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Focus on the professional use of ozone also in reference to COVID-19

Working group ISS-INAIL

Version of July 23, 2020

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This document is aimed at providing the technical-scientific evidence currently available on ozone in the COVID-19 epidemic context. For this purpose, it reports state of the art with particular reference to regulatory status, available assessments at the national and international level, information on the dangers and risks associated with the use of ozone, information on toxicity and the impact on human health and environment, effectiveness of the substance as a virucide, safety of use and precautions to be taken in the *in-situ* generation of ozone in the field of prevention and control of SARS-CoV-2. It also addresses the various applications of ozone, from sanitizing environments and devices to the food sector, to water treatment. The report, based on scientific evidence, also examines the therapeutic efficacy of ozone therapy by assessing its safety of use, critical issues and upcoming developments. The paper does not consider exposure to ozone as an involuntary product from UV radiation from the atmosphere or from air purification systems. The document does not examine other *in-situ* generated substances with disinfectant or sanitizing action or other processes in use in the COVID-19 epidemic context, and therefore this elaborate does not allow an exhaustive evaluation of the cost/benefit ratio compared to the other available systems.

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Target

The main recipients of this document are the competent Authorities, Healthcare Professionals, Supervisory Bodies, Companies, Workers and the Population.

Acronyms

BPR	Biocidal Products Regulation (Regulation (EU) 528/2012)
CLP	Classification, Labelling and Packaging Regulation (EC) 1272/2008
COVID-19	Coronavirus Disease 2019
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
IARC	International Agency for Research on Cancer
MERS	Middle East Respiratory Syndrome
PMC	*Presidio Medico-Chirurgico*
PT	Product Type
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals Regulation (EC) 1907/2006
SARS	Severe Acute Respiratory Syndrome
SARS-CoV-1	Severe Acute Respiratory Syndrome CoronaVirus 1
SARS-CoV-2	Severe Acute Respiratory Syndrome CoronaVirus 2
v/v	volume/volume
w/w	weight/weight
CDC	Centers for Disease Control and Prevention
WHO	World Health Organization

Executive summary

This document presents and discusses the technical and scientific evidence currently available on the safety and efficacy of ozone for sanitization treatments in reference to the ongoing COVID-19 epidemic.

Currently in Italy, from a regulatory point of view, ozone can be marketed and used exclusively as a sanitizer; for any use as a disinfectant, i.e. as a product specifically designed to combat (reduce, eliminate, render harmless) microorganisms, it is necessary to wait for the completion of the assessment at European level pursuant to Regulation (EU) 528/2012 (BPR) on biocides.

With regard to the identification and characterization of hazards, ozone is a hazardous substance both for its intrinsic physical, toxicological and ecotoxicological properties. The main toxic effects of ozone can be traced back to the same properties that determine its effectiveness, namely the ability to act as an oxidizing agent. This toxicity mechanism causes damage to cell membranes with directly exposed tissue lesions: respiratory system, eyes, mucous membranes, skin. Epidemiological studies have documented inflammatory effects and increased susceptibility to respiratory infections associated with the use of ozonisers for air purification, especially by vulnerable subjects such as children. The available data indicate that the toxic effects of ozone can arise following prolonged exposure to ozone concentrations in the air above 0.1 mg / m^3.

Regarding long-term effects, ozone is one of the components of air pollution, which is classified as a Group 1 carcinogen (Carcinogenic for humans) by IARC (2016). On the other hand, IARC has not so far carried out any systematic assessment of the carcinogenic potential of ozone as such. In 2020, the US EPA concluded that there is no adequate evidence to conclude whether or not there is a causal relationship between ozone exposure and cancer risk. However, in water containing bromide ion, ozone treatment can induce the formation of bromate, which is a potentially carcinogenic compound. Considering the potential criticality where there is prolonged human exposure to ozone-treated waters, the formation of bromate must not exceed the provisional guide value of 10 µg / L, proposed by the WHO (2017) and adopted in the proposal for the recast of the Directive EU on the quality of water intended for human consumption (2019).

Other dangers include oxidative damage to food, equipment and other materials present in the treated environments: in particular, altered functionality of the work equipment can be associated with health risks. In addition, toxicity to environmental organisms (ecotoxicity), especially aquatic ones, should be mentioned.

Irrespective of its use as a sanitizer, there is exposure to ozone in indoor environments: concentrations depend on internal sources (e.g. printers, photocopiers), air exchange, reactions with other indoor pollutants and outdoor contribution. In indoor environments, ozone can interact with Volatile Organic Compounds (VOCs) emitted by materials and consumer products. This can lead to the secondary production of toxic substances (e.g. formaldehyde) and PM_{10} and $PM_{2.5}$, contributing to the inhalation toxicity of ozone. In the absence of specific indoor sources, the indoor/outdoor ozone ratio was estimated in a range between 0.2 and 0.7.

With regard to the hazard classification and guiding values in the European Union and Italy, to date, within the framework of Regulation (EC) 1272/2008 (CLP), concerning the hazard classification of substances and mixtures, no harmonized classification of ozone has been carried out in the European Union. Also in Europe, in the context of Regulation (EC) 1907/2006 (REACH), concerning the registration, evaluation, authorization and restriction of chemical substances, companies interested in the registration of ozone have submitted a classification proposal (self-classification) as a substance which can cause or aggravate a fire; lethal if inhaled; causes serious skin burns and serious eye injuries; damages organs in case of prolonged or repeated exposure by inhalation; and very toxic to the aquatic environment with long-lasting effects.

Also, in reference to the health of workers, the European Union has not set any Indicative Occupational Exposure Limit Values (IOELV) although some Member States have established national limit values for both short- and long-term occupational exposure.

Nationally, Annex XXXVIII of Legislative Decree 81/2008 does not include any limit value for occupational exposure for ozone. However, in the absence of national and EU values, in the Italian regulatory framework, the reference (similar to what has been adopted by the other EU Member States) is represented by the *TLV®–TWA* of the *American Conference of Governmental Industrial Hygienists* (ACGIH) which has recommended different values for ozone in relation to the workload and cumulative exposure duration, in view of the volumes of air breathed. For example, the limit value for an 8-hour workday ranges from 0.1 (heavy work) to 0.2 (light work) mg/m^3.

The WHO guidelines for outdoor air quality recommend a guide value of 100 μg (0.1 mg) / m³ for 8 hours, substantially overlapping with the parameters recommended by ACGIH.

This document reviews the use and effectiveness of ozone in different contexts, from sanitizing environments to devices, to the food sector, to water treatment.

Ozone is able to rapidly degrade organic compounds (sanitization); at generally higher concentrations, it is also able to quickly deactivate a wide range of pathogens (bacteria, including spores, viruses, protozoa). Operating conditions should be carefully selected as the effectiveness of ozonation processes varies significantly depending on the characteristics of the environment to be sanitized, as well as possible contraindications (e.g. oxidative damage to products or equipment).

As to the effectiveness against SARS-CoV-2, a disinfectant action is entirely plausible considering the mechanisms of ozone action. On the other hand, there is currently no direct evidence of efficacy obtained in controlled studies. This lack of validated information is not limited to ozone but is common to several active substances being evaluated, such as biocides.

Multiple therapeutic indications have also been proposed for ozone, those supported by stronger evidence. A beneficial effect in COVID-19 patients is also theoretically possible. However, while waiting for evidence from clinical trials, it should be recalled that it is difficult to predict the overall effect of treatment, especially in the case of patients in clinically serious conditions, especially given the toxicity and oxidant potential of ozone.

Ozone generators are currently promoted as work-friendly sanitization devices. On the market, there is a wide availability of products with different characteristics and production capacity depending on the use to which they are intended. Low-capacity equipment is suitable for small environments and is typically equipped with non-modifiable pre-defined programs and often an ozone conversion device at the end of delivery. The equipment with the highest production capacity is obviously intended to operate in large environments. In any case, before using ozone for the treatment of premises, it is necessary to assess the risk of exposure to both the operators responsible for sanitization operations and the staff who benefit from the sanitized premises. Operators must be trained and experienced and equipped with suitable Personal Protective Equipment (PPE).

The toxicity and oxidizing action of ozone is a limiting factor in its use. This has stimulated the development of systems for the instant decomposition of its residues found in indoor environments where they are used. Conventional air removal technologies are based on the use of activated charcoal filters or catalysts based on noble metals or oxides from other transition metals. Photocatalytic oxidation is particularly promising among emerging technologies.

Conclusions and recommendations: in light of the available information, the application of ozone for sanitization can be useful in different environmental contexts. However, in relation to its dangerous properties and associated risks, ozone generators should be used after a thorough risk assessment, taking appropriate organizational measures in order to carry out the sanitization process safely. For the reasons mentioned above, it is therefore not recommended that non-professional operators use it in the home.

Considering both the dangers associated with ozone and the benefits of proper use, it is entirely desirable to have timely regulation at EU level, on the basis of the conclusion of the ongoing evaluation process under the Biocide, REACH and CLP Regulations.

An effective disinfectant action against SARS-CoV-2 is fully plausible; however, further studies would be useful to define protocols for effective and safe “sanitization” of environments/surfaces, so that essential parameters such as concentration and contact time can be assessed. A beneficial effect in COVID-19 patients is theoretically also possible, but controlled clinical trials on safety and efficacy are essential, especially in the case of patients in clinically serious conditions.

Introduction and purpose of the document

This document aims to provide the technical and scientific evidence available to date on ozone in the COVID-19 epidemic context with reference to regulatory status, internationally available assessments, information on ozone-related hazards and risks, toxicity and impact on human health and the environment, substance efficacy such as virucide, the safety of use and precautions to be taken in the *in-situ* generation of ozone in the field of SARS-CoV-2 prevention and control, as well as the different applications, from sanitization of environments to devices, to the food sector, to water treatment.

It should be noted that pending approval as a disinfectant under Regulation (EU) 528/2012 on Biocidal Products Regulation (BPR), ozone can currently be used exclusively as a sanitizer.

This document also examines the therapeutic efficacy of ozone therapy by assessing its safety of use, as well as critical issues and developments in the making. The paper does not consider ozone exposure as an involuntary product from UV radiation in the atmosphere.

However, this document does not look at other substances generated *in-situ* with possible disinfectant or otherwise sanitizing action or other processes in use in the COVID-19 epidemic context and therefore does not allow a comprehensive assessment of the cost-benefit ratio compared to other available systems.

1. Substance, hazard classifications, evaluations of international institutions and organizations and reference values

Ozone (O_3) is naturally generated by dissociation of molecular oxygen into atomic oxygen from electrical discharges into the stratosphere, and in particular in the ozone sphere, concentrated about 25 km above sea level, producing a shielding effect from more than 90% of UV radiation harmful to life on our planet. It is an oxidizing gas and spontaneously decomposes in the presence of oxidable materials, moisture and solid surfaces, it can easily explode in the liquid phase, and therefore it is essential to avoid liquefaction.

The classification of hazards and related ozone assessments are the subject of attention by government and international bodies and agencies such as the European Chemicals Agency (ECHA), the United States Environmental Protection Agency (US EPA), the US Occupational Safety Health Administration (OSHA) and independent scientific institutions such as the International Agency for Research on Cancer (IARC) an autonomous agency within the World Health Organization (WHO). The assessments issued by the US EPA and IARC are not at the national level a cogent provision, nor do they have legal value, but represent a scientific reference in the management of health and environmental issues.

Ozone is a hazardous substance both for its intrinsic chemical-physical, toxicological and ecotoxicological properties. The main effects related to short-term human exposure from a cause-effect relationship is irritation of the eyes and the upper respiratory tract and are hypothesized but no proven to have cardiovascular effects, while repeated long-term exposures are causally associated with effects to the respiratory system. To date, there is no harmonized classification under Regulation (EC) 1272/2008 concerning the hazard classification of substances and mixtures (Classification, Labelling and Packaging, CLP) (Europe, 2008), and the substance is, therefore, self-classified by the manufacturer, importer or downstream user, responsible for market entry, and notified under Article 40 of the CLP, to the ECHA Inventory of classifications and labelling. Please note that the classification is based on the intrinsic properties of a substance ('hazard') and not on the probability of exposure and on considerations of health and environmental risk. In fulfilling registration obligations under the Regulation (EC) 1907/2006 (Registration, Evaluation, Authorization and Restriction of Chemicals, REACH) (Europe, 2006), registrants have self-classified ozone as a substance that may cause or intensify the fire, oxidiser; fatal if inhaled, causes severe skin burns and eye damage, causes damage to organs through prolonged or repeated inhalation exposure, very toxic to aquatic life with long-lasting effects. Some notifiers self-classify ozone as a suspected of causing genetic defects mutagenic suspect; DNA damage would not result from direct interaction with DNA but would be indirectly linked and secondary to ozone-induced oxidative stress. In this regard, the competent German authorities expressed in 2016 to ECHA the intention to propose for ozone a classification and labelling harmonized also as mutagen in category 2 and carcinogen in category 2 (suspected human carcinogens)[1]. The scheduled date for the submission of the classification proposal was 30 December 2018 but, to date, the proposal has not been submitted to the Agency.

[1] The **Registry of Intention** (RoI), managed by ECHA and available with free access on the ECHA website, gives an indication of the classification that will be proposed from the parties who intend to submit a dossier for the harmonization of classification and labelling to the Agency .

The ECHA website also reports that the German Competent Authorities themselves are carrying out for ozone, as required by the evaluation program of all biocidal active substances, pursuant to Regulation (EU) 528/2012 (BPR) (Europe, 2012), an evaluation of any properties as an endocrine disruptor, but even in this case, the evaluation has not yet been completed.

1.1. Available information on ozone carcinogenicity

The possible carcinogenic effect of ozone was assessed by evaluating the association between tumour development and the intensity of exposure in epidemiological studies and on experimental animal models exposed, in general, to high gas concentrations. Human studies are difficult to interpret because the level of specific exposure to ozone is generally not measured. In fact, they are related studies of air pollution of which ozone is only one component.

The IARC has so far failed to systematically assess the potential carcinogenicity of ozone, except as a component of air pollution, classified as a Group 1 carcinogen (Carcinogenic for humans) (IARC, 2016). Some studies have assessed the incidence of tumours in areas with different concentrations of ozone in the atmosphere, trying to isolate the effect of different agents present in extremely complex mixtures, with obvious uncertainties related to concomitant exposure to many risk factors that act as confounders.

A large cohort study by the American Cancer Society (Cancer Prevention Study II, ACS CPS-II) found no association between ozone levels in the air and incidence of lung cancer (the organ most exposed to a gaseous agent).

A previous study assessing the carcinogenic effect of smog on a cohort of Seventh-Day Adventists (characterized by the absence or lower frequency in the distribution of certain risk factors such as smoking and alcohol – AHSMOG study), instead showed an association of ozone levels with incidence and mortality from lung cancer but only in males (Abbey *et al.*, 1999). The results are, therefore, mixed and difficult to interpret.

In terms of studies in experimental animals, some works are available in the literature. In particular, a two-year carcinogenesis study in GLP *(Good Laboratory Practice),* conducted by the US National Toxicology Program (NTP) in which F344/N rats and B6C3F1 mice inhaled ozone, identified a marginal increase in the incidence of bronchioloalveolar adenomas or carcinomas (combined) in female mice and increased incidence of bronchioloalveolar carcinomas in male mice and bronchioloalveolar adenomas in female mice (NTP, 1994).

The IARC Advisory Group to Recommend Priorities, which recommends priorities in the framework of the IARC Monographs programme as to ensure that the Monographs evaluations reflect the current state of the scientific evidence relevant to carcinogenicity, assigned to ozone “medium priority” in the Report for the period 2020-2024 (IARC, 2020).

Priority is assigned on the basis (i) of evidence of human exposure and (ii) of the extent of available evidence for evaluating carcinogenicity (i.e. the availability of relevant human cancer, experimental animal bioassay, or mechanistic evidence to support a new or updated evaluation). In particular, the “medium priority” attributed to ozone is based on epidemiological cohort studies, and case-control studies cited in IARC Monographs Volume 109 (IARC, 2016) demonstrated null associations between exposure to ozone and cancer risk; moreover most of the studies published after 2016, including several cohort studies and two meta-analyses, found no evidence of association between ozone exposure and cancer risk.

The US EPA periodically updates scientific knowledge of the six major air pollutants, including ozone, on the mandate of the *Clean Air Act.* To this end, the US EPA's Center for Public Health and Environmental Assessment (CPHEA) develops Integrated Science Assessments (ISA) that summarize, for each pollutant, knowledge of health and wellness effects.

In April 2020, the US EPA published an updated ISA related to ozone, concluding that "evidence describing the relationship between ozone exposure and cancer remains inadequate to understand the presence or absence of a causal relationship" (US EPA, 2020).

1.2. Direct and indirect ozone toxicity

At the cellular level, the main toxic effects of ozone can be traced back to its ability to oxidize and per-oxidize biomolecules both directly and indirectly. Ozone, in fact, by rapidly decomposing in the watery phase can give rise to a number of Reactive Oxygen Species (ROS) including free radicals and hydrogen peroxide (H_2O_2 oxygenated water) that cause alterations in the structure and function of biological macromolecules. The main mechanism of action of ozone is the lipid peroxidation. ROS and free radicals attack lipids containing double bonds and in particular, polysaturated fatty acids, causing damage at the cellular level, especially to membrane phospholipids. The toxicity of ozone also depends on its ability to oxidize amino acids by irreversibly altering the structure and function of proteins. The resulting oxidative stress can also cause indirect damage to nucleic acids.

With regard to the possible impact on human health, although low-concentration ozone is not particularly toxic, at high concentrations it may be responsible for alterations of epithelial permeability, resulting in a reduction in lung function. It can also worsen bronchitis or asthma symptoms and cause other disorders such as burning eyes, headache, weakness, side effects that need to be carefully monitored, especially in the case of operators in charge of cleaning indoor environments. Exposures of a few hours to ozone levels from 0.2 mg/m^3 (expressed as an hourly average) are able to cause irritating symptoms on the eye mucous and the first airways; transient reductions in respiratory function have been observed in children even at lower levels of ozone (0.12 mg/m^3). Short-term effects are generally reversible, meaning they cease once individuals are no longer exposed to high levels of ozone. However, it is possible that damage from repeated exposures can induce permanent changes in the lung, with long-term effects, and reduced lung function.

The environmental risk, as a result of the use of ozone for the treatment of surfaces/environments, etc. appears at present negligible, given the high percentage of ozone normally present in the atmosphere. The only scenario assessed to date, as part of the authorization procedure as a biocide under the BPR, is that of spillage into the sewer system for which the possible long-term effects are under consideration.

Unlike other *in-situ* generated active substances, such as chlorine, which release polluting residues, ozone decomposes to oxygen, and this could be beneficial to the environment.

Ozone has a characteristic pleasant odour in concentrations of less than 2 ppm that becomes pungent and irritating at higher concentrations (ACGIH, 2019), with a hint of cloves, hay or chlorine (its name comes from the Greek "smell"). The olfactory perceptibility threshold for humans is between 0.02 ppm and 0.05 ppm, so it is already recognizable at very low concentrations and therefore, those potentially exposed are warned in advance of exposure to high concentrations that are potentially harmful to health. The smell, however, is not a reliable indicator of the concentration in the air as, in a short time of exposure, adaptation to the odour itself occurs.

The WHO Guidelines for Outdoor Air Quality (WHO, 2005) recommend a guide value expressed as the average daily maximum concentration calculated at 8 hours of 100g/m^3 (0.05 ppm).

The National Institute for Occupational and Safety Health (NIOSH) points to an IDLH (Immediately Dangerous to Life or Health) of 5 ppm (10 mg/m^3) based on acute toxicity data for inhalation in humans (Deichmann & Gerarde, 1969; Kleinfeld *et al.*, 1957). IDLH, a level to be used in emergencies, is defined as the maximum concentration of a toxic substance to which a healthy person can be exposed for 30 minutes, without suffering irreversible effects for their health or without the effects of exposure preventing escape (NIOSH, 1994).

Under real conditions, the natural decay time required to make the premises accessible is at least 2 hours; and still, until it reaches a residual concentration below the threshold of olfactory perceptibility for humans, between 0.02 and 0.05 ppm, and equal to about 1/10 of the threshold of 0.2 ppm defined as safe in work environments for a maximum exposure time of 2 hours.

1.3. Workplace benchmarks, safety and precautions to take

Based on information about the intrinsic properties of ozone, its use in work environments requires specific precautions.

In particular, it is necessary to assess the risk of exposure to both sanitization treatment workers and workers who have to do their work in treated environments.

In fact, since it is a dangerous substance used in a workplace, it is necessary to apply the provisions of Legislative Decree 81/2008, Title IX - Dangerous Substances, Cape I - Protection from chemical agents, carrying out a risk assessment and taking general and specific measures of protection and prevention, providing provisions in case of accidents or emergencies, and carrying out correct information and training of workers.

In particular, for the purposes of reducing chemical risk, it is always recommended to consider the possibility of using substances and/or processes that are not dangerous or less dangerous (replacement principle) (Legislative Decree 81/2008, Art. 15, co.1, lett. f). Workplace sanitization procedure.

Sanitization refers to the complex of cleaning and/or disinfection procedures and maintenance of good air quality. Ozone generators are currently promoted as work-friendly sanitization devices. Manufacturers and dealers of ozone generators often present ozone as a natural disinfectant and ozone itself as a healthy variant of oxygen.

As previously seen, ozone is a gas capable of causing adverse effects on human health with properties very different from oxygen. Due to its toxicological properties and its ability to oxidize different materials, the sanitization of work environments by ozone must take place in the absence of people, using a sufficient dose and time of use to eliminate microorganisms and viruses but with minimal deterioration effects on materials. It is well known that environmental concentrations of ozone acceptable to human health may not be sufficiently effective as sanitizers.

Therefore, all possible precautions must be taken including operations aimed at reducing the residual concentration of ozone and the concentrations of pollutants that can form by a secondary reaction between ozone and Volatile Organic Compounds (VOCs) (e.g. formaldehyde and other substances of particular sanitization interest).

There is a wide availability of products with different ozone production capacity on the market. The time required to reach the desired concentration in the air has to be calculated according to the volume of the environment to be treated.

Typical stages of a course of treatment are:

- the conditioning phase, in which ozone is injected into the environment to achieve the planned ozone concentration;
- the treatment phase, which lasts the time it takes to obtain an effective treatment compared to the expected results;
- ozone conversion phase, which ensures the elimination of ozone from the air in the treated environment and continues until ozone concentrations are met to protect workers' health.

In this regard, some ozone generators are equipped with a catalyst that, after treatment, turns all residual ozone into oxygen.

If according to the supplier's guidance, it is not possible to determine the time and manner of environmental aeration necessary to reach safe concentrations for workers, depending on the environmental volume and the amount of ozone used, measures will need to be taken to determine them.

1.4. Occupational exposure limit values

For ozone, the European Union has not set any Indicative Occupational Exposure Limit Value (IOELV), but some Member States have set national limits for both long-term and short-term occupational exposures.

Even at the national level, Annex XXXVIII of Legislative Decree 81/2008 does not include any limit value for occupational exposure to ozone. However, in the absence of national and EU values, in the Italian regulatory framework, the reference is the TLV®–TWA[2] of the American Conference of Governmental Industrial Hygienists (ACGIH) which recommended different TLVs for occupational exposure to ozone in 1999 that incorporate the effects of ozone concentration, workload and cumulative exposure duration (as volumes of breathed air change) (ACGIH, 2019).

In the REACH ozone registration dossier, a DNEL[3] (Derived No Effect Level) was identified for long-term inhalation for workers of 24 μg/m^3 (ECHA, 2020).

Table 1 shows the occupational exposure limit values, which are available in the IFA GESTIS database[4] adopted in several European and non-European countries. Values range from a minimum of 0.05 ppm (about double the olfactory threshold of 0.02 ppm) for long-term exposures to a maximum of 0.3 ppm for short-term exposures. Ireland and Spain have adopted the same limit values as ACGIH.

[2] TLV®-TWA (Threshold Limit Value - Time Weighted Average): the concentration limit for a normal 8-hour workday and a 40-hour workweek to which nearly all workers may be repeatedly exposed, day after day, without adverse effect.

[3] Generated under REACH, DNEL is the level of exposure to a substance above which humans should not be exposed. DNELs are established by manufacturers and importers on their own responsibility and are published in this form by the ECHA.

[4] Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung (IFA, Institute for Occupational Safety and Health of the German Social Accident Insurance). IFA https://limitvalue.ifa.dguv.de/WebForm_ueliste2.aspx

Table 1. Occupational exposure limit values in several European and non-European countries

Country or Agency	Value limit for 8 hours		Value limit for short-term exposures (15 minutes)	
	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 work. moderate or light < 2 hours 0.2	Heavy work. moderate or light < 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 work. moderate or light < 2 hours 0.2	Heavy work. moderate or light < 2 hours 0.4
Sweden	0.1	0.2	0.3	0.6
Switzerland	0.1	0.2	0.1	0.2
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 work. moderate or light ≤ 2 hours 0.2	Heavy work. moderate or light ≤ 2 hours 0.4
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)	0.1	0.2		
New Zealand			0.1	0.2
People's Republic of China			0.15	0.3
Singapore			0.1	0.2
South Korea	0.08	0.16	0.2	0.4

1.4.1. Risk due to electromagnetic field

Ozone generators are high voltage electrical equipment, with all the consequent safety implications. The crown effect that is generated, whatever the frequency of the current applied, produces a wide spectrum of radiofrequency dependent on many parameters (Moongilan *et al.*, 2009).

Protection of the health of workers exposed to electromagnetic fields is also regulated by Legislative Decree 81/2008, which in art. 209, co.5, lett d) states that the employer should pay attention to the health effects of particularly sensitive workers, such as those carrying implanted, active or passive medical devices, or medical devices brought to the body and pregnant workers.

The REACH ozone registration dossier, available for free access on ECHA's website, in the section "Guidance on safe use – Exposure controls / personal protection" lists among the preconditions for safety the prohibition of entry to premises where ozone generators are present to pacemakers and other electrical devices (ECHA, 2020). In the risk assessment, the extent of the area in which the electromagnetic field exceeds the benchmarks set for the protection of human health should be taken into account, which, depending on the configuration of the generator, can range from a few centimetres to a few meters. However, too high a radio emission frequency can compromise the proper functioning of other critical devices.

1.4.2. Risk mitigation measures

For the safe use of ozone in occupational environments, appropriate technical and organizational prevention and protection measures, including Personal Protective Equipment (PPE) must be adopted. Overall, these measures must cover both treatment workers (professional workers who are subject to dedicated health surveillance plans, specific training on the characteristics and use of ozone, equipped with PPE if action is needed, etc.) and, where necessary, peoples working in ozone-treated environments.

Recommendations on the safe use of ozone (ECHA, 2020) are available on the ECHA website. In particular, the following risk mitigation measures are recommended.

With regard to the information available on the ECHA website, it is noted that the data in the REACH registration file, provided by manufacturers and importers as part of the registration process, relate in particular to the industrial use of ozone. Further out, it is stressed that such data should be used with great caution as there is no guarantee that the information contained in the file is correct or that the file is in accordance with REACH. In fact, such data is hosted on the ECHA website but not necessarily verified by the Agency, which currently only examines 20% of the registration files relating to each tonnage band. In addition, the contents of the files can be changed by registrants without any notice.

Technical measures

- Ozone generation systems must be placed in closed, lock-in rooms.
- The premises where ozone generation systems are located should not be used as permanent workplaces. If this is not possible for reasons related to the process, it is necessary to ensure that the concentration of ozone in the air in the workplace does not exceed the limit value of occupational exposure.
- The rooms, where ozone loss can occur in the event of a failure, should be monitored with optical and acoustic ozone detectors that stop ozone generation when triggered. This is not necessary for rooms where ozone-containing pipes contain no removable connections, which have been examined for possible losses by a qualified person.
- In premises with ozone generation systems, appropriate signage must be present.
- The rooms where the ozone generation systems are placed must be equipped with an installed ventilation exhaust so that the suction is positioned at floor height and automatically ignites when the gas detector is activated; at least three air parts must be guaranteed per hour.
- Use ozone destruction units (thermal and/or catalytic) to remove residual ozone from the air after treatment.
- Ability to monitor ozone concentrations in the air: control in air quality rooms through gas concentration measurement systems.

Organizational measures

- Instructions for the use of ozone generators must be provided before use and then at least once a year.
- An escape and rescue plan must be put in place if the workplace requires it.
- Only procedure workers can access the areas during treatment or after completion to ensure that the conditions for the return of workers in these areas are met.
- Replace garments that have been in contact with or absorbed ozone and aerate clothing away from any source of ignition.
- People with heart pacemakers or other electrical devices do not have to log into an environment with an ozone generation system.
- Given the length of the treatment procedure and the time required for the complete decay and/or elimination of residual ozone, interventions must be planned in such a way as to ensure the safe return of the workers occupying the premises and treated areas. This can be achieved, for example, by using programmable automated systems that allow operations during the downtime of production activities.

Personal protection

In view of the danger associated with the use of ozone, as has already been pointed out, the treatment of environments must take place in the absence of staff. Therefore, in general, the use of PPE is limited to any emergencies where access to the environments being treated is necessary and, therefore, with dangerous concentrations of ozone in the air.

In addition to PPE for the protection of hands (gloves), body (chemical protection suits) and eyes (mask glasses), respiratory tract protection devices are of fundamental importance.

In the case of low concentrations or short-term operations, the use of a filtering device with a NO-P3 gas filter (blue-white code) or CO (black code) is recommended. In the case of long-term operations, the use of a breathing apparatus (e.g. air systems or compressed air breathing apparatus) is recommended.

1.4.3. Impact of ozone on materials and equipment in workplaces

Ozone is a strong oxidizing agent; therefore, it can damage both functionally and aesthetically (colour, resistance, durability, etc.) materials and equipment present in the workplace, creating new risks in relation to the altered functionality of work equipment or generating additional costs for companies in relation to the need to restore/replace damaged materials or equipment.

1.5. Conclusions

From the above, it appears that, even in the absence of community values, benchmarks are available in the occupational environment (e.g. ACGIH). The risk mitigation measures described above, particularly related to the industrial use of ozone, can also be adapted to different occupational exposure scenarios.

2. Impact on indoor environments and materials: purification from chemical pollutants

Considering the general indications that derive from Legislative Decree 81/2008, indoor environments intended as workplaces (e.g. schools, offices, banks, post offices, hospitals, libraries, gyms, means of transport), have substantial differences in terms of structural characteristics, types of activities, expected levels of exposure and sensitivity of the potentially exposed population (e.g. asthmatic subjects or with respiratory problems) or other vulnerable categories (such as children).

As is well known, the situation of Indoor Air Quality (IAQ) or, more generally, air quality in the typical indoor workplace is extremely low although a number of initiatives are underway to make it possible to enact specific legislation on the subject and to increase the use of specific WHO references.

One of the critical issues is the absence of an integrated national indoor air quality policy, with specific regulatory references including national numerical values (e.g. guidance values, reference values, action values, etc.) while, for a correct strategy of monitoring and evaluation of results, some volumes of the series *Rapporti ISTISAN* (reports published by the ISS working group on Indoor Pollution) are available taking into account the outcome reported in the WHO documents. In the absence of national references, one can use those in the WHO's Air Quality Guidelines, which in the case of ozone have a guide value that has been reduced from 120 µg/m^3 to 100 µg/m^3 as the maximum daily average calculated at 8 hours (WHO, 2005). Alternatively, by analogy, other standards such as those relating to ambient air for which specific legislative references are available (such as Legislative Decree 155/2010 and subsequent amendments and additions) can be referred to for a limited number of pollutants including ozone with a value of 120 µg/m^3 as a daily average over an 8-hour period for the protection of human health, information threshold[5] of 180 µg/m^3 and an alarm threshold[6] of 240 µg/m^3, both as an hourly average.

Europe in addressing the complex issues related to indoor environments has implemented, as part of the concerted European Collaborative Action (ECA) Indoor Air Quality and its Impact on Man (currently Urban Air, Indoor Environment and Human Exposure), a multidisciplinary collaboration between scholars who deal in an integrated way with the aspects related to indoor environments (sources, quality and quantity of chemicals and biological products, thermal comfort, energy consumption and ventilation) by creating a series of reports. Report 26 "Impact of Ozone-initiated Terpene Chemistry on Indoor Air Quality and Human Health" reports the status of ozone research and its impact on IAQ and human health as well as products that form as a result of ozone reactivity leading to degradation of Volatile Organic Compounds (VOCs) (oxidation) with the formation of stable products such as aldehydes and peroxides, some of which are irritating to the eyes and airways. The Report indicates the need for greater knowledge of risk characterization for acute and long-term exposure effects (upper and lower eyes and airways) (EC, 2007).

Several countries such as Canada (Health Canada: Residential Indoor Air Quality Guideline Ozone, 2016) have developed a benchmark for ozone in indoor environments of 40 g/m^3 as an average over an 8-hour period. This concentration is considered more representative of overall ozone exposure. The paper shows that the value of 40 µg/m^3 represents half of NOAEL (No Observed Adverse Effect Level) derived from a controlled population exposure study (Adams, 2002).

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[5] Information threshold: level beyond which there is a risk to human health in the event of short-term exposure for some particularly sensitive groups of the population as a whole and the achievement of which requires ensuring adequate and timely information.

[6] Alarm threshold: level beyond which there is a risk for human health in the event of short-term exposure for the population as a whole and the achievement of which requires immediate measures to be taken.

The values are used by government control bodies to identify/evaluate the sources present and to collect specific information to be used for the necessary solutions/mitigations or to assess the effectiveness of the actions taken.

In the case of ozone in indoor environments, several studies have shown that concentrations depend on internal sources (e.g. printers, copiers and many others and devices designed for internal use that release ozone intentionally or unintentionally), air turnover, reactions with other indoor pollutants and *outdoor* contribution. In the absence of specific indoor sources, the indoor*/outdoor* ratio of ozone is generally between 0.2-0.7 (Kirchner *et al.*, 2002).

Ozone can interact with the VOCs emitted from the different sources (e.g. consumer materials and products such as terpenes) present in indoor environments leading to the production of VOCs that can be more toxic and also carcinogenic (e.g. formaldehyde) and PM_{10} and $PM_{2.5}$ or through homogeneous and heterogeneous reactions with indoor materials (Poppendieck *et al.*, 2007; Nicolas *et al.*, 2007).

Several studies related to the use of ozonisers for air purification by chemical pollutants document inflammatory effects and increased susceptibility to respiratory infections related to the presence of ozone, especially in the most vulnerable population such as children (Salonen *et al.*, 2018; Kunkel *et al.*, 2010; Trich *et al.*, 2006; Mudway *et al.*, 2000; Levy *et al.*, 2001).

Studies of ozone-generating devices for air purification and ozone levels find that exposures to concentrations of 120 ppb (0.12 ppm^3) are associated with a variety of acute effects (e.g. respiratory irritation, cough, headache, etc.) (Boeniger, 1995). In addition, ozone has been associated with school absenteeism due to respiratory diseases, drug use, respiratory problems associated with asthma, decreased respiratory function and increased hospitalizations for asthma (Salonena, 2018).

It has been estimated that an increase of 10 $\mu g/m^3$ of ozone (1 hour) leads to an average increase in mortality of 0.21% (Levy et *al.*, 2005)

The French *Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail* (ANSES) published the document "Identification et analyse des différentes techniques d'épuration d'air intérieur émergentes", which examines the main techniques used for the purification (from chemicals) of indoor air, catalysis and photocatalysis, ozonation and ionization (ANSES, 2017). These chemical air purification devices are intended for the whole population and can cause adverse effects on sensitive individuals with asthma and/or allergies. The bibliography collection also shows that these devices, used to reduce chemical pollutants, can purify IAQ by generating new pollutants (PM_{10}, $PM_{2.5}$ formaldehyde, etc.). ANSES, based on the scientific literature examined, reports that ozone can be effective on chemical and biological contaminants but at concentrations in the air that can have an impact on human health. On the contrary, ozone concentrations that have no effect on human health are of little effectiveness. Ozone generators would produce concentrations in the air above the guide value of 100 $\mu g/m^3$ set by the WHO (ANSES, 2017).

Also, the *Institut National de Santè Publicque du Quebec* (INSPQ) in the document "Analyse de l'efficacité des dispositifs d'épuration de l'air intérieur en milieu résidentiel. Revue De La Littérature Scientifique" of 2019 reports, in the case of indoor environments, how health organizations generally recommend eliminating ozone sources and avoiding unnecessary exposures to this gas (INSPQ, 2019).

The use of this type of device to control indoor air contaminants is therefore not recommended as individuals using these devices (ozonisers to purify the air from chemicals) are generally exposed to concentrations that exceed the exposure thresholds recommended by health organizations (EPA, 2014; Gouvernement du Canada, 2012; Britigan *et al.*, 2006; Zhang *et al.*, 2011).

2.1. Conclusions

Considering the sanitization implications of using ozone indoors for air purification from chemical pollutants, some authors express concerns about the potential health risks that need to be investigated (ANSES, 2017; INSPQ, 2019). In view of the studies currently available and in view of the possible concentrations of ozone and secondary products (VOCs, PM_{10}, $PM_{2.5}$) that are formed in indoor air, the further investigation continues.

3. Biocide efficacy: regulatory framework and applications

3.1. National, European, International Regulatory Framework

Ozone generated *in-situ* from oxygen is an active ingredient with "biocidal" action and is under review (assessment) under Regulation (EU) 528/2012 on biocides (BPR) (Europe, 2012) by the Member States of Germany and the Netherlands, as a disinfectant for surfaces (PT2 and PT4), for disinfection of drinking water (PT5) and for use in the cooling towers of industrial plants (PT11). Although the evaluation of the relevant dossier has not been completed, a large database is available, confirming its microbicide effectiveness that also includes viruses (ISS, 2020).

Pending the outcome of the evaluation process at European level as a biocide, the issue of the authorization to market in Italy as a "disinfectant" is not possible, since the national authorization as Presidio Medico Chirurgico (PMC) – disinfectant involves the only production in manufacturing plant that must be authorized by the Ministry of Health. This condition, in the case of *in-situ* generation (out-of-manufacturing plant), is not practicable. Pending the finalization of the European assessment, it may be present on the national market as a product with a "sanitizing" and non-disinfectant action. The above is clearly stated in the note of the Ministry of Health of 22 February 2019 (Ministry of Health, 2019) which reads:

> "All products claiming disinfectant action on the label must be classified as biocide products – and are only marketed after obtaining a specific marketing authorisation from the Ministry of Health or the European Commission. Products that claim the term "sanitizing/disinfectant" fall within the definition of biocide products and are therefore subject to the relevant authorization regime."

The same problem also applies to other products generated *in-situ,* such as active chlorine generated by electrolysis, by different precursors, using mobile/portable devices. Although BPR assessments are also ongoing, at national level marketing is currently only allowed as environment sanitizers and therefore in accordance with Regulation (EC) 648/2004 of the European Parliament and the Council of 31 March 2004 relating to detergents (Europe, 2004).

With regard to the virucidal activity of ozone, it is known that inactivation of viruses occurs quickly as a result of ozonation, although it requires the administration of gases at higher concentrations than is necessary for bacteria.

The International Ozone Association itself, a non-profit organization for the information of ozone specialists around the world, on its website (www.ioa-pag.org), while confirming that ozone is effective for inactivating numerous viruses, states that it is not aware of any research and tests conducted specifically on the SARS-CoV-2 coronavirus.

The absence of specific data with the tests planned to demonstrate efficacy as a biocide is common to several disinfectants on the market today, both as PMCs and Biocides, for which no efficacy data is available against SARS-CoV-2.

A single study to date has assessed the effect of some disinfectants specifically on the suspended SARS-CoV-2 virus, noting that all those tested – bleach (sodium hypochlorite) for domestic use at various dilutions, 70% ethanol, 7.5% iodine povidone, 0.05% chloride and benzalkonium chloride at 0.1% – made the virus undetectable after 5 minutes of incubation *(*Chin *et al.*, 2020).

In this regard, it is necessary to represent that the evidence of efficacy for the authorization as a disinfectant, valid and applicable for all the active substances so far authorized or under review – such as ethanol (in review as active substance sodium hypochlorite (already authorized as a biocide), quaternary admonitions, etc. – must be carried out according to the two technical standards (UNI EN 14476:2013 and UNI EN 16777:2019), provided both nationally and in Europe, for the evaluation of the effectiveness of products to be authorized as PMC/Biocides. The two rules require efficacy tests to be carried out on species that have been identified as representative of enveloped viruses and naked viruses. Specifically, in the suspended test according to UNI EN14476, poliovirus, adenovirus and mouse norovirus (*Murine Norovirus*, MNV). For a *claim* against enveloped viruses only, the effectiveness against the vaccine virus is evaluated. Adenovirus, norovirus and vaccine viruses are used to test the effectiveness on surfaces according to UNI EN16777 (poliovirus is excluded as it is highly sensitive to drying). Depending on the viruses examined, and whether the tests were carried out according to both rules, against a logarithmic reduction > 4, one can claim on the label an action against enveloped viruses, limited virucide or complete virucide.

It is often difficult to compare the following in published scientific studies with data obtained according to the technical standards EN (standard) required by the provisions, not being clearly defined, in published studies all the parameters that are taken into account in the application of the standard protocols provided for the demonstration of effectiveness and authorization as a disinfectant.

For example, as indicated, studies are available indicating the effectiveness of ozone on norovirus (Hudson *et al.*, 2007). Mouse norovirus is one of the species considered in the standard EN protocols but, always in view that what is considered in a scientific study is not always uniquely comparable to the conditions of a standard protocol, in the study cited the norovirus in question was generically identified as feline calicivirus – also in the family of norovirus but not the same used in EN standards. For example, it is known that mouse norovirus is more stable at large pH intervals than feline calicivirus, but both have a similar inactivation curve between 63 and 72°C (Cannon, 2006).

During this period, the US EPA's N-list is often cited, which includes authorized biocide products in the US with an indication of which are active against viruses. Ozone is not mentioned among these, as it is included in the list of *Pesticide Devices* and therefore falls into a different regulatory framework. As such it is classified as "tool/machine used to destroy, ward off, trap or mitigate harmful agents, including bacteria and viruses".

3.2. Applications in decontamination of environments

The biocide action of ozone for the treatment of environments is evidenced by several studies available in the literature. An initial report indicated that ozone treatment was not sufficient for the decontamination of hospital environments against Methicillin-Resistant *Staphylococcus Aureu*s (MRSA) (Berrington *et al.*, 1998). Subsequent laboratory studies have shown that it was possible to achieve an $R_{log}4$ (99.99%) *E. coli* and *S. aureus* after exposure to ozone concentrations between 300 and 1500 ppm, in a time interval between 10 seconds and 8 minutes (Kowalski et *al.*, 2008). At the time of the SARS outbreak, Kenneth (2003), to test the efficiency of ozone in the disinfection of environments, showed how keeping an ozone level of 2.5 ppm for 30 minutes resulted in a 93% decrease in microbial charge. The same study inferred from this data that, since viruses are much more sensitive to ozone than bacteria, an important clearance of viruses could be obtained at the same concentrations and contact times.

In addition, in one study, *in-situ* generated ozone was effective in inactivating norovirus in hotel rooms, ship cabins and healthcare environments (Hudson et *al.* 2009) and, in 2020 Dubuis *et al.* reported that ozone used at low concentrations in combination with high relative humidity is effective against bacteria and noroviruses present in the air with log reductions of at least two orders of magnitude with low concentrations

of ozone (1.13-0.26 ppm and 0.23-0.03 ppm, respectively, at various levels of relative humidity for an exposure of 70 minutes).

However, further studies carried out to predefined standards, would be useful in order to technically define a protocol for the “sanitization” of environments/surfaces, with indications of concentration, contact time and other parameters determining the effectiveness of sanitizing treatment.

3.3. Applications in the decontamination of reusable medical devices

The use of ozone as a “sterilizing” agent (destruction of all microbial forms, including spores) was declared suitable by the FDA in 2003 for the treatment of reusable medical devices. Ozone is compatible, as shown above, with a large number of materials, including steel, titanium, anodized aluminium, ceramics, glass, silicone, PVC, PTFE Teflon®, polypropylene, polyethylene, etc., and was deemed suitable for the treatment of approximately 4 hours at 30-35°C was able to cause a 6 log decrease in one of the most resistant organisms, *Geobacillus stearothermophilus.*

The Food and Drug Administration (FDA) recently granted an emergency use authorization of the STERIZONE VP4 Sterilizer (Stryker) device that uses hydrogen peroxide vapour and ozone sequentially in a multi-phase process for sterilization of medical devices and also for decontamination of N95 respirators for up to two cycles. The application system must, of course, be adapted from time to time in relation to target materials and microorganisms (FDA, 2020).

Similar awards do not appear in the EU regulatory framework.

3.4. Conclusions

In light of the available information on the effectiveness against viruses, the application of ozone for sanitization can be useful in different environmental contexts. However, in relation to its dangerous properties and associated risks, ozone generators should be used after an appropriate risk assessment and appropriate organizational measures in order to carry out the sanitization process safely.

For the reasons mentioned above the use is not recommended for non-professional users in domestic settings.

4. Food applications: regulatory framework and applications

4.1. Aspects and assessments by regulatory bodies

4.1.1. At EU level

There is no specific regulation at the time for the direct use of ozone on food, with the exception of natural mineral waters (Europe, 2003). The use of substances other than drinking or clean water, to reduce surface microbial contamination in food, was allowed with the entry into force of Regulation (EC) 853/2004 and subsequent changes, which did not affect this general approach. In the European Union (EU), a dossier must be submitted for the use of these substances and the European Food Safety Authority (EFSA) has to assess its potential benefits and risks (e.g. persistence of toxic residues in food) under the proposed conditions of use (Hugas & Tsigarida, 2008).

EFSA has so far not produced a specific opinion on the effectiveness and safety of ozone use; moreover, it does not appear that at present, pending evaluation, neither the European Commission nor the Member States have asked any questions on the subject. In 2012, EFSA discussed ozonation as a disinfection treatment for bottled seawater (considered as a niche product) (EFSA, 2012). In short, EFSA concluded that ozone is generally effective against viruses and bacteria in water, but as gas is toxic and in seawater, it can interact with traces of bromide, forming bromate, which is a carcinogenic substance. To minimize this problem, the concentration in water should not exceed 0.5 mg/L, slightly higher than the usual concentration of 0.4 mg/L. In addition, EFSA notes that ozone treatment is commonly used for water disinfection in bivalve shellfish sewage plants. Since ozone can be toxic to animals, the excess amount must be eliminated from water by forced aeration or filtration with activated charcoal.

Ozone treatment was also considered by EFSA (2011) as part of an opinion on the decontamination of chicken carcasses potentially contaminated with Campylobacter (EFSA, 2011). The opinion did not highlight specific problems for ozone. In general, and in accordance with other opinions, EFSA stressed that decontamination treatments should be considered complementary, not substitutes, to good hygiene practices in slaughter.

4.1.2. In Italy

The Ministry of Health, with the protocol of 31 July 1996 24482, recognized the use of ozone in the treatment of air and water, as a natural garrison for the sterilization of environments contaminated with bacteria, viruses, spores, mould and mites. In 2010, the National Committee for Food Safety (*Comitato Nazionale per la Sicurezza Alimentare,* CNSA) issued an opinion on the use of ozone for the disinfection of cheese ageing chambers (CNSA, 2010). The CNSA, which refers to the Ministry of Health protocol of 31 July 1996 24482, expressed its support for the ozonation of ageing chambers and/or storage environments, provided that there is no food and as long as the treatment is not inconsistent with specific production disciplines. Apart from the well-known oxidizing power of ozone, the opinion did not show against specific indications.

4.1.3. In the USA

Ozone has been recognized as a GRAS (Generally Recognized As Safe) chemical additive since 1997; it is permitted as an additive according to CFR (*Code of Federal Regulations)* title 21 vol 3 – Ref

21CFR173.368. In 2001 it was approved by the FDA as an antimicrobial additive for direct contact (for meat, eggs, fish, cheeses, fruits and vegetables).

4.2. Safety aspects of ozone use in the food sector

With regard to **user exposure** as described above (Chapter 1) ozone has dangerous characteristics which must include measures to minimize exposure of the respiratory tract and mucous membranes. The use must, therefore, be made according to protocols and good practices that keep the exposure of users below the parameters specified in step 1.4.

Of course, the use of ozone on food does not give rise to residues: the "excess" ozone is quickly transformed into oxygen (Prabha *et al.*, 2015). However, use at excessive levels in contact with certain matrices can lead to the formation of toxic compounds.

The scientific literature highlights possible indirect problems related to an impact on nutritional qualities, in particular the reduction of vitamin C and other antioxidants and the increase in peroxides. The available literature shows that these effects exist, that their presence and extent vary in relation to the type of food (and therefore the content of antioxidants, the number of substances, such as polysaturated fats, vulnerable to oxidation, etc.) as well as the extent of treatment. On the other hand, it is very difficult to draw information from the literature for a relationship between the ozone dose and the effect (probably different ratio depending on the type of food). In addition, the published works do not provide any indication of a possible health risk associated with these effects, which are still undesirable. However, these considerations lead to an element of further caution in relation to indiscriminate ozone treatments (Sachadyn-Kr'l *et al.*, 2020; Zhu *et al.*, 2019; Goffi *et al.*, 2020; Singh *et al.*, 2019; Ismail *et al.*, 2018; Ianni *et al.*, 2019)

4.3. Effectiveness of ozone use in the food sector

The use of ozone in the food industry has been extensively investigated for the treatment of biological, chemical and toxicological risks of specific food products that are not suitable or effective with conventional treatments.

Among the possible applications of ozone or ozonated water, there are, for example: i) the reduction of the surface microbial load of ready-to-eat products such as fresh-cut fruit and vegetables in order to extend their shelf-life; ii) the killing of potentially present pathogenic microorganisms, such as *Salmonella*, *Listeria*, *Campylobacter*, pathogenic *E. coli*, etc. on raw materials of animal origin (e.g. meat and fish) or on animal carcasses; iii) the degradation and detoxification of specific mycotoxins in cereals; iv) the degradation and removal of pesticide residues from fruit and vegetables; v) use as an alternative to fumigants for the control of pests; vi) the use for sanitizing surfaces and plants/machinery.

Several studies have specifically assessed the effectiveness of ozone or ozonized water as a virucide in food or agri-food production:

- In a comparison of different virucide treatments (acetic acid, hydrogen peroxide, hydrochloric acid, sodium hypo-chloride, dry heat treatment, treatment with water at 65°C, ultraviolet), ozone has been among the most effective, reducing on average 32% of the concentrations of different plant viruses – *Tomato Mosaic Tobamovirus* (ToMV), *Cucumber Mosaic Cucumovirus* (CMV), *Soybean Mosaic Potyvirus*(SMV), *Lettuce Mosaic Potyvirus* (LMV) – used in the study (Paylan et *al.*, 2014).
- The effectiveness of gas ozone has been assessed in the deactivation of some viruses commonly transmitted by food – human noroviruses, tested through their surrogate, MNV, and hepatitis A virus – inoculated on the surface of raspberries. The study showed that a concentration of 3 ppm with

exposure times of 1 minute was suitable for a reduction >3 log of surrogate norovirus. On the other hand, neither the same concentration/time of application nor higher concentrations and times showed significant efficacy on the hepatitis A virus, thus showing considerable variability in viral resistance to ozone treatments (Brie *et al.*, 2018).

- Similar to the previous study, the study by Predomore *et al.* (2015) assessed the effectiveness of gas ozone (6% in oxygen) for the deactivation of two human norovirus surrogates (MNV and *Tulane Virus*, TV) used on lettuce and strawberries, carrying out treatments ranging from 10 to 40 minutes. Results showed that gas ozone inactivated noroviruses dose-dependently, with a reduction in the infecting virus present on the food surface >3 logs after 40 minutes (Predomore *et al.*, 2015).
- The use of ozonized water (6.25 ppm) was evaluated for the deactivation of noroviruses (surrogates: MNV and TV) in alfalfa shoots (with treatment times ranging from 30 seconds to 30 minutes), highlighting a reduction in viral infectivity ranging from 1.7 to 5.6 log (Wang *et al.*, 2015).
- The effectiveness of ozonized water (6.25 ppm) in the deactivation of noroviruses (surrogates: MNV and *Feline Calicivirus*, FCV) has also been confirmed by Hirneisen *et al.* (2011) which, in experiments on lettuce and shallots experimentally inoculated and treated for times ranging from 30 seconds to 10 minutes, showed a reduction in viral infectivity >2 logs after 1 minute per MNV and after 5 minutes for FCV. The study also showed that the food matrix has a protective effect compared to treatment and confirmed variability in the resistance of different viruses (Hirneisen *et al.*, 2011).

5. Ozone water treatment: regulatory framework and applications

5.1. Effectiveness of ozone against microorganisms in water

Ozone can quickly inactivate a wide range of pathogens (bacteria, spores, viruses, protozoa) (Wang, 2018). Generally, the dose required to ensure effective disinfection of water is higher than that which allows the degradation of organic compounds that, therefore, are co-removed (Xi, 2017). Operating conditions should be carefully selected because the effectiveness of ozonation processes varies significantly depending on the characteristics of treated water (Ried, 2009). According to the US EPA (US EPA, 1999), ozone is more effective than chlorine in water disinfection, with shorter reaction times.

Several studies have assessed its effectiveness in removing human viruses (adenovirus, polyomavirus, coxsackievirus, norovirus, astrovirus and parvovirus) from wastewater (Ried, 2009; Wolf, 2018; Tondera, 2015). The treatment of ozonation leads to the oxidation of viral proteins of the capsid, which results either in the destruction of that structure or in its ability to bind to the cell receptor thus preventing the viral infection of sensitive cells (Ried, 2009). As other disinfectants, its effectiveness is calculated quantitatively in terms of C·t, that is the product between the residual concentration of disinfectant in water and the contact time necessary to remove a predetermined number of the microorganism in logarithmic terms. Table 2 summarizes some of the C·t values for the removal of 2 logs of bacteria, protozoa and viruses following the treatment of ozone-spiked wastewater. Bacteria and viruses are more susceptible than protozoa to the biocide action of ozone as they require lower doses and/or contact times. This implies the need to design the disinfection system with the minimum operating conditions necessary for the removal of protozoa that are the limiting factor of the process.

Table 2. Some C·t values for the removal of 2 logs of bacteria, protozoa and viruses following the treatment of ozonation of artificially contaminated wastewater

Pathogen	Type	C·t (mg O_3·min/L)
E. coli	bacteria	$6{,}0 \cdot 10^{-3}$
C. parvum	protozoa	$3{,}1 \cdot 10^{-0}$
G. lamblia	protozoa	$0{,}65 \cdot 10^{-0}$
G. muris	protozoa	$0{,}24 \cdot 10^{-0}$
Echovirus	virus	$1{,}9 \cdot 10^{-3}$
Adenovirus	virus	$4{,}1 \cdot 10^{-3}$

5.2. Application in the treatment of surface and underground waters to be intended for human consumption

In Italy, water resources used as drinking-water supplies are mostly groundwater (48.0% well water, 36.3% spring water) and, to a lesser extent, surface water (9.9% reservoir water, 4.8% river water, 0.9% natural lake water, 0.1% sea or brackish waters) (ISTAT, 2017).

Water with good characteristics derived from deep springs and wells generally requires simple treatments (such as, for example, sand filtration and/or disinfection) as they benefit from natural self-purification phenomena that occur during the permeation of rainwater through subsurface layers. Surface water and some shallower groundwater require more complex treatments due to their intrinsic characteristics

and their vulnerability to accidental pollution. They typically include sedimentation (in which coarse solids separate from water by gravity), clarification (through which suspended particles are aggregated into larger and more sedimentable flakes by aluminium or iron salts), oxidation, filtration through sand, activated charcoal, alumina and/or other means, as well as post-disinfection. In all cases, drinking-water treatments (to which collected waters can be subjected before being distributed to consumers) have the purpose of guaranteeing water health and cleanliness by reaching and maintaining, up to the users' taps, chemical, physicochemical, and microbiological requirements set up by the current legislation (Legislative Decree 31/2001 and subsequent amendments).

The use of ozone, alone or in association with hydrogen peroxide or UV irradiation in advanced oxidation processes, within the drinking-water treatment chain is a useful tool for pre-disinfection (that implies reduction of the presence of pathogens), removal of suspended particles and oxidation of organic substances of natural and anthropogenic origin and organic and inorganic micropollutants, as described below.

5.2.1. Using ozone in particle removal

Before their purification, all surface waters contain suspended solids of different origin, size and composition, often contaminated by infectious cysts and parasite oocysts (*Giardia*, *Cryptosporidium*). Depending on the quality of raw water at the inlet of the treatment plant, particle removal can be achieved by rapid or slow filtration, coagulation, flocculation and deep bed filtration, decantation or flotation followed by rapid filtration, tangential filtration through a membrane (such as nano, micro and ultrafiltration). It has been seen that a pre-ozonation treatment at rather low dosages (0.5-2.0 mg/L) near the inlet of the particle abatement unit can significantly improve the efficiency of the latter and substantially reduce the consumption of reagents while increasing the flow rates of the treated water (Jekel, 1998). Overall, reductions in final turbidity of about 20-90% of those measured in the absence of ozone were observed.

Pre-ozonation can be effectively combined with flotation in the removal of algae present in water collected at surface reservoirs during algal blooms. The process also allows the concomitant destruction of both intracellular and extracellular toxins.

5.2.2. Using ozone in pre-disinfection

Surface water can contain a multitude of bacteria, viruses and parasites, excreted in animal and human faeces, which must be effectively removed to ensure the minimum health and cleaning requirements by current legislation. Achieving this objective is guaranteed by the implementation of a complex multi-barrier system of which ozone is a key component. Its effectiveness is often superior to that exhibited by other reagents (free chlorine, chlorine dioxide, hydrogen peroxide and chloramines), especially against the most resistant microorganisms, such as protozoa.

The short half-life of dissolved ozone (which makes it virtually unusable during water distribution to the consumers) and its ability to produce biodegradable organic substances (which must be removed before the inlet of the distribution network) place its use immediately before sand or activated charcoal filtrations.

5.2.3. Using ozone in inorganic micropollutants oxidation

The direct use of ozone in the oxidative removal of inorganic micropollutants is a relatively rare application as there are other more effective methods for achieving this. The ability to break down certain inorganic species is therefore considered as a side effect of ozonation implemented for other purposes (particle removal, pre-disinfection, Natural Organic Matter oxidation).

A particularly critical reaction from the health point of view is the formation of bromate from water containing the bromide ion. Since this is a potential carcinogen, WHO has proposed a provisional guide value of 10 µg/L (WHO, 2017), recently adopted in the proposed recast of the EU Directive on the quality of water intended for human consumption (EU, 2019). The strategies currently implemented to limit the formation of this by-product are based on the optimization of ozone dosage, on the correction of water pH before ozonation, or on the dosage of small amounts of ammonia or hydrogen peroxide (Jarvis *et al.*, 2007; von Gunten, 2003). The use of activated charcoal filters only partially contributes to its removal from treated water (Hoigné *et al.*, 1985; Haag *et al.*, 1983; von Gunten & Hoigné, 1994; Koudjonou *et al.*, 1994).

Ozone can rapidly decompose other disinfectants: the latter can only be used after the removal of ozone residues, immediately before water distribution (post-disinfection). A particular case is represented by the interaction with hydrogen peroxide, used profitably in advanced oxidation processes (AOP) following the formation of hydroxyl and peroxylic radicals, highly reactive towards persistent target organic compounds.

5.2.4. Using ozone in Natural Organic Matter (NOM) oxidation

All water sources contain a complex mixture of natural organic substances in concentrations between 0.2 and 10 mg/L. The presence of NOM at concentrations of more than 0.4-0.6 mg/L can be a problem in achieving the minimum quality requirements of drinking water as it is jointly responsible for a series of negative phenomena such as the alteration of the organoleptic characteristics of water, the formation of disinfection by-products for interaction with biocides, bacterial regrowth in the distribution system, reduced efficiency in the treatment of particle removal and early saturation of activated charcoal filters. The removal of NOM or its transformation into less reactive substances with chlorine is a priority task, especially in the treatment of surface water. To this end, various technological processes can be used, including ozone-mediated chemical oxidation, to be placed between the decantation/floatation and rapid filtration processes, or between rapid filtration and adsorption on activated charcoal (Camel & Bermond, 1998).

5.2.5. Using ozone in organic micropollutants oxidation

Organic micropollutants can be found in surface water and groundwater in concentrations in the range 0.01-100 µg/L depending on the pollution level of the supply source. The degradation of these compounds to oxidized metabolites or their total decomposition through the use of ozone is a complex process influenced by the characteristics of treated water (pH, inorganic and organic carbon, etc.). For this reason, the oxidative action of ozone on organic micropollutants present in drinking water is, to date, considered a positive side effect found in ozonation treatments implemented for other purposes. The removal of organic micropollutants by ozone is rarely complete, so it is necessary to implement a subsequent treatment unit such as biological degradation and/or absorption on activated charcoal.

5.3. Application in the treatment of public pools

The State-Regions Agreement of 16 January 2003 (Italy, 2003) and the subsequent Interregional Agreement of 16 December 2004 (Italy, 2004) set hygienic criteria and requirements for swimming pools. In Annex I of the aforementioned State-Regions Agreement, ozone is listed among the substances allowed for the treatment of the water entering the pool in order to guarantee the control of the microbiological risk in swimming facilities (Bonadonna *et al.*, 2007) minimizing, at the same time, the formation of by-products of chlorine disinfection (Glauner, 2006). Ozonation is implemented outside the swimming pool on the recirculation water, before the subsequent re-chlorination. The concentration of ozone added to the treated water is such that the C·t value is between 2.4 and 15.0 mg/L (i.e. 0.8-1.5 mg/L of ozone for 3-10 minutes of contact) (DIN, 1997). Immediately before returning to the pool, the treated water must not contain ozone

residues at concentrations greater than or equal to 0.01 mg/L in order to ensure compliance with this limit even in the water contained in the swimming pool (Italy, 2003). At the same time, an outdoor air exchange of at least 20 m^3/h per m^2 of the pool area is prescribed for indoor swimming pools (Italy, 2003) with the aim of reducing the risk associated with exposure to volatile by-products deriving from ozone (acetaldehyde) or from the ozone-chlorine mixture (trialoacetaldehydes).

5.4. Application in the treatment of natural mineral and spring water

Natural mineral waters, regulated by Legislative Decree 176/2011 and Ministerial Decree 10 February 2015, are defined as "water that, originating from an underground aquifer or reservoir, come from one or more natural or drilled springs and which have particular hygiene and possibly properties favourable to health". They differ from drinking water for their original purity, the absence of any disinfection treatment and the level of protection from potential pollution risks. They are generally more pleasant from an organoleptic point of view and ensure the absence of disinfection by-products. The nature and hydrogeographic configuration of the source mean that these waters always have the same chemical, physical and organoleptic characteristics in all seasons.

The spring waters, also governed by the legislation mentioned above, are, on the other hand, defined as "waters intended for human consumption, in their natural state and bottled at the source, which, originating from an underground aquifer or reservoir, come from a source with one or more natural or perforated emergencies". Despite being subjected to the same organoleptic, physical and chemical quality criteria of drinking water, unlike the latter, they cannot be subjected to standard purification treatments, including post-disinfection. They must therefore meet the microbiological purity requirements for natural mineral waters.

Both mineral and spring water must be bottled at the source without undergoing processes that can modify their characteristics by changing the composition in essential components. Some specific treatments are exceptions, such as that with ozone-enriched air for the oxidative separation of iron, manganese, sulphur and arsenic. Such treatment must be carried out without causing the formation of residues at a concentration above the maximum limits set up by the current legislation (50 µg/L for dissolved ozone, 3 µg/L for bromate and 1 µg/L for bromoform) or, in general, at levels that may represent a risk for the consumer's health. Ozonation must in any case be expressly authorized by the Ministry of Health, after consulting the Board of Health.

5.5. Application in urban wastewater treatment

Urban wastewater consists of a complex and variable combination of domestic sewage (from household activities and human waste), runoff water (from street washing and rain collection) and industrial wastewater that can be considered qualitatively similar to domestic sewage. One of the main characteristics of urban wastewater is the presence of a high quantity of dissolved and suspended organic solids with high biodegradability, that allow their removal through biological processes.

The treatment of sewage consists of several phases (i.e. screening, sand removal, oil removal, primary sedimentation, aeration and secondary sedimentation, nitrification, denitrification, dephosphating and final disinfection) during which chemical contaminants and pathogenic microorganisms are removed, giving rise to a final effluent of such quality as to be compatible with the self-purifying capacity of the receiving body (land, lake, river or sea), in compliance with the provisions of the current legislation (Legislative Decree 152/2006 and subsequent amendments and additions). The contaminants removed, but not completely degraded during the wastewater treatment, are pre-concentrated in the form of sludge subjected to a series

of further treatments (such as thickening, biological stabilization, conditioning and dehydration) to make it suitable for disposal in special landfills, for incineration in waste-to-energy plants and / or for reuse in agriculture as it is or after composting.

In the urban wastewater treatment chain, ozone is very effective in both primary treatment and disinfection prior to discharge into the receiving body.

During primary treatment, ozone can be injected directly into the tanks preceding secondary treatments to correct BOD (Biochemical Oxygen Demand) and COD (Chemical Oxygen Demand), eliminate unpleasant odours, reduce the concentration of microorganisms and pathogens such as *E. coli*, *Listeria*, *Pseudomonas*, *Salmonella* and *Penicillium*, oxidize elements (iron, manganese, etc.), inorganic compounds (cyanides, sulphites, nitrates, nitrites, etc.) and organic compounds (phenols, detergents, pesticides, derivatives of quaternary ammonium, metal-proteins, aromatic compounds

In post-disinfection, ozone can reach levels of efficacy higher than those of chlorination or UV irradiation in killing bacteria, protozoa and viruses after contact times ranging from 10 to 30 minutes. At the end of the treatment, there is no algal regrowth in the soluble fraction of the effluent.

Ozonation is often implemented as part of a multistage chemical biological process (CBP) in which the biodegradation process operated by microorganisms is preceded and sometimes followed by the chemical oxidation phase with ozone that increases the biodegradable organic fraction by oxidizing the most stable organic micropollutants.

5.6. Application in the treatment of industrial waste

The effectiveness of ozone-based technologies has been established for several production sectors, such as textiles (Moraes *et al.*, 2000), electroplating and petroleum, phenol and paper industries (Li *et al.*, 2006).

In particular, the combined use of UV/H_2O_2 photocatalysis with ozonation has allowed phenolic compounds to be completely removed from industrial wastes (Kusic *et al.*, 2006). Organic pollutants such as phthalate esters and other Persistent Organic Pollutants (POPs) in raw water have been efficiently eliminated through various processes that combine the use of TiO_2-based catalysts, UV radiation, ozone and biological activated charcoal (Ciardelli *et al.*, 2001).

Effluents from the pharmaceutical industry show a low biodegradability due to the presence of active substances (antibiotics, anticancer agents and analgesics) that are poorly degradable and absorbable in the sewage sludge. The use of ozone in association with UV and/or H_2O_2 allows to completely oxidize the most recalcitrant compounds, making them less harmful and forming readily biodegradable by-products (Hernando *et al.*, 2007).

6. Ozone therapy and guidance for medical use

6.1. Properties and mechanisms of action

Ozone therapy combines a mixture of oxygen and ozone for therapeutic purposes and is proposed for the treatment of different clinical conditions.

The mechanisms underlying the effects of ozone are quite complex and not yet fully known, as once introduced into the body, this compound activates a cascade of multiple multifaceted events whose final outcome can vary considerably depending on the concentrations and oxygen-ozone ratios, endogenous antioxidant capacities and the patient's immune state. This complexity was well described by Sunnen, who observed that, if the biochemical configuration of the serum (with its enzymes, immunoglobulin, clotting factors, hormones, vitamins, lipoproteins, carbohydrates, electrolytes, etc.) was compared to an orchestra, the introduction of ozone would result in the inclusion of a powerful new musical instrument that influences the harmony and integration of all others (Sunnen, 2013, rev 2014). The literature reports that the main properties of ozone are oxidative action (induction of a moderate and transient oxidative stress that induces an antioxidant effect), stimulation of the trans membrane passage of oxygen with improved mitochondrial respiration, complex effects on the immune system (stimulation of the production of inflammatory cytokines resulting in the induction of an anti-inflammatory regulatory response), and many others *(Smith et al.*, 2017; Martinez-Sanchez *et al.*, 2020).

6.2. Routes of administration

With the exception of the inhalation route (which cannot be used due to its airway toxicity), the administration of ozone for therapeutic purposes is carried out through virtually all delivery routes: intravenous (both for direct administration and by autohemotransfusion (O3-AHT)), intramuscular, intrarectal, local infiltration (cutaneous, subcutaneous, transdermal, interarticular, paravertebral). In particular, O3-AHT consists of the sampling of 180 ml of the patient's blood and the immediate re-infusion into the vein after being treated with an oxygen and ozone mixture at concentration of 10-80 µg/mL of gas per mL of blood.

6.3. Therapeutic uses

A 2011 review states that ozone can be used to treat "114 diseases" (Elvis & Ekta, 2011). According to the Guidelines and Good Practices in Oxygen-Ozonotherapy art. 6 (Law 8 March 2017, No. 24, rev. 05/10/2019), however, of the multiple therapeutic indications that have been proposed, those supported by more robust evidence (type A evidence, i.e. based on systematic reviews of randomized controlled clinical trials, homogeneous cohort studies and case-control studies) are represented by orthopedic pathologies of the spine and knee. Other conditions for which there is lower level (type B) evidence are other orthopedic indications, skin ulcers, diabetic foot, skin infections supported by viruses, fungi and bacteria. Other indications (including respiratory distress syndrome) provide only C-type evidence, based on expert opinions, case reports, laboratory and epidemiological studies.

6.3.1. Use of ozone therapy in SARS-CoV-2 infection

Ozone therapy, and in particular, autohemotransfusion (O_3-AHT), has also been proposed in the treatment of COVID-19 patients. This use is largely based on the observation that (*via* the induction of lipid and phosphoprotein peroxidation resulting in capsid damage), ozone can have direct antiviral activity (Murray *et al.*, 2008). It has also been hypothesized that this effect could be specifically relevant to coronaviruses such as SARS and MERS (Sunnen, 2013, rev. 2014). However, how much antiviral activity of ozone is really observable *in vivo* remains to be defined, since the induction of a transient oxidative stress and the initial release of cytokines could theoretically stimulate viral reproduction, impairing the direct virucide effect (Bocci *et al.*, 1998). In fact, it has been reported that endogenous antioxidant properties effectively protect the integrity of viral particles (Martinez-Sanchez *et al.*, 2020). Clinical trials in patients with viral infections show conflicting results. A clinical study run several years ago in patients with HIV infection showed no antiviral effect of autohemotransfusion (Bocci *et al.)*., 1998), while a reduction in viral load was reported in patients with hepatitis C following treatment with autohemotransfusion plus rectal insufflation of ozone (Zaky, 2011). An immediate clinical response was also reported in 5 West African Ebola patients treated by two clinicians upon invitation of Sierra Leone's president. (Rowen, 2019).

In addition to the hypothetical antiviral activity, mechanisms that would underlie the possible effectiveness of ozone in COVID-19 include the antioxidant, anti-inflammatory, immunostimulant, oxygenating and cytoprotective effects (Martinez-Sanchez *et al.*, 2020; Valdenassi *et al.*, 2020).

Although most of these mechanisms can theoretically result in a beneficial effect in COVID-19 patients, at the moment there is still a lack of direct evidence obtained in controlled studies. Two cases of COVID-19 patients treated with ozone therapy have recently been described. Patients received for 7 days 100 ml of blood treated with oxygen-ozone in a 1:1 ratio with a final concentration of 20 µl/ml of blood. Oxygen-ozone therapy was added to therapy with antivirals, antibiotics, immunoglobulin and omeprazole and oxygen supplementation (3 L/min). Both patients improved their symptoms and radiological lung appearance and showed nasopharyngeal swab negative for nucleic acids of SARS-CoV-2 and were discharged 18 and 7 days after hospitalization. Comparing the course of disease of these two patients treated with oxygen-ozone therapy in addition to standard therapy (see above) with 2 patients with COVID-19 of comparable severity but treated with standard therapy, in the latter ones a longer period of time of viral *shedding* and hospitalization was observed (Zheng *et al.*, 2020).

It seems that ozone therapy (in the form of autohemotransfusion) is currently being used in some Italian hospitals to treat patients with SARS-CoV-2 infection. As far as we know, however, the results of these treatments have not yet been published.

6.4. Conclusions

Waiting for evidence from clinical trials, it should be pointed out that it is difficult to predict the overall effect of treatment (due to the multiple mechanisms of action of ozone treatment) especially in the case of patients in critical condition. In fact, the basic reactivity and immune response seem to be very important variables, able to dramatically influence (both positively and negatively) the outcome of the treatment. The dosage and duration of treatment in the various indications must also be appropriately defined with dose-response clinical studies. Finally, some of the effects of ozone (e.g. its ability to release pro-inflammatory cytokines) require obvious caution and appropriate time to be used during the course of SARS-CoV-2 induced disease.

7. Methods for ozone decomposition

The toxicity of ozone at concentrations of more than 0.1 mg/m^3 in the gas phase has stimulated the scientific community's interest in the development of systems for the instant decomposition of its residues found in indoor environments when in use (photocopiers, laser printers, sterilisers, etc.).

Conventional air removal technologies are based on the use of activated charcoal filters or catalysts based on noble metals or oxides from other transitional elements (Dhandapani *et al.*, 1997; Batakliev *et al.*, 2015).

Activated charcoal filters are commonly used in air treatment for the removal of both ozone and other contaminants (such as VOC) from indoor environments (Weschler *et al.,* 1993; Gundel *et al.*, 2002; Beko *et al.*, 2008). They require periodic replacement as a result of the progressive reduction in their effectiveness by gradual saturation of active sites by organic contaminants and for the concomitant damage of the structure by ozone (Lee & Davison, 1999). This makes them unsuitable for the treatment of high airflows, such as those generated by centralized ventilation/air conditioning systems.

A viable alternative to activated charcoal filters is catalytic converters capable of decomposing ozone at room temperature (20-50°C) with yields of more than 95%, even in high airflows. The active component of the converter consists mainly of a catalyst based on noble metals (Pt, Pd and/or Rh) or, alternatively, from oxides of other transition metals belonging to the type "p" (in particular: MnO_2, Co_3O_4, NiO, Fe_2O_3, Ag_2O, Cr_2O_3, CeO_2, V_2O_5, CuO, MoO_3). The latter have progressively replaced the former because of the lower cost. Among the different oxides that can be used, manganese dioxide has been the most active (Dhandapani & Oyama, 1997): this justifies its prevalence in the devices currently on the market. In both cases, the catalyst is deposited on a matrix with high surface development (γ-Al_2O_3, SiO_2, TiO_2 ZrO_2 or activated charcoal), supported by a monolithic substrate that can ensure adequate contact with treated air in the absence of substantial load losses.

Emerging technologies in the field of air purification include PhotoCatalytic Oxidation (PCO) as it is considered very promising in the removal of ozone residues (Jacoby *et al.* 1996; Hager & Bauer 1999; Ohtani *et al.* 1992; Mills *et al.* 2003; Cho *et al.* 2004; He *et al.* 2006; Lin & Lin 2008; Lu *et al.* 2014; Lee *et al.* 2015). PCO uses semiconductor materials such as titanium dioxide, possibly with noble metals, which are activated by absorbing ultraviolet light and generating positive gaps within the semiconductor. Subsequent interaction with water determines the formation of hydroxyl radicals that induce, among other things, the decomposition of ozone. Advanced prototypes based on the use of this technology have recently been tested (Ohtani *et al.* 1992; Mills *et al.* 2003; Lu *et al.* 2014; Cho *et al.* 2004; He *et al.* 2006; Lin & Lin 2008; Kadribegovic *et al.* 2011).

8. Monitoring and analytical methods

8.1. Methods for determining ozone in indoor environments

The concentration of ozone in the air in indoor environments is affected, in a complex way, by several parameters, such as the emission levels of the devices that can generate it, the presence and mode of ventilation/air conditioning systems, the background concentration outside the buildings (variable during the day in operation, among other things, solar radiation and vehicular traffic), the speed of air exchange between the interior and exterior of the building, the kinetics of chemical and physicochemical interactions with internal surfaces and other environmental micropollutants (Weschler, 2006). The difficulty of predicting the trend of its concentration over time and the consequent level of human exposure made it necessary to develop analytical methods to be used in monitoring its concentration near the ground, both continuously and in integrated and mediated over a predefined period of time.

The main reference documents are those drawn up by standardization bodies such as CEN and ISO, which have long been engaged in the development of standardized methods with which to make measurements (sampling, analysis and evaluation methods of measured levels) and concern the standards of the EN ISO 16000 series “Air in indoor environments”, standards that have been partly implemented in Italy by UNI (Italian Unification Body). In addition to these standards, UNI EN 13528 “Ambient Air Quality. Diffusive samplers for the determination of concentrations of gases and vapours. Requirements and test methods. General Requirements.”, UNI EN 14625 “Ambient air quality - Standard method for the measurement of the concentration of ozone by ultraviolet photometry”, and ISO 10313:2017 “Environment air - Determining ozone mass concentration - Chemiluminescence method” should be used.

Continuous determination in the air is usually carried out at fixed or portable stations using mainly one of the following types of direct measurement devices, through which the air taken from the environment under monitoring is made to flow continuously:

- Photometric analysers, which can measure the absorption of UV radiation at 254 nm by the ozone molecule. In order to correct interference produced by numerous other environmental micropollutants with chromophore groups sensitive to the same wavelength, the flow of the air sample is analysed a second time after ozone decomposition by a catalytic converter containing MnO_2. The difference between the two measures results in the concentration of ozone present in the original flow of the sample unless a potential overestimation for the presence of hydrocarbons, mercury vapours and SO_2, also removed by the converter (Andersen *et al.)*., 2010; 2005). This method of measurement (UNI EN 14625 “Normalized method for measuring ozone concentration in air by ultraviolet photometry”) was chosen as the official reference method for monitoring ozone in the ambient air (Legislative Decree 155/2010).
- Chemiluminescence analysers, capable of measuring emission of radiation generated as a result of the reaction between ozone and a specific gas (e.g. ethylene or NO) or an active surface (disc soaked in organic dyes such as Rhodamine B). This type of instrumentation is not affected by interference from other micropollutants (Eipel *et al.*, 2003; Takeuchi *et al.*, 1990).
- Electrochemical analysers, able to measure the variation of the potential between two electrodes immersed in an electrolyte following the diffusion of ozone through a porous membrane. Since their response is also affected by the presence of Cl_2 and NO_2, this type of sensor is used almost exclusively in indoor and industrial monitoring (Cho, 2015; Pang *et al.*, 2017).
- Semiconductor analysers of the HMOS (Heated Metal Oxide Sensor) type, able to measure the electrical resistance variations of a gas-sensitive metal oxide following the passage of ozone at a

given temperature (Cao *et al.*, 2016; Piedrahita *et al.*, 2014). The response of this type of sensor is affected by interference from volatile organic micropollutants.

Other possibilities for the continuous measurement of ozone near the ground have been offered by the recent introduction of optical cavity spectroscopy (CRDS) (Washenfelder *et al.*, 2011), differential optical absorption spectroscopy (DOAS) (Hoffman *et al.*, 1995) and sensors for detecting delay in the reflection of surface acoustic waves (SAW) (Westafer *et al.*, 2014).

The calibration and subsequent verification of the reliability of the continuous measuring devices are carried out by applying, concomitantly, indirect methods to determine the concentration of ozone in the air. An aliquot of the air under examination is made to flow through an aqueous solution containing a specific reagent capable of interacting and fixing the ozone present (e.g. indigo carmine or potassium iodide); subsequently, the remaining amount of the reagent or its reaction product are measured spectrophotometrically or volumetrically (Tjahjanto *et al.*, 2012; Bergshoeff *et al.*, 1984).

Unlike continuous determination, integrated passive monitoring of ozone in air is performed with sampling devices worn directly by exposed personnel, in order to evaluate the average amount of inhaled ozone over a predefined period of time (8 hours, 1 day or 2 weeks) (Demirel *et al.*, 2014; Koutrakis *et al.*, 1994; Liard *et al.*, 1999; Liu *et al.*, 1995). In this case, sampling is done passively, that is, in the absence of suction pumps or other moving parts, exposing test strips or badge indicators that require subsequent laboratory analysis at the end of the measurement period. Personal sampling devices are treated with indicators that can react, more or less selectively, with ozone (such as 10,10'-dimethyl-9,9'-biacacridilidine, sodium nitrite, 3-methyl-2-benzothiazolone hydrazone, p-acetamidophenol or indigocarmine). Recently, personal sampling devices based on the use of monitoring systems that are adequately miniaturized and capable of mediating instant response have been proposed on the market.

8.2. Methods for determining ozone in water

The use of ozone in water and waste purification involves the on-site generation of the oxidant and its concomitant introduction into the water flow through an appropriate gas-liquid contact system. The control of this process and the evaluation of its performance is based both on the measurement of the ozone dose in the flow of gas entered into the water and on the periodic determination of its residual concentration in the water after a predefined contact time.

The latter is usually carried out using one of the direct measurement devices described in paragraph 10.1 (particularly UV photometric analysers) or, alternatively, the colorimetric method with indigotrisulfonate, free from interference from secondary oxidants (hydrogen peroxide and chlorite) and chlorine residues when operating in the presence of masking agents (Bader *et al.*, 1982).

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Appendix

Ozonisers and security procedures

Ozone cannot be stored or transported due to its high reactivity but is generated *in-situ* from air, oxygen or water. On the market, ozonisers are available based on different operating principles and different production capacity depending on the use to which they are intended. Those with lower production capacity are suitable for small environments and are generally equipped with predefined programs that are not modifiable and often ozone conversion device at the end of the dispensing. Those with higher production capacity are intended for large environments, including production environments. They allow for the adoption of custom use protocols and therefore, are intended for professional use. Heat production, being connected to the amount of ozone formed, is intense and requires the presence of techniques for cooling the cell (as the temperature rises the ozone formed tends to decompose).

The most important and most widespread process for the production of ozone is obtained by means of generators that operate on similar principles or derived from the generator designed by Welsbach in 1950 using cells that follow the Werner von Siemens 1857 crown-effect (or crown discharge) patent and from which variants have developed over time. Among their advantages, the possibility of building generators of even small size, the longevity of the cells (several thousand hours) and high productivity.

Another process available for ozone production uses ultraviolet lamps with an emission band focused on 185 nm. This mode generally has several disadvantages compared to crown-effect cells: much lower ozone productivity, increased electricity consumption, the short operational life of lamps. This process will not be covered in this document.

A third process is based on water electrolysis and is used for ozone production for use in watery solution.

The purpose of this annexe is to provide some guidance on the correct and safe use of *in-situ* ozone generators from air/oxygen as a precursor.

A. Precursor

Ozone cells can be powered by air or *oxygen* (Valeri, 2004)

1. Using oxygen in the ambient air

Most of the *in-situ* ozone generators available on the market today use the oxygen present in the ambient air as an ozone precursor. The advantages are represented by the absence of costs for the raw material and the ease of use. On the other hand, the direct ozonation of the ambient air itself involves the possible production of harmful substances, mainly **nitrogen products.**

The production of nitrogen oxides is strongly determined by the technology used or by the type of ozone cell and how it operates. The use of closed-cell generators, rather than generators that use surface-plate crown discharge, offers fundamental quality and durability benefits.

The air that powers the cell must be purified and dehydrated. Generators must be equipped with a special filter system of the ambient air source in order to retain the possible contaminants present (atmospheric particulate matter PM_{10}, $PM_{2.5}$, VOCs, etc.) and avoid or limit the generation of reaction by-products (secondary pollutants) harmful to the human body. Environment air as a precursor should therefore not be used in the presence of work activities involving the release of chemical pollutants.

The filter system must also be able to break down air moisture to avoid a decrease in relative ozone yield and the possible production of nitric acid that can damage the cell and vaporize and disperse into the environment through the dispensing flow.

In the absence of information about this in the manual of use, it will therefore be essential to acquire from the manufacturer a specific statement on the guarantees taken to avoid the unwanted phenomena mentioned above.

2. Oxygen use from cylinders or portable oxygen concentrators (oxygen-powered oxygen cell > 95%)

Such a solution has the advantage of having a controlled oxygen source that does not generate secondary pollutants as well as offering the possibility of achieving higher concentrations of ozone in the flow delivered and higher production yields.

These are equipment that incur additional costs and are more complex. In this case, the risk of fire/explosion should also be checked. In particular, the operation of the cell must be such as to avoid the formation of electric discharges. The user manual should provide useful elements to highlight the danger and measures taken to prevent risk. The use of concentrators is usually preferred to cylinders because they are safer.

B. Treatment cycle for environmental sanitization

The typical stages of a course of treatment are (SSICA, 2010):

- *Conditioning phase*:
  This phase coincides exactly with the one in which ozone is started to be dispensed in the room to be treated. This disbursement must enable the planned ozone concentration to be achieved in a short time as necessary in relation to the target to be reached.

- *Ozone action phase:*
  This phase is intended to ensure the effectiveness of the cycle by prolonging the necessary time of sanitization by keeping doors and windows closed.

- *Phase of elimination of residual ozone:*
  This phase must ensure the elimination of ozone from the air in the treated room and continues until the required ozone concentrations for occupant safety are reached.

In order to develop the treatment cycle, it is crucial to know:

- features of the generator,
- characteristics of the environment to be treated.

The process must also be able to reach all surfaces and hotspots by distributing ozone gas in a homogeneous and constant way to carry out its activity.

Technical data of the ozone generator to consider:

- ozone production (g/h);
- delivery rate of the air (or oxygen) / ozone mixture (m^3/h);
- ozone concentration at the point of delivery (ppm or mg/m^3).

These parameters determine the concentration and distribution of ozone in the environment and the time of action required to achieve the target set in relation to the size of the room. Most machines are equipped with only one-timer, while some equipment has approximate programs that define the duration of the cycle in relation to the m^2/m^3 of the room concerned with the treatment and, sometimes, also of control/alarm devices for safe use.

For example, if we have a generator with a production capacity of 2 g/h of ozone, for the treatment of a room of 100 m^3, we will need 20 minutes of dispensing to reach a concentration of 2.5 ppm (keeping in mind that in the air 1 ppm is 2.14 mg/m^3). As the volume of the environment increases, with the same production capacity of ozone, more dispensing time will be required, or for smaller environments, it will be possible to use shorter times or, at the same time of action, higher concentrations.

During the ozonation process, the concentration of ozone increases very slowly in the initial period of time. The delay in the accumulation of ozone concentration is due to ozone consumption due to the presence of pollutants in the initial period of time. Subsequently, after oxidating the main pollutants, the concentration of ozone inside the room rapidly increases to the desired level. In order to ensure the “biocide” action, the concentration of ozone must therefore be maintained for the expected time (action time). Relative humidity, temperature, chemicals, present microorganisms determine the real amount of ozone needed to reach the desired concentration and maintain it for as long as necessary. In this regard, it underlines the importance of pre-cleaning the surfaces for the removal of dirt.

When the generator is turned off, the ozone concentration gradually decreases due to spontaneous conversion of ozone into oxygen.

The relative temperature/humidity environmental conditions have an important influence on the susceptibility of ozone microorganisms. Literature data show, in particular, that susceptibility increases in the presence of high levels of relative humidity (>70%): the combined effect of ozone and OH radicals strengthens the effectiveness of treatment.

For large environments (e.g. sheds) generators with long-range fans should be used to ensure a homogeneous distribution of gas.

During treatment, it is necessary to close doors and windows, and it is forbidden to stay in the room.

C. Elimination of environmental residual ozone

Ozone is an unstable gas and spontaneously decays to oxygen. The time it takes to bring the ozone concentration back to the adequate concentration for occupant safety is always a function of the concentration of ozone used in the treatment. The time of ozone decomposition also depends on temperature, relative humidity and levels of chemical and biological contamination of the environment.

In real conditions, the natural decay time required to make the premises accessible is at least 2 hours (remaining concentration below the threshold of olfactory perceptibility for humans, between 0.02 ppm (40 µg/m^3) and 0.05 ppm (100 µg/m^3), and equal to about 1/10 threshold of 0.2 ppm defined as safe in work environments for a maximum exposure time of 2 hours.

If possible, it is preferable to perform treatments at night so that when work resumes, the amount of environmental ozone is within health safety limits.

To speed up conversion times, however, UV-C 254 nm ultraviolet rays or chemical catalysts that some manufacturers already install within their equipment can be used in the final phase of the cycle, with the ozonogenous cell turned off.

Avoid eliminating residual ozone by using forced ventilation to convey it to the outside environment: Legislative Decree 155/2010 sets limits and quality targets for ozone ambient air concentrations as well (Italy, 2010)

D. Treatment cycle validation

It should be stressed that for sanitization with gas ozone, all treatment parameters must be adapted to the different environmental conditions of the production realities considered. It is therefore desirable, especially in the case of contexts important for dimensions or characteristics of furniture such as the presence of fabric furniture that technical tests of concentration, homogeneity of distribution and safety are carried out in order to transfer the use into the operational reality.

The most advanced equipment is equipped with a system that manages the operation of the generator in order to control the production of ozone, the concentration in the environment and the timing of the treatment and subsequent decay of ozone. However, it is always a good practice to validate the entire treatment cycle by monitoring ozone concentrations using special ozone-measuring sensors capable of storing and returning the detected values (evidence of maintaining concentrations, diffusion and ozone decay/elimination).

Any certification of successful environmental treatment requires the data of ozone concentration and relative humidity detected in the environment (report or graph) as well as the reference protocol adopted and validated by the Laboratory accredited to achieve the set goal that determined the definition of the concentration and time of action of ozone, relative humidity conditions and air temperature. The certification must also specify the type of generator used, the precursor adopted (i.e. whether oxygen or ambient air), indicating the measures to avoid the formation of secondary pollutants. The use of ozone is indicated for environments not contaminated with chemicals. Environments with a large influx of people or with an important presence of volatile chemicals require a preliminary assessment; some classes of organic compounds can react quickly with ozone to form, as a result of incomplete oxidation, new pollutants that have adverse effects on human health. In such cases, it may be useful to measure the amount of VOCs before and after treatment using special instruments. In this respect, adequate air turnover at the end of treatment is of particular importance in any case.

E. CE marking

According to the EU directives on electrical and electronic equipment, ozone generators must comply with the following directives:

- LOW TENSION Directive 2014/35/CE;
- ELECTROMAGNETIC COMPATIBILITY Directive 2014/30/CE;

- Directive 2011/65/CE (RoHS) restriction of the use of certain hazardous substances in electrical and electronic equipment

Manufacturers must therefore provide appropriate EC compliance statement on this issue and comply with labelling requirements.

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Rapporti ISS COVID-19 (ISS COVID-19 Reports)

ISS COVID-19 Reports are mainly addressed to healthcare professionals to cope with different aspects of the COVID pandemic. They provide essential and urgent directions for emergency management and are subject to updates. All reports have an English abstract.

The complete list is available at **https://www.iss.it/rapporti-COVID-19**.

Some reports (highlighted below) are also translated in English and are available at **https://www.iss.it/rapporti-iss-COVID-19-in-english**

1. Gruppo di lavoro ISS Prevenzione e controllo delle Infezioni. *Indicazioni ad interim per l'effettuazione dell'isolamento e della assistenza sanitaria domiciliare nell'attuale contesto COVID-19.* Versione del 24 luglio 2020. Roma: Istituto Superiore di Sanità; 2020 (Rapporto ISS COVID-19, n. 1/2020 Rev.)
2. Gruppo di lavoro ISS Prevenzione e controllo delle Infezioni. *Indicazioni* ad interim *per un utilizzo razionale delle protezioni per infezione da SARS-CoV-2 nelle attività sanitarie e sociosanitarie (assistenza a soggetti affetti da COVID-19) nell'attuale scenario emergenziale SARS-CoV-2.* Versione del 10 maggio 2020. Roma: Istituto Superiore di Sanità; 2020 (Rapporto ISS COVID-19, n. 2/2020 Rev. 2)
3. Gruppo di lavoro ISS Ambiente e Gestione dei Rifiuti. *Indicazioni* ad interim *per la gestione dei rifiuti urbani in relazione alla trasmissione dell'infezione da virus SARS-CoV-2.* Versione del 31 maggio 2020. Roma: Istituto Superiore di Sanità; 2020 (Rapporto ISS COVID-19, n. 3/2020 Rev. 2)
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