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ANTIMICROBIAL EFFECTS OF OZONE

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Abstract: Antibiotic resistance is considered a public health problem with greater clinical relevance, as it makes it difficult to control infectious diseases; favors the increase of morbidity and mortality; decreases therapeutic efficacy; promotes the transmission of infections to other individuals; poses a risk to patient safety. The development of microorganisms resistant to the action of disinfectants and antimicrobial drugs, associated with the emergence of new pathogens, represent one of the greatest threats to the health of humans and animals. The World Health Organization (WHO), in 2017, released for the first time the classification of bacteria or bacterial families resistant to most of the drugs used, considering that the production of antimicrobials has been restricted to a small amount of chemical compounds. Ozone causes inactivation of bacteria, viruses and fungi, disturbing the integrity of the bacterial cell envelope due to oxidation of phospholipids and lipoproteins. In fungi, O3 inhibits cell growth at certain stages, and the forming cells are more sensitive. With viruses, O3 damages the viral capsid and disrupts the reproductive cycle, interrupting virus-cell contact with peroxidation. The use of ozone as an antiseptic method begins to gain great prominence in scientific research: An advantage of molecular ozone is the permeation of the gas through cell membranes and the destruction of pathogenic microorganisms, such as bacteria, fungi and protozoa, being able to replace chlorine and others disinfectants. Despite the high lethality of ozone, even when inhaled at low concentrations, its use has been suggested as a germicidal, bactericidal and chemical cleaning agent. In addition, it has low cost and ease of operation of ozonators.

Keywords: Antimicrobials; ozone; ozone therapy; effect of ozone on the body.

INTRODUCTION

Antimicrobials correspond to a class of drugs used worldwide in the treatment of numerous pathologies triggered by microorganisms. In recent years, as a result of the increase in bacterial resistance, mainly due to pathogens potentially harmful to health, the need for the search for new substances in order to contain the infections triggered in hospital environments and in the community has gradually been aroused. 2018).

FURTADO (2019), adds that antimicrobials are substances of natural or synthetic origin that act on microorganisms to inhibit their growth or cause their destruction, being used in a prophylactic and/or therapeutic way in order to constitute a pharmacological advance of paramount importance and of wide use. They are also capable of preventing the multiplication or causing the death of fungi or bacteria, these classified as microbicides, which cause the death of microorganisms, or “static”, which favor the blocking of microbial development (TEIXEIRA, 2019).

Today, the phenomenon called “superbugs” is usually one of the main topics discussed in many developed and developing countries.

According to World Health Organization (WHO), the *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and *Treponema pallidum* are notorious examples of bacteria that have shown resistance to antimicrobials (SILVA, 2018).

It is therefore noteworthy that the excessive and mistaken use of antibiotics by the population is one of the most significant factors related to the dissipation of resistant bacteria (SILVA, 2018). Bacterial resistance is associated with the indiscriminate use of drugs. The search for immediate effects leads the patient to resort to unnecessary and distorted prescriptions, or to self-medication (GARCIA, 2021). Therefore, antibiotic resistance is considered a public health problem with greater clinical relevance, since it makes it difficult to control infectious diseases, favors an increase in morbidity and mortality, decreases therapeutic efficacy, promotes the transmission of infections to other individuals and brings risk to the patient safety (RODRIGUES, 2018).

In this sense, the World Health Organization (WHO) issued an imminent and worrying notification to public health agencies, requesting that several countries develop conducts in the conflict to this problem. In its Global Antimicrobial Resistance Surveillance Report released in 2014, the WHO highlighted seven species of microorganisms of extreme global relevance (Table 1).

Pathogen	Resistance antibiotic
Escherichia Coli	Third-generation cephalosporins and fluoroquinolones
Klabsiella pneumoniae	Third-generation cephalosporins and carbapenems
Staphylococcus aureus	Methicillin
Staphylococcus pneumoniae	Penicillin
Salmonella spp.	Fluoroquinolones
Shigella spp.	Fluoroquinolones
Neisseria gonorrhoeae	Reduced susceptibility to third-generation cephalosporins

Table 1. Antibiotic-resistant microorganisms according to the World Health Organization's global alert.

The World Health Organization (WHO), in 2017, released for the first time the classification of bacteria or bacterial families resistant to most of the drugs used, considering that the production of antimicrobials has been restricted to a small amount of chemical compounds (NASCENTE, 2019). The development of microorganisms resistant to the action of disinfectants and antimicrobial drugs, associated with the emergence of new pathogens, represent one of the greatest threats to the health of humans and animals.

The early and excessive use of antibiotics combined with the lack of resources, rapid and accurate diagnoses generate the need for additional antibiotics in case the patient presents clinical worsening, contributing to the use of broad-spectrum antimicrobials and, possibly, to an increase in bacterial resistance. or its potential implications, both in the hospital environment and in the community (PELUSO, 2021).

Therefore, bacterial resistance may be a natural evolutionary factor given to the evolution of the microorganism in question, "whose genetic adaptation constitutes a change in the environment" (DAVID, 2021).

In Brazil, the National Health Surveillance Agency published, in the edition of the Official Gazette in 2011, the Resolution of the Collegiate Board of Directors (RDC) nº 20, establishing, on the services provided by pharmacists, the dispensation of antimicrobial drugs in pharmacies and drugstores in the national legislation, as well as establishing a new period of ten days for the validity of prescriptions for these drugs (antibiotics) in order to limit their indiscriminate use by the population.

Aiming to broaden the discussion on this topic of extreme social relevance, since it also directly interferes with human health, the objective of the present work is to highlight the use of ozone in the search to explore its antimicrobial potential, aiming to combat multidrug-resistant bacteria.

At the beginning of the 20th century, ozone therapy emerged, which consists of exploring possible beneficial pharmacological and clinical effects associated with ozone. The initial applications were aimed at treating wounds and skin infections caused by anaerobic bacteria, being an alternative to traditional methods that did not have the desirable effects.

Velio Bocci was one of the greatest scholars in the world of ozone therapy, being considered the "Father of modern ozone therapy". Physician, specialist in respiratory diseases and physiologist, Bocci has written numerous articles on ozone and even books on the subject, especially "A New Medical Drug", in which the professor explains the biological bases of ozone therapy, in line with classical biochemistry, physiology and pharmacological knowledge (PHILOZON, 2021).

Although the formation of O3 involves a series of complex chemical reactions, such reactions can be synthesized by equations 1 and 2. Carbon monoxide or VOC react and form the hydroxyl (OH-) or hydroperoxyl (HO2•) radical, which, by therefore, react with NO to form NO2. NO2 undergoes photodissociation in the presence of radiation (hν) of wavelengths lower than 430 nm, according to equation 1:

$$(1)\ NO_2 + h\nu \rightarrow NO + O^{\bullet}$$

The oxygen atom (O•), resulting from the reaction shown in equation 1, reacts, in turn, with molecular oxygen (O2), in the presence of a third molecule (M), thus forming the ozone molecule (O3) (Eq. 2).

$$(2)\ O^{\bullet} + O_2 + M \rightarrow O_3 + M$$

The number resulting from equation 1 reacts quickly with O3 to form a molecule of NO2 and O2 (Eq. 3), continuing the cycle.

$$(3)\ NO + O_3 \rightarrow NO_2 + O_2$$

The production and concentration of O3 is governed both by meteorological conditions, such as speed, wind direction, air temperature and solar radiation (SANTOS, 2018) and by the location of emission sources of precursor pollutants. These factors cause spatial and temporal variability, both in the production and in the concentration of the gas in the atmosphere (YAMAMOTO, 2021).

Some researchers, such as MAGALHÃES (2019), emphasize that molecular ozone is the main inactivator of microorganisms, while others claim that antimicrobial activity is carried out by ozone decomposition by-products such as OH-, O2 and HO3•.

NEUTEGEM (2018) states that there are compounds susceptible to reaction with ozone, which are mainly those that contain nucleophilic sites, such as oxygen, nitrogen, sulfur, phosphorus and activated carbon. During the degradation process, many molecules are cleaved, giving rise to products that are more oxidizable, more polar, with lower molecular weights and sometimes more biodegradable than their parent molecules.

The radicals, which are highly unstable, react with oxygen molecules to form ozone, as in the equation (NEUTEGEM 2018).

$$O^{\bullet} + O_2 \rightarrow O_3$$

OZONE

Ozone (O3) is an allotropic compound of oxygen (O2), it is formed through electrical discharges on the oxygen molecule, in which it breaks to release atoms, where it binds to another oxygen molecule, forming O3. Because it is extremely oxidizing and unstable, it reverts to its molecular form of oxygen with ease (NESI 2018).

Ozone, in high concentrations, is a slightly bluish gas. In the liquid state, in which it assumes an explosive character, it is blue, while in the solid state it is dark violet. Its melting and boiling points are respectively -192 °C and -112 °C (LIMA, 2021).

OLIVEIRA (2018) cites that ozone gas (O3), or triatomic oxygen, is an allotropic form of oxygen, which can be produced naturally as a result of lightning, or ultraviolet radiation, or synthetically by the corona discharge method. In ozone generation, oxygen molecules (O2), when exposed to electrical discharge, are dissociated, producing highly reactive free radicals. These free radicals react with other oxygen molecules, forming O3, as shown in figure 1.

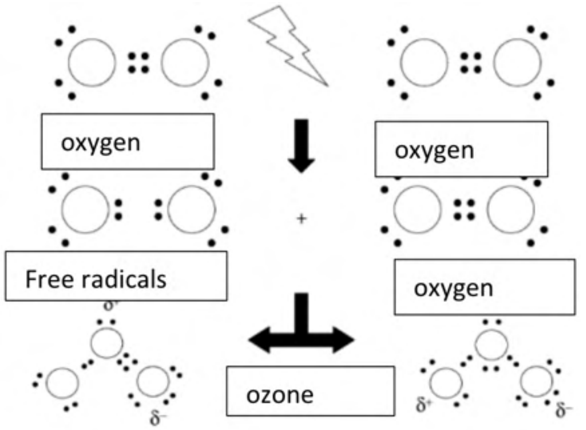

Figure 1 - Mechanism of formation of ozone gas from oxygen molecules (OLIVEIRA, 2018).

The author PAULA (2018) describes that ozone production can be carried out in three main ways: by electrolysis, ultraviolet and the corona effect. The most common and easily accessible for medicinal treatments and production via the corona effect is when ozone is generated by an electrical discharge in a stainless steel tube called an ozone reactor. In this tube, a high voltage electrical discharge is applied, together with an air flow that generates ozone gas, causing a gas with high oxidizing power, and when generated by pure oxygen, its production efficiency is increased.

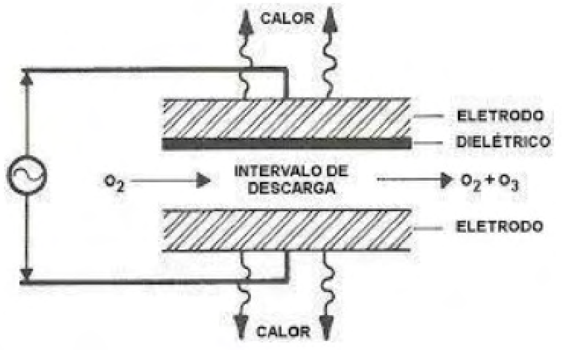

Figure 2. Ozone generation by corona discharge (PAULA 2021).

Ozone is detectable even at concentrations as low as 0.02-0.05 ppm. Its half-life varies with temperature variation. At 20°C, it has a half-life of 40 minutes, and at 0°C, about 140 minutes. Ozone causes the inactivation of bacteria, viruses and fungi, and further disrupts the integrity of the bacterial cell envelope by oxidizing phospholipids and lipoproteins. The low concentration (0.1 ppm) is sufficient to inactivate bacterial cells. In fungi, O3 inhibits cell growth at certain stages, and the forming cells are more sensitive. With viruses, O3 damages the viral capsid and disrupts the reproductive cycle, interrupting virus-cell contact with peroxidation (BELEGOTE, 2018).

Considering the antimicrobial action, CAETANO (2021) explains that O3 acts in the oxidation of glycopeptides, glycoproteins and amino acids of the cell wall, modifying permeability and causing cell lysis. When penetrating inside the cell, O3 recombines with cytoplasmic elements, causing the oxidation of amino acids and nucleic acids, consequently cleavage and cell death. In addition, O3 promotes the breakdown of cellular enzymatic activity by attacking the sulfhydryl groups of enzymes, as well as modifying the purine and pyrimidine bases of nucleic acids.

The author JUNIOR (2021), clarifies that O3 acts in the defense mechanism against fungi, bacteria and reactive oxygen species (ROS), one of these ROS is ozone (O3), a gas formed in the troposphere from photocatalytic reactions between hydrocarbons, nitric oxides (NOx) and carbon dioxide (CO2). ozone is a reactive oxygen species, with high oxidizing potential capable of oxidizing several molecules present in foods, oxidizing olefinic groups, through the mechanism proposed by Criegee in 1975, producing aldehyde and ketone as main products (RIBEIRO 2021).

Therefore, the use of ozone as an antiseptic method begins to gain prominence in scientific research. An advantage of molecular ozone is the gas permeation through cell membranes and the destruction of pathogenic microorganisms, such as bacteria, fungi and protozoa, being able to replace chlorine and other disinfectants (VECCHIO, 2019).

When ozone decomposes into water, the hydrogen peroxide (H2O2) and hydroxyl (OH-) free radicals formed are of great importance in the disinfection process. Thus, ozonation is effective against microorganisms, including gram-negative and gram-positive bacteria, molds, yeasts, viruses, protozoa, including sporulated forms and cysts of protozoa, which are more resistant (NEUTEGEM, 2018).

In contact with the skin, ozone gas acts on the phospholipid layer of erythrocytes, resulting in reactive oxygen species, such as hydrogen peroxide (H2O2), which increases ATP production and also promotes greater oxygen transport. production of cytokines and interleukins, stimulating platelet activity, increasing cell growth factors and, finally, acting on tissue repair (MONTEIRO, 2021).

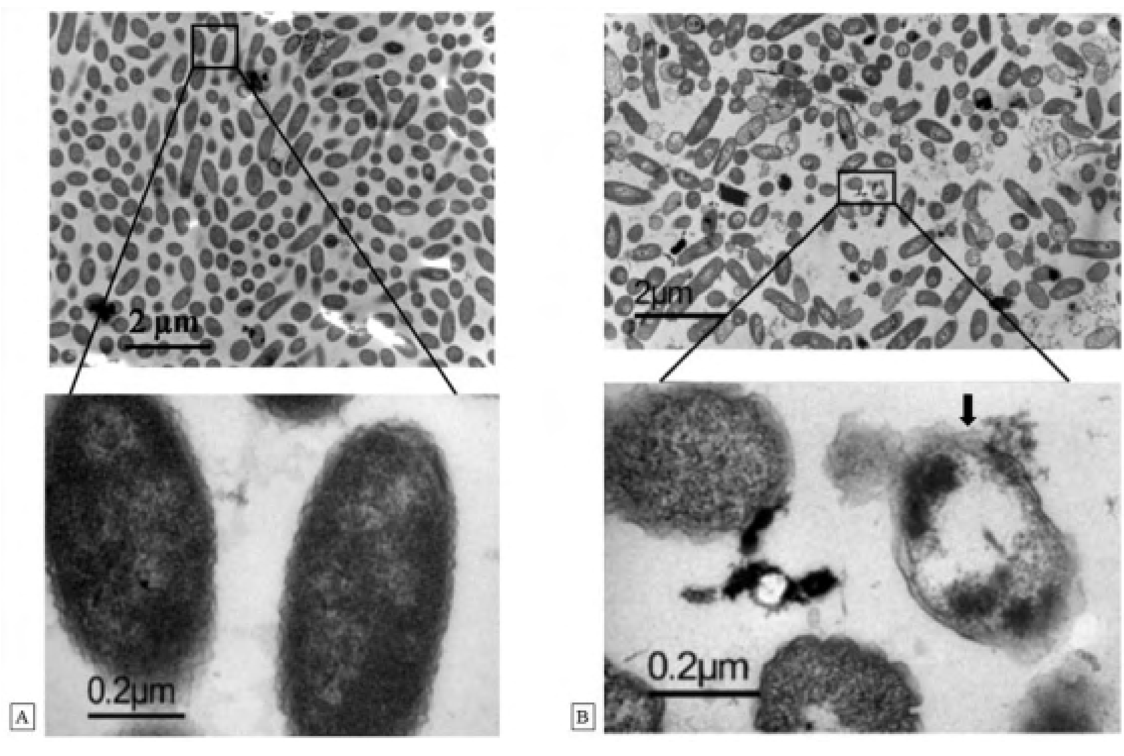

Figure 3. Transmission electron microscopy micrographs of *Vibrio parahaemolyticus* without exposure to ozone (A) and with exposure to ozonated water (B), showing lower bacterial density and changes in cell morphology, such as vesicle formation and cell wall rupture (black arrow). Source: Feng et al. (2018), p. 28.

When in contact with the blood, ozone causes the formation of reactive oxygen species (ROS), free radicals that activate the endogenous antioxidant system, constituting the reactive species that overcomes the defense system and gives the organism the onset of oxidative stress, presenting the subsequent degradation of lipids, proteins and DNA with consequent cell damage (ESPADA, 2020).

In the form of systemic application, CARVALHO (2018) explains that ozone also has an immunomodulatory action, since, according to its dose, it provides an increase in the production of endogenous antioxidants in the cell and the release of some substances that act as messengers. in the immune system, therefore stimulating and having a direct influence on the control of oxidative stress. Its administration can be done subcutaneously (SC), intramuscularly (IM), intradiscal, intracavitary, intravaginal, intraurethral, vesical and by ozonated autohemotherapy (SILVA, 2018).

O3 acts as an evidence of the immune system that activates neutrophils and stimulates the synthesis of some cytokines, increases the transport of oxygen in the blood, through changes in cellular metabolism, activating aerobic processes and using energy resources. As a result, it improves the metabolism of inflamed tissues and reduces inflammatory processes. It allows a slight healing of wounds, as it causes the accelerated migration of cells which increases the activity of fibroblasts, the synthesis of collagen and cytokines (BELEGOTE, 2018).

For CARVALHO (2018), when ozone comes into contact with organic fluids, it reacts immediately and ceases to exist. Ozone reacts with polyunsaturated fatty acids, antioxidants (such as ascorbic and uric acid) and thiol compounds of the -SH group (such as cysteine, glutamine and albumin). All these reagents function as electron donors and can undergo oxidation, forming molecules of reactive oxygen species (ROS) and lipid oxidizing products (POL). Depending on the concentration, they can cause a therapeutic or harmful biological effect.

POLs are smaller, more stable and more diffusing molecules, depending on the concentration they can be more toxic than ROS. These POLs are easily distributed among body tissues and fluids. Its metabolism is mainly regulated by glutathione transferase and aldehyde hydrogenases (HAYASHI 2018).

POLs exert a strong immunomodulatory effect, being highlighted by a feeling of well-being reported by patients during ozone therapies. This well-being effect declared by most patients, according to Bocci (2012), is probably due to the bands of alkenes that bind to glutathione (GSH), stimulating neurons in the hypothalamus, with consequent release of adrenocorticotropic hormones, corticotropin and cortisol. (CARVALHO 2018). Its excess can cause toxic effects that damage various cellular components, including lipids, proteins, DNA 11 and even induce cell death.

ROS react with molecules derived from oxygen, generated by endogenous or exogenous sources (heat, ultraviolet rays, therapeutic drugs, ionizing radiation and pollutants). In excess, it can induce cell death by apoptosis and necrosis (HAYASHI 2018).

ROS influence several biochemical events of cellular metabolism, providing some antimicrobial effects, in addition to benefits in the repair and balance of the target organism (CARVALHO 2018).

At low concentrations, reactions with POLs can be beneficial because they cause acute oxidative processes that act as signals to the body of other existing oxidative stresses, stimulating antioxidant mechanisms such as the antioxidant enzymes superoxide dismutase, glutathione-reductase, glutathione-peroxidase, heme-oxygenase I and catalase. The action of these enzymes can increase the release of stem cells, favoring tissue reconstitution (HAYASHI 2018). The healing property is explained by the increased migration of fibroblasts to the lesion and the increase in collagen synthesis and the expression of cytokines, especially TGF-β1.

O3 stimulates immunoglobulin synthesis, can activate macrophages and increases the sensitivity of microorganisms to phagocytosis, causes increase in interferon, tumor necrosis factor, interleukin IL-2 (HAYASHI 2018).

Founded in 2006, the Brazilian Association of Ozone Therapy (ABOZ), was created from the need to legalize the practice in a conscious and ethical way. It offers two theoretical-practical courses per year (NESI 2018). In Brazil, the Ministry of Health includes ozone therapy among the integrative and complementary practices that are funded by the Unified Health System. Ordinance N° 702, March 21, 2018 of the Ministry of Health, describes this practice as "low cost, proven and recognized safety, which uses the application of a mixture of oxygen and ozone gases, through various routes of administration (...) (LIMA, 2021).

OZONE CAUTION

Despite the high lethality of ozone, when inhaled even at low concentrations, its use has been suggested as a germicidal, bactericidal and chemical cleaning agent. In addition, it has low cost and easy operation of ozonators (LIMA 2021).

According to HAYASHI (2018), ozone therapy causes an extremely low number of side effects, but four deaths from pulmonary embolism have already been recorded after intravenous administration of O2/O3. Direct inhalation of ozone (0.1 to 1ppm) can be toxic to the upper respiratory tract, causing upper respiratory tract irritation, rhinitis, headaches, and occasionally nausea and vomiting, however these effects are infrequent, with occurrence of less than 0.0007%.

In the body, according to ALVES (2020), the first target of ozone is the respiratory tract, and the symptoms are headaches, vertigo, burning sensation in the eyes, throat and cough. The appearance of irritation in the eyes, respiratory tract and the aggravation of preexisting respiratory diseases, such as asthma, are the most reported occurrences in the exposure of human beings to O3, however, this depends on the concentration of the gas and the exposure time. The effects can assume an acute or chronic character, both found in epidemiological studies.

The damage caused by inhaled ozone is related to the release of arachidonic acid from the cell membranes of the lungs, producing an increase in the levels of leukotrienes, primarily responsible for the chemotaxis processes. As a result, neutrophils are attracted to the lung tissue causing local damage.

Although ozone is toxic when used pure, in therapeutic doses, it has been shown to be safe and effective. When used correctly, there will hardly be any adverse reaction. However, some adverse effects can occur, to prevent this toxicity from happening and at the same time for the ozone to offer enough oxidative stress to fight bacteria, such as the ozone concentration range that must be 40-70 μg/ml, depending on the progression of the disease and the systemic condition of the patient (BELEGOTE, 2018).

It must be noted that sensitive groups of people (for example, the elderly and individuals with cardiovascular problems, asthma or with diseases that compromise the respiratory system) are directly affected by the variability of air quality and are more susceptible to the devastating effects of the presence of O3, either by punctual or chronic exposure. These factors increase the levels of hospitalization, in addition to being related to deaths, due to the worsening of more critical clinical conditions. In this sense, studies relate the presence of O3 in the environment to the undue growth of the lungs and the decrease in lung function. Adverse effects are reported even for preterm infants, who are at greater risk of respiratory problems and appear to be substantially more vulnerable to the effects of pollution (LIMA, 2021).

The most important effects of acute exposure are: cellular damage (mainly in the alveolar region); lung cell death and increased replication rates (hyperplasia); decrease in lung activity; inflammation of the airways and the appearance of symptoms such as coughing; chest pain; difficulty in performing deep breathing movements; and sometimes headache and nausea. Among the chronic effects, the most studied are the incidence of asthma and lung cancer. According to Velio Bocci, the incorrect use of ozone due to lack of knowledge generated the dogma that ozone is toxic and must not be used in the health area. It is known that any drug or substance, depending on its dosage, can be therapeutic or toxic (PHILOZON 2021).

METHODS

This is an integrative literature review by analyzing relevant research on the subject.

The search for scientific articles took place in a first phase using the following descriptors in Portuguese and English: "Ozônio", "new antimicrobial alternatives", "Ozonetherapy", through the Scielo, Publish or Perish, Bireme, Medline and Pubmed databases, in addition to searching the Science Direct database. The time frame was from 2017 to 2022.

The selection of articles was weighted through the titles of the works, as well as the abstracts of the works, in order to verify if the literature covered the purpose of this review. Subsequently, there was a mapping of scientific productions, based on their year of publication; periodicals in which they were indexed; type of study employed and objective of the work in question.

CONCLUSION

Ozone is a gas with great potential for use in improving biological safety, because it is capable of annihilating a wide spectrum of pathogens that can endanger humans. It reacts with a variety of naturally occurring organic compounds and can be man's ally in destroying unwanted compounds that plague modern society in the form of pollutants. These properties suggest that ozone has great potential for diverse applications. However, they are also the biggest indication that the handling of ozone requires special care to avoid contact of the gas with humans, animals and plants. The handling of ozone must be carried out by well-trained people and all safety conditions must be maintained. The need arises for a regulation for the use of ozone, based not only on the purpose of use, but also on the individual and collective safety of the user and the population, as happened with radioactive elements and antibiotics, which, although they are widely used, their risks have been minimized by specific regulations.

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