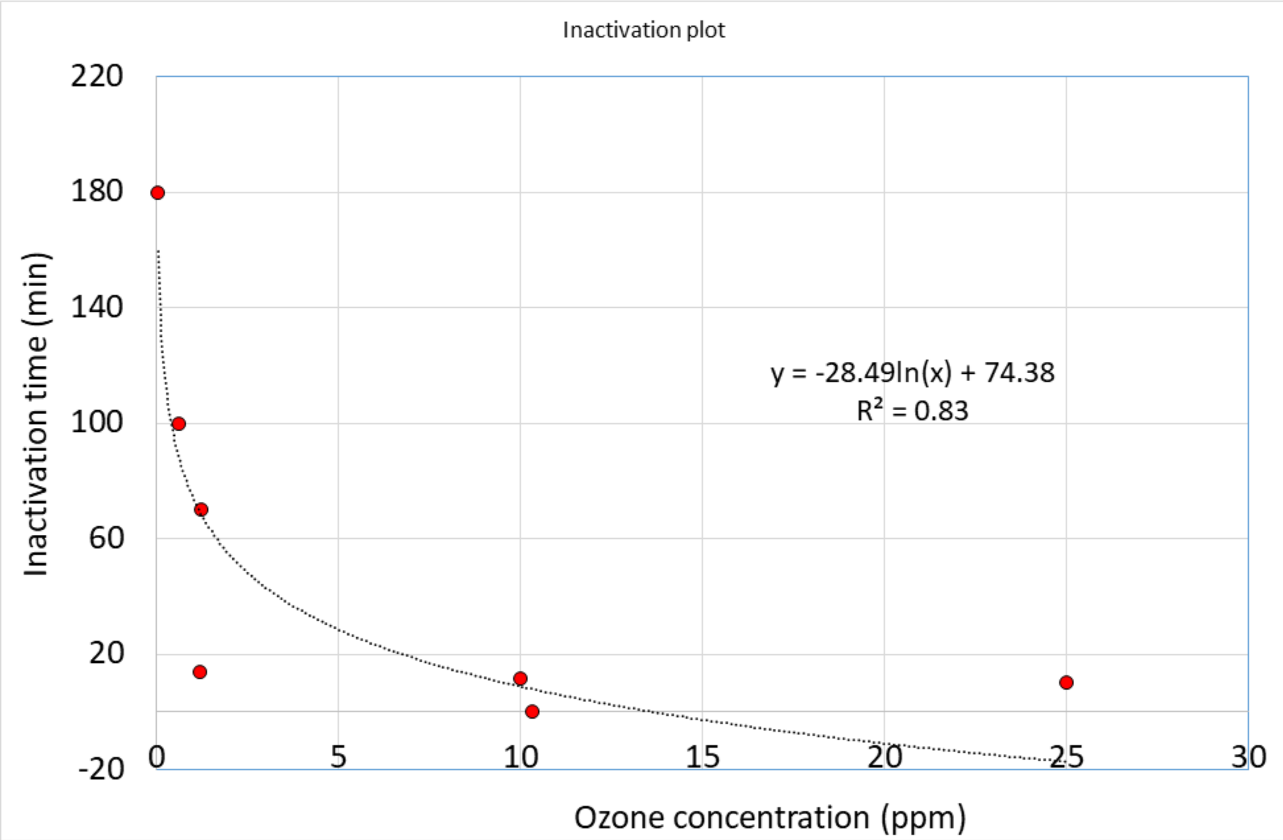

Fig. 1: relationship between ozone concentrations and contact time for viral inactivation.

From these data we could extrapolate that the time needed in minutes (y) is – 28.49 ln (x) + 74.4, where x is the ozone concentration expressed in ppm, with a good correlation (R^2=0.83, p= 0.013).

The last point of the curve (25 ppm) derives from the experiment of Hudson [Hudson J., et al., 2009] 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 viceversa should be based on the specific issues of the location to be disinfected.

Safety issues

Ozone generators are currently promoted as an effective method to clean indoor air pollution and odours. However ozone is associated with adverse health effects. Available scientific evidence shows that ozone concentrations that are safe to breathe are unlikely to be effective in controlling indoor air pollution. Manufacturers and sellers of ozone devices use a variety terms that suggest that ozone is a "healthy" kind of oxygen. However, ozone is a toxic gas with very different properties to oxygen [EPA 2015].

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 [Moonligan D., et al., 2009].

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, it could range from centimeters to meters, however a too high radio frequency emission could impair the correct functioning of other critical devices than medical equipment.

Toxicological Information

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 labeling (CLH) Intentions, a proposal to classify ozone even for mutagenicity (Muta. 2) and carcinogenicity (Carc. 2) has been submitted by Germany on 2016.

A comprehensive evaluation of human health effects has been lately performed by the US EPA as part of the "Integrated Science Assessment for Ozone and Related Photochemical Oxidants" [US EPA, 2020]. 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 hours 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 results from toxicological studies further characterize potential mechanistic pathways and provide continued support for the biological plausibility of ozone-induced respiratory effects. There is emerging evidence that short-term ozone exposure contributes to metabolic disease, including complications related to diabetes. Specifically, animal toxicological studies demonstrate that ozone exposure impaired glucose tolerance, increased triglycerides in serum, induced fasting hyperglycemia, and increased hepatic gluconeogenesis. The available evidence was inadequate to determine whether there was a causal relationship between exposure to ambient ozone and cancer. Very few epidemiologic and toxicological studies had been published examining ozone as a carcinogen, but collectively the results of these studies indicated that ozone may contribute to DNA damage.
Due to its high reactivity, toxic effects of ozone reaction products should be considered too.

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

The Directive 2008/50/EC 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) set two ozone concentration thresholds: Information threshold (SI) per hour of 180 µg /m^3 and Long-term objective (OLT) for the protection of human health of 120 µg /m^3 (0.24 ppm), calculated as the daily maximum of the moving average of 8 hours.

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.

Occupational exposures

For ozone, no IOELVs (Indicative Occupational Exposure Limit Values) 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 occupational exposure to ozone.

Limit values in use in several countries can be found on the IFA database GESTIS [IFA https://limitvalue.ifa.dguv.de/WebForm_ueliste2.aspx]

Alternatively, the values indicated by the American Conference of Governmental Industrial Hygienist (ACGIH) [ACGIH, 2020] can be taken into account, which are related to the physical activity carried out. 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. 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/m³**	**ppm**	**mg/m³**
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 moderate work 0.08 light work 0.1	heavy work 0.1 moderate work 0.16 light work 0.2	heavy, moderate and light works < 2 hours 0.2	heavy, moderate and light works < 2 hours 0.4
Latvia	0.05	0.1		
Poland	0.075	0.15		
Romania	0.05	0.1	0.1	0.2
Spain	heavy work 0.05 moderate work 0.08 light work 0.1	heavy work 0.1 moderate work 0.16 light work 0.2	heavy, moderate and light works < 2 hours 0.2	heavy, moderate and light works < 2 hours 0.4
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	heavy work 0.05 moderate work 0.08 light work 0.1	heavy work 0.1 moderate work 0.16 light work 0.2	heavy, moderate and light works < 2 hours 0.2	heavy, moderate and light works < 2 hours 0.4
USA - NIOSH			0.1	0.2
USA - OSHA	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)	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

Exposure control/Personal protection

A guidance on the safe use of ozone provided by manufacturers and importers of ozone generators is available [ECHA, 2020]. The following risk mitigation measures are recommended:

Preconditions for safe operation of an ozone device

- Ozone generation systems must be placed in closed, lockable rooms.
- Rooms which ozone generation systems are placed in must not be used as permanent workplace. If this is not possible for process related reasons, it must be assured that ozone concentration in ambient air at the workplace does not exceed the occupational exposure limit value.
- Rooms in which, in case of failure, ozone leakage can occur must be effectively monitored with gas detectors with optical and acoustic signal which interrupt ozone generation when triggered. This is not required for rooms in which ozone bearing piping without detachable connections, which was tested for leakage by a qualified person, is present.
- Rooms with ozone generation systems must be labeled accordingly.
- Rooms which ozone generation systems are placed in must be equipped with technical exhaust ventilation. It must be installed in such a way that the suction opening of the sucking ventilation is placed directly above the floor and is switched on automatically when the gas detector is triggered, at least a three-fold air exchange must be assured.

Technical and organizational protection measures

- Use ozone destruction units (thermal and/or catalytic) for off gassing ozone.

- Instruction must be provided before employment and then at a minimum of once per annum thereafter.
- An escape and rescue plan must be prepared when the location, scale, and use if the work-site so demand.
- Only employees are permitted to enter the work areas.
- Change clothing that has been in contact with or taken up any of the gas and air the clothing far from any sources of ignition.
- People with cardiac pacemakers or other electric implants are not permitted to enter a room with a ozone generation system.

Personal protective equipment

Hand protection:

- Wear protective gloves.

Body protection:

- Wear protective clothing.
- Depending on the risk, wear tight protective clothing or suitable chemical protection suit.
- Protective suits have to be checked for embrittlement after each use.

Eye protection:

- Wear eye protection.
- If there is a risk of gases escaping eyes should be protected.
- When handling solutions containing ozone, chemical safety goggles must be used as well as a protective shield.

Respiratory protection:

- In case the threshold limit value (TLV) is exceeded, use appropriate breathing protection when rescuing injured persons.
- In case of low concentrations / short term rescue operation: Filter apparatus with gas filter NO-P3 (code color blue-white) or CO (color code black).
- In case of longer term rescue operations / line breakage: Self-contained breathing apparatus (e.g. airline systems or compressed air breathing apparatus)
- Possibility of analysis: Breathing air check by gas detection tubes.

Ozone negative impact on consumer goods and other materials

- Being a strong oxidizing agent, ozone can cause substantial damage to a variety of materials such as rubber, plastics, fabrics, paint and metals. Exposure to ozone progressively damages both the functional and aesthetic qualities of materials and products, and shortens their life spans. Damage from ozone exposure can result in significant economic losses because of the increased costs of maintenance, upkeep and replacement of these materials.
  [https://ww2.arb.ca.gov/sites/default/files/2017-10/ozone-fs.pdf accessed June 08, 2020].
- The limited data available indicate significant damage to rubber products and surface coatings but either insignificant or unquantifiable damage to textiles and other polymeric materials at the range of atmospheric concentrations. [David S. Lee, et al., 1996].

- Moreover, ozone has been shown to fade dyes on nylon and acetate, and many of the natural dyes and dye-based pigments used by artists. It also participates in the chemistry of corrosion of metals like copper and aluminium. [Druzik James R., 1985].

Practical uses of Ozone generators in COVID 19 emergency

Ozone is used in air care products and biocides, and is being proposed as a means for the disinfection of workplaces and public places atmosphere, and also for disposable masks and personal protective equipment disinfection for reuse (in the face of shortages) in the phase of restarting of productive and social activities following the COVID-19 pandemic outbreak. Ozone can be generated in-situ by using a small compact ozone generator using dried ambient air as precursor.

Due to its toxicological properties and to its capability of degrading several materials, the optimal use of ozone for the disinfection of air and surfaces is in the absence of human presence and using a sufficient dose of ozone for a time period that will destroy viruses, but have minimal degradation effects on materials.

Disinfection of workplaces and public places atmosphere

There are many commercial products, with different capacity of production of ozone, and they can deliver different air concentrations, also depending on the volume of the environment to be disinfected.

The typical phases of a treatment cycle are; the conditioning phase, in which ozone is injected into the room to be disinfected to reach the programmed ozone concentration; the treatment phase, lasting for the time necessary for the disinfection; the ventilation or the ozone conversion phase, which guarantees the elimination of ozone from the air in the treated room and continues until the ozone concentrations required for workers' safety are reached.

To this regard, the most recent ozone air cleaners are equipped with a catalytic converter, that, after the treatment, transforms all the residual ozone into oxygen.

If it is not possible to establish on the basis of the supplier's indications or good practices what are the time and mode of aeration of the environment, based on its volume and on the amount of ozone used, to achieve the concentrations that guarantee the safety of the worker, it will be necessary to take measures to determine them.

Measurement method

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.

Disposable masks and personal protective equipment disinfection for reuse

In times of emergency, the introduction of disinfection and sterilization protocols, even of disposable PPE (gloves, glasses, face shields, gowns, filtering facepieces respirators) may be necessary. The use of ozone for the sterilization of disposable PPE is still something to investigate. A brief overview of the issues for each device is reported here below.

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 by the same individual;
- that the acceptable number of reuses has been determined and has not been reached.

Most non-reusable PPE is 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 Criegge [Criegee R., 1975]. Ozone, therefore, has greater affinity with unsaturated polymers or with high presence of double bonds. 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 [ISO 1431-1, 1989; ISO 1431-2, 1994; ISO 1431-3, 2000; ISO 3011, 1997].

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 [Jaffe L.S., 1967]. The same author also states that the main factors that influence the action of ozone on the rubbers are the degree of stress, the nature of the rubber compound, the concentration of ozone, the period of exposure, the speed of contact ozone with the surface and the temperature. Simultaneous action of stress and ozone has been observed to be a necessary condition for the manifestation of visible cracks [Van Rossem A., Talen H. W., 1951]. It is also known as the combined effect between ozone and other agents such as UV [Peeling J., David T. Clark, 1985; Walzak M. J., et al. 1995] or chemicals [Druzik, J. 1985] 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 exposed at the same time to an atmosphere containing 0.01-0.02 ppm of ozone [Crabtree J. and Malm F., 1956]. Instead, butyl rubbers, neoprene and polyurethane, exposed to the same ozone concentrations, withstand almost triple stress compared to natural rubbers. Finally, rubbers such as silicone, polyacrylic, chlorosulfonated polyethylene, ethylene-propylene copolymer, characterized by saturated chemical structures, are the most resistant [Jaffe L.S, 1967].

Gloves

Gloves are among the most common and cheapest protective devices. The disposable gloves used in healthcare 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 the sterilization procedures, even other than those with ozone.

Goggles, safety glasses and face shields

Droplets of liquid and, potentially infected, splashes directed towards the eyes can be blocked by specific glasses (goggles or safety glasses) or by a shield covering the face up to the chin. For their excellent optical and mechanical characterics, these PPE are normally composed of polycarbonate which offers excellent ozone resistance. Therefore the use of ozone sterilization procedures could adapt very well to this type of PPE.

Filtering facepiece respirators

Disposable filtering facepieces respirators (FFR) are designed to reduce exposure by inhalation of particulate contaminants (such as droplets or aerosols). The filtering action is carried out through the particular non-woven fabric (polyethylene and / or polypropylene) of which the FFR is composed. These types of rubber are normally resistant to the chemical action of ozone. Regarding the sterilization of FFR, Zhang et al. [Zhang, et al., 2004] studied the inactivation of SARS-CoV-1 by applying different concentrations of ozone and discovering that this virus can be inactivated using a high concentration of ozone equal to 27.73 mg / l for 4 min. A recent study [Manning Edward P., et al., 2020] showed that exposure to ozone concentrations of 400 ppm for up to 10 cycles of 2 hour (at room temperature and RH 75-90%) did not degrade the filtration and fit of the FFR but caused an unclear residual odor that needs further more detailed investigation. In another study [Dennis R., et al., 2020] 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 minutes and 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 the fit: in fact, in some FFR models, straps can be made of latex and they could lose tensile strength or even could break.
In any case, the fact that ozone is a gaseous virucide makes it a particularly effective method for reaching shadows and crevices and sterilize porous and fibrous materials better than other methods (for example UV).

Gowns

The medical gowns can be made of different materials depending on their reusability. The reusable disposable 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 is that with hot water, soap and disinfectants (chlorine) [WHO, Interim guidance, 2020]. 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 [Bogaty H., et al., 1952] as well as a progressive whitening of dyed fabrics [Prabaharan M, et al., 2001; Perincek S.D., et al., 2007]. The reactions between ozone and dirty used clothes can lead to the production of harmful volatile organic compounds like aldehydes and acetone [Rai A.C., et al., 2014].

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

Conclusions

The review of the scientific literature on ozone virucidal activity shows that an opportune combination of ozone concentration in the range 1-25 ppm and contact times between 10 minutes and 3 hours are able to efficiently inactivate very different kind of viruses at room temperature and medium relative humidity.

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 for the sterilization of disposable PPE is still something to investigate. However, on the basis of the available information, the use of ozone sterilization 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.

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