CHAPTER

27 Chlorine Dioxide

Zhao Chen and Mark A. Czarneski

CHEMICAL AND PHYSICAL PROPERTIES

Chlorine dioxide is a yellow-green gas that was first prepared by Chenevix in 1802.[4] Humphrey Davy independently prepared this compound in 1811, elucidated its composition, and proposed the name of euchlorine.[5] Although chlorine dioxide is the most widely accepted English name for this compound, the names chlorine oxide, anthium dioxide, chlorine(IV) oxide, chlorine peroxide, chloroperoxyl, and chloryl radical also have been used. The Chemical Abstracts Service Compound Registry Number (CAS RN) for chlorine dioxide is 10049-04-4.

Structural Properties

Chlorine dioxide contains one atom of chlorine and two atoms of oxygen and exists entirely or almost entirely as a free radical monomer (Figure 27.1).[6,7] Microwave spectra of chlorine dioxide in the gaseous phase have given chlorine-oxygen distances of about 0.147 nm; electronic diffraction indicates 0.149 nm. This chlorine-oxygen distance is approximately that of an average chlorine-oxygen double bond. The angle formed by the oxygen-chlorine-oxygen bonds is in the range of 117.7 + 1.7 degrees.[7,8] Chlorine dioxide has considerable unsaturated bond character,[9] but, in solution, there is no evidence of dimerization or polymerization,[10] and at neutral pH, it does not hydrolyze.[11] Crystalline hydrates of chlorine dioxide have been reported, including a hexahydrate, an octahydrate, and a decahydrate.[7,12-14]

Chlorine dioxide is one member of a series of oxides that also includes chlorine monoxide (Cl_2O); chlorine peroxide [$Cl(O_2)$], which has the same molecular formula as chlorine dioxide but a different structure; chlorine trioxide (ClO_3); chlorine tetroxide (ClO_4); chlorine heptoxide (Cl_2O_7); as well as dimers and mixtures of these oxides. Although all are sometimes called chlorine oxide, each has distinctly different properties from chlorine dioxide.

Spectral Properties

The ultraviolet adsorption spectrum of chlorine dioxide dissolved in carbon tetrachloride shows maxima at 375 and 355 nm with a minimum at 263 nm.[11] The absorption spectrum in aqueous solution has a broad band near 360 nm.[7] The molar extinction coefficient is frequently reported as 1150 M cm^{-1},[15] but when using high-resolution, narrow bandwidth spectrophotometers, it has been determined to be 1250 cm^{-1}.[14,16] The extinction coefficient is temperature, acid, and ionic strength independent from 25°C to 50°C, 0.2 to 4 N, and 2 to 4 M, respectively,[7,14] and is unaffected by chloride concentration up to 0.3 M.[14,17] The chlorine dioxide gas-phase spectrum is the same as that in aqueous solution,[7,14] providing a convenient method for monitoring concentration in various processes.

In the infrared, the fundamental vibrational frequencies of chlorine dioxide in the gaseous phase, measured as wave numbers, are 946 cm^{-1}, 448 cm^{-1}, and 1110 cm^{-1}, corresponding to the symmetric stretch, bend, and asymmetric stretch, respectively.[8]

Physical Properties

Under standard pressure, chlorine dioxide freezes at a temperature of −59°C and boils at 11°C.[18] Thus, it is a true gas at room temperature, where its density is about 1.6.[19] The gas has an odor similar to that of chlorine.

Chlorine dioxide is soluble in water, and a formula has been developed to predict solubility under various conditions.[15,20] Solubilities at 0°C, 15°C, and 30°C were demonstrated to be linear,[18] and extrapolated solubility

FIGURE 27.1 Structure of chlorine dioxide, indicating its radical character.

curves have been published.[11] At 20°C, chlorine dioxide gas present at a concentration of 4% by volume has a solubility of about 4 g/L. The partition coefficient of chlorine dioxide between water and the gaseous phase is expressed as:

$$L = \frac{C_{ClO2\ (aq)}}{C_{ClO2\ (g)}}$$

and is equal to 70 ± 0.7 at 0°C, 45 at 15°C, and 26.5 + 0.8 at 35°C.[11]

Chlorine dioxide in the gas phase is stable in concentrations of less than 10% in air at atmospheric pressure. The gas tends to be unstable at higher concentrations and can be destabilized further by contact with light or with substances that catalyze its decomposition. When decomposition occurs, the volume increase is relatively small, and the resulting explosion has sometimes been described as a "puff." Using chlorine dioxide in gas form for area disinfection/sterilization, the concentrations are typically 0.04% to 0.18% (1-5 mg/L). These concentrations are far below the "puffing" threshold for chlorine dioxide. Detonation has never been observed, even at much higher concentrations at temperatures less than 42°C.[21] Attempts to store chlorine dioxide in a compressed form, with or without other gases, have been unsuccessful[15] until recently when CDG Environmental obtained US Department of Transportation permission to ship 3000 ppm solutions (CDG Solution 3000 liquid concentrate). Recent studies have confirmed the lower limit for explosive decomposition at 9.5% (chlorine dioxide/air).[22] Thus, when the concentration of chlorine dioxide gas in air is below 9.5%, there is no explosion hazard.

METHODS OF PREPARATION

Because chlorine dioxide is not sufficiently stable to be stored, it is typically produced at the site of use. Many methods exist for the preparation of chlorine dioxide, and the method chosen for a specific application will depend on the amount required, the amount of side products that can be tolerated, and whether the gas is required in solution or the gaseous form. The large-scale commercial production of chlorine dioxide, as is required in the bleaching of paper, generally involves the reduction of sodium chlorate by a suitable acid.[7] Several alternative processes use this method, and the details can be found in the extensive compilation of Masschelein and Rice.[11]

Sterilization and disinfection applications are smaller scale processes in which production quantities do not exceed 2000 kg/d. For such processes, sodium chlorite is the preferred starting material.[14] In potable water–treatment facilities, all the chlorine dioxide used is generated from sodium chlorite.[15] As pointed out by Gordon et al,[7] the advantages of using chlorite in the generation of chlorine dioxide are its ease of use and the purity of chlorine dioxide that is produced. In some methods, acid is added to the chlorite/hypochlorite mixture.[23] Chlorine dioxide also has been generated by oxidation of chlorite with nitrogen trichloride.[7] Today, most small generators use an oxidative process in which chlorine (either as a gas or in solution) is mixed with sodium chlorite solution (either as a solid or in solution).[15] This reaction is rapid, readily goes to completion, and proceeds with following stoichiometry:

$$2NaClO_2 + Cl_2 \rightarrow 2ClO_2 + 2NaCl$$

A convenient application of this method of production consists of passing diluted chlorine gas through a column or tower of sodium chlorite to yield uncontaminated chlorine dioxide.[25] Chlorine dioxide generated by this method can be used immediately or dissolved in a solvent (typically water) for temporary storage. This method also can be used to remove traces of chlorine from a stream of chlorine dioxide gas prepared using another method.[7]

Numerous alternative methods for generating chlorine dioxide for smaller scale applications have been reported. Some examples include the electrolysis of chlorite solutions,[7,11] by passing nitrogen dioxide, obtained from the effluent gasses of an electric arc machine, through a column of sodium chlorite.[26] For the preparation of small quantities of chlorine-free chlorine dioxide, the oxidation of chlorite with persulfate, as illustrated by the following reaction, has been used[7,11]:

$$2NaClO_2 + Na_2S_2O_8 \rightarrow 2ClO_2 + 2Na_2SO_4$$

CHEMICAL REACTIONS OF CHLORINE DIOXIDE

Chlorine dioxide is a strong oxidizing agent and, in contrast to chlorine, does not tend to react with organic materials to form chlorinated species or with ammonia to form chloramine. The inorganic chemistry and reactions of chlorine dioxide with organic matter have been well studied and can be found in the comprehensive references written by Gordon et al,[7] Masschelein and Rice,[11] and Aieta and Roberts.[27]

Decomposition

As a free radical species, chlorine dioxide is not stable for long periods in storage and can decompose. Whereas the

gas can be stable in concentrations of less than 10% by volume in air, decomposition is facilitated at higher concentrations. Although its instability precludes storage of chlorine dioxide in compressed form,[15] it is sufficiently stable to permit its routine use, and studies have been done to elucidate the factors that influence its stability.

The decomposition reaction of high concentration of chlorine dioxide has been studied in depth.[28-31] For concentrated chlorine dioxide gas (15%-30% by volume), the ignition temperatures are approximately 130°C; the presence of light, dust, petroleum-based lubricants, and sulfur all lower the decomposition temperature.[32] Further, chlorine dioxide can undergo autocatalytic decomposition, which may or may not involve explosion, and the ratios of products formed during decomposition depend on the concentration of water vapor and temperature.[28] Surface area can accelerate the decomposition of chlorine dioxide, but sufficiently large surface areas appear to inhibit catalytic decomposition by adsorption of the intermediates.

Exposure to light leads to decomposition of chlorine dioxide,[11,29] and in the gas phase, the primary photochemical reaction is the homolytic fission of the chlorine-oxygen bond to form $ClO^{\bullet}$ and $O^{\bullet}$.[11] The reaction mechanism for the light catalyzed decomposition of gaseous, dry chlorine dioxide is postulated as:

$$ClO_2 + h\nu \rightarrow ClO^{\bullet} + O^{\bullet}$$

$$ClO_2 + O^{\bullet} \rightarrow ClO_3$$

$$2ClO^{\bullet} \rightarrow Cl_2 + O_2$$

Interestingly, when moisture is present along with gaseous chlorine dioxide, exposure to light may induce the formation of a visible mist that does not contain chlorine but rather consists of a complex mixture of acids.[29] The following mechanism has been proposed for the photolytic decomposition of chlorine dioxide in the presence of moisture[10]:

$$ClO_2 + h\nu \rightarrow ClO^{\bullet} + O^{\bullet}$$

$$ClO_2 + O^{\bullet} \rightarrow ClO_3$$

$$2ClO_3 \rightarrow Cl_2O_6$$

$$ClO^{\bullet} + ClO_2 \rightarrow Cl_2O_3$$

$$Cl_2O_6 + H_2O \rightarrow HClO_3 + HClO_4$$

$$Cl_2O_3 + H_2O \rightarrow 2\ HClO_2$$

$$2\ HClO_2 \rightarrow HClO + HClO_3$$

In solution at neutral pH, in the absence of light, and at room temperatures (18°C-25°C) or cooler, chlorine dioxide is quite stable. The primary decomposition process, when it occurs, is hydrolysis and disproportionation of chlorine dioxide into chlorite and chlorate ions:

$$2ClO_2 + 2OH^- \rightarrow ClO_{2^-} + ClO_{3^-} + H_2O$$

The rate of hydrolysis is impacted by temperature and pH, increasing rapidly at elevated temperature and at pH values above 10, and is more rapid in the presence of chlorine and hypochlorite, producing chlorate and hydrochloric acid.[14]

In reducing environments, chlorine dioxide may undergo single electron transfer processes, which ultimately result in the formation of chloride. The standard potential (E°) for chlorine dioxide is 1.511 V,[33] although in solution, the electron potential is dependent on the pH and the number of electrons transferred.

Reactions With Organic Compounds

Chlorine dioxide is a selective yet versatile oxidant for many organic compounds. It does not act via chlorination, and thus, trihalomethane formation does not occur.[15] The chemistry and mechanisms of reactions of chlorine dioxide with organic compounds have been reviewed extensively,[11,34,35] and a compilation of kinetic data is also available.[36] The oxidation of amines with chlorine dioxide was studied in detail by Rosenblatt et al[37,38] and Hull et al[39,40] who found that, in aqueous solutions, primary and secondary amines react slowly or not at all with chlorine dioxide but that tertiary amines were readily oxidized, producing a secondary amine and an aldehyde.

Chlorine dioxide does not react with saturated aliphatic hydrocarbons, whereas alcohols, aldehydes, and ketones are oxidized to form carboxylic acids.[14] Chlorine dioxide reacts with carbohydrates, such as glucose, to oxidize the primary hydroxyl groups, first to aldehydes and then to carboxylic acids.[11] The reaction with lipids is mainly an oxidation at the double bond. Although most amino acids do not react readily with chlorine dioxide, tyrosine, tryptophan, and cysteine are exceptions.[35,41] Peptides and proteins are subject to oxidation, substitution, and addition reactions.[42] Chlorine dioxide solutions have been shown to denature proteins,[43] and the gas phase has been shown as an effective way of inactivating β-lactams from old production facilities.[44] Additionally, chlorine dioxide can cause destruction of lysozyme function via protein denaturation and degradation.[45]

Chlorine dioxide rapidly oxidizes phenolic compounds[46] and has been used to oxidize chlorinated phenolic compounds to reduce their toxicity.[14] Occasional chlorination of aromatic or unsaturated aliphatic hydrocarbons has been reported,[47] but no trihalomethanes were formed. Because chlorine dioxide does not tend to form dioxins or trihalomethanes or react with ammonia to form chloramines, it has great appeal for the treatment of water and wastewater.

BIOCIDAL PROPERTIES

The antimicrobial properties of chlorine dioxide solutions were documented in the 1930s by Schaufler[48] and Kovtunovitch and Chemaya.[49] Later, Ridenour and Ingols[50] reported on the addition of chlorine dioxide along with chlorine to commercial water supplies to produce better tasting water, a practice that continues today. Numerous studies have been carried out since then to validate the biocidal properties of chlorine dioxide in an aqueous environment and have demonstrated broad-spectrum biocidal properties. Chlorine dioxide has several registered uses in water treatment, disinfection, and sterilization in both dissolved in water and applied as a gas.

Biocidal Activities

Studies have proved the efficacy of aqueous and gaseous chlorine dioxide on a wide variety of microorganisms, including bacteria, fungi, spores, viruses, and protozoa. The effect of chlorine dioxide has been proved in laboratory conditions on major bacterial pathogens responsible for principal outbreaks, such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica*.[51] Meanwhile, some early studies have shown that chlorine dioxide is capable of inactivating enteroviruses, polioviruses, rotavirus, and human immunodeficiency virus (HIV).[52-59] The increasing interest in the potential biocidal uses of chlorine dioxide has also stimulated studies into the activity of this agent against various protozoal, fungal, and algal species, such as *Cryptosporidium parvum* oocysts, *Streptomyces griseus*, and yeasts.[60-63]

With the current concerns about prions and prion-associated diseases, it is not surprising that studies into the inactivation of these agents by chlorine dioxide have been undertaken. Brown et al[64-66] studied the inactivation of prions by chlorine dioxide and other disinfectants. Sodium hypochlorite produced consistently marked inactivation (3-4 log within 15 minutes), whereas chlorine dioxide (50 ppm) exhibited moderate to substantial inactivation. It was suggested that doubling or tripling the concentration of chlorine dioxide would be more effective in inactivating the agent of Creutzfeldt-Jakob disease.

Microorganisms differ greatly in the sensitivity to chlorine dioxide (Figure 27.2).[51] One of the most distinct features of some bacteria is the ability to produce stress-resistant spores. Because of sporulation, resistance to heat, radiation, desiccation, extreme pH, chemicals, enzymes, and high pressure are largely increased.[67] This resistance enables the bacteria to survive many antisepsis and disinfection (eg, pasteurization) processes. As noted in the previous sections, the broad-spectrum activity of chlorine dioxide in solution was well established in the 1940s and the years following; however, the sporicidal activity of gaseous chlorine dioxide and, thus, its efficacy as a gas sterilant were not demonstrated until the 1980s. The lethal activity of chlorine dioxide gas on spores of *Bacillus subtilis* was first reported by Rosenblatt et al[68] in their patent of the use of chlorine dioxide as a gas sterilant. Sporicidal activity was present at concentrations as low as 11 mg/L. In a work reported subsequently, it was found that, analogous to ethylene oxide, adequate hydration of the spores was required for optimal activity.[69] Activity was also demonstrated against the anaerobic spore former *Clostridium sporogenes*.[70] In 1988, chlorine dioxide gas was registered as a sterilizing agent by the US Environmental Protection Agency (EPA) under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Work by Jeng and Woodworth[71] confirmed the sporicidal activity of chlorine dioxide and provided further information about the conditions required for its effective use.

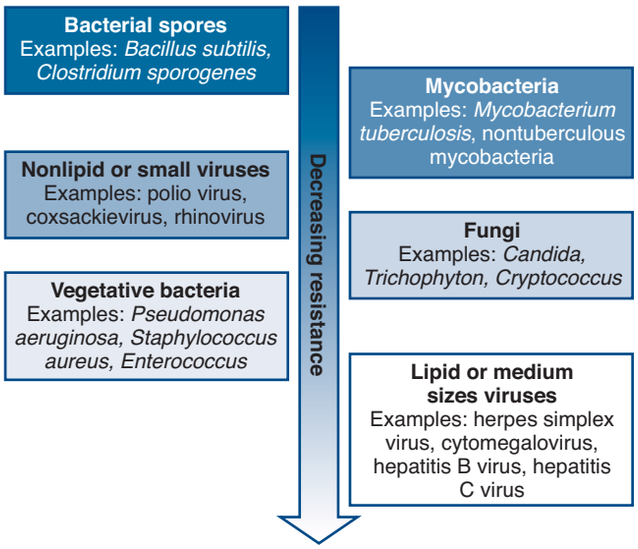

FIGURE 27.2 Typical resistance of microorganisms to chlorine dioxide.

Biofilm can be defined as a community of microorganisms, generally associated with or attached to abiotic or biological surfaces (see chapter 67).[73] These cells are frequently embedded in exopolymeric substances. The tolerance of vegetative bacterial cells in biofilms to environmental stresses, such as routinely used disinfectants, can be profoundly increased than planktonic bacteria. The exopolysaccharide (EPS) matrix restricts the access of antimicrobial substances to cells within the biofilm by reducing diffusion and by maintaining cells at slow growth rate. For example, the survival of *Pseudomonas fluorescens* M2 cells after exposure to a chlorine dioxide solution was apparently enhanced by the presence of *P fluorescens* DL5 in binary biofilms.[74] Vaid et al[75] reported that a 10-minute treatment of 0.3 mg/L gaseous chlorine dioxide and 7 mg/L of aqueous chlorine dioxide resulted in reductions of 3.21 and 3.74 log CFUs/cm^2 of *L monocytogenes* within a biofilm matrix on stainless steel coupon, respectively.

Interestingly, Martin et al[76] isolated a *B subtilis* strain from a washer-disinfector whose vegetative form demonstrated unique resistance to 0.03% chlorine dioxide. It has been postulated that additional efficient intracellular mechanisms may be involved to explain its significant resistance to in-use concentrations of commonly used high-level disinfectants.

The effectiveness of chlorine dioxide on inactivating microorganisms also largely depends on treatment conditions, such as chlorine dioxide phase, concentration, contact time, temperature, pH, total suspended solids, and organic matter.[77] The efficacy increases at higher concentrations and treatment times but decreases with a decrease in temperature and pH. Concerning total suspended solids, their presence promotes pathogen aggregation and interferes with the disinfection performance of chlorine dioxide.[78] Nonetheless, when compared to chlorine, chlorine dioxide is a more effective biocide in the presence of high levels of organic matter, depending on the concentration.

Another factor associated with treatment conditions is the phase of chlorine dioxide. Studies have demonstrated that the application of chlorine dioxide in a gaseous form is more effective in inactivating microorganisms compared with its aqueous form, presumably due to its higher penetrability.[51]

Biocidal Mechanism

The possible modes of action of chlorine dioxide over microorganisms have been widely studied (see Figure 27.2). Research into the mechanism of the biocidal action of chlorine dioxide has involved the identification of specific chemical reactions between chlorine dioxide and biomolecules and evaluation of the effect of chlorine dioxide on physiological functions. The most widely reported mechanism is the disruption of cell protein synthesis primarily due to the oxidations of cell surface membrane proteins[15,79] and free fatty acids[80] and the increase in the permeability of the microbial cell.[81] Cho et al[82] reported that chlorine dioxide caused some levels of both surface damage and inner component degradation in *E coli*. Furthermore, Cho et al[82] explained that only after a certain level of surface damage, intracellular components were attacked because the initial lag phase in enzyme degradation was noticeable and was related to the time required for chlorine dioxide to penetrate the cell.

Chlorine dioxide has also been related to membrane damage on bacterial spores.[83,84] Chlorine dioxide caused damage to the inner cell membrane, change in cell permeability, and interruption of the complete germination of *B subtilis* spores.[83] In *Bacillus cereus*, chlorine dioxide caused surface roughness, indentations, and elongation of cells that resulted in the inhibition of division and associated metabolic damage of bacterial cells.[83] With respect to viruses, chlorine dioxide inactivated poliovirus by altering viral capsid proteins and reacting with RNA separated from the capsids, which impaired RNA synthesis.[52] Li et al[85] reported that the inactivation mechanism of hepatitis A virus (HAV) by chlorine dioxide was due to the loss of the 5′nontranslated regions (5′NTR) (the sequence from bp 1 to 671) and/or destruction of the antigenicity, which is different from that of chlorine.

However, most of the published studies have been focused on the inactivation mechanisms of chlorine dioxide on bacteria and viruses. And there are very few studies on the fungicidal mechanisms of chlorine dioxide. Zhu et al[86] has showed that the metal ion leakage, the inhibition of enzyme activities, and the alteration of cell structure were critical events in *Saccharomyces cerevisiae* inactivation by chlorine dioxide.

TOXICOLOGY AND SAFETY

Chlorine dioxide is used in large quantities, and experience has demonstrated that it can be used safely. Because it is a chemical disinfectant and sterilizing agent, however, it is toxic to living systems. Its application, therefore, should be carefully managed, and steps should be taken to prevent unacceptable exposure. Smith and Willhite[87] indicated that although there are concerns about chlorine dioxide's acute toxicity, they concluded that the human experience with chlorine dioxide in both prospective studies and in actual use situations has failed to reveal adverse long-term health effects.

The threshold limit value (TLV) time-weighted average (TWA) signifying the level at which a human can be safely exposed for up to 8 hours has been established as 0.1 ppm for gaseous chlorine dioxide, and the short-term exposure level (STEL) has been set as 0.3 ppm, as required by the Occupational Safety and Health Administration (OSHA). The STEL is the 15-minute exposure safety level. This TWA concentration is also the most frequently cited odor threshold, which means that exposure to this gas is self-warning.[88]

Chlorine dioxide is considered a mucous membrane irritant, and inhalation of excessive amounts can lead to pulmonary edema.[89] Meggs et al[90,91] describe the nasal pathology of persistent rhinitis with chemical sensitivity after chlorine dioxide exposure.

Because chlorine dioxide is used for water treatment and in the food industry, most of the toxicity studies that have been done have involved ingestion of chlorine dioxide or its metabolites. Whereas most of these studies involved animals, some human studies have been reported. A few studies involving topical application[92,93] also have been done.

When ingested, chlorine dioxide is metabolized and excreted as chloride and chlorite.[94,95] It is difficult to separate effects attributable solely to chlorine dioxide from

those of its metabolic products; therefore, many of these studies compared the effects of the administration of chlorine dioxide and chlorite.

Studies in rats and mice on the ingestion of chlorine dioxide in water[96-98] found little other than a transient increase in serum glutathione. By contrast, studies involving chlorite ingestion[96,99] found methemoglobinemia, decreases in glucose-6-phosphate dehydrogenase (G6PD) activity, and increased erythrocyte fragility. At the highest dose, a few deaths and significant erythrocyte abnormalities were observed in animals. Studies on the ingestion of chlorine dioxide conducted with rats,[100] monkeys,[101] and pigeons[102] all demonstrated decreases in thyroid function. In their work, Bercz et al[101] found that the effect was reversible on withdrawal of chlorine dioxide.

Animal studies to delineate the teratogenic potential of chlorine dioxide and chlorite showed no significant association,[103-105] except for the study on chlorine dioxide by Suh et al.[106] The evidence for mutagenicity of either chlorine dioxide or chlorite is contradictory.[72,107,108] Available data support that chlorite is not considered carcinogenic.[107] There appears to be no published research on the carcinogenicity, if any, of chlorine dioxide.

Studies with human subjects have not detected any serious toxicities associated with the acute or chronic ingestion of chlorine dioxide and chlorite. A wide-ranging, EPA-sponsored study determined the effect of administering discrete doses of increasing concentration of chlorine disinfectants, including chlorine dioxide, to normal healthy volunteers.[109-111] The impact on normal human subjects of daily ingestion of the disinfectants at concentrations of 5 mg/L over 12 weeks also was studied. Lastly, chlorite, at a concentration of 5 mg/L, was administered daily to G6PD-deficient subjects. The physiologic impact was assessed by a large battery of qualitative and quantitative tests. The study affirmed the relative safety and tolerance of normal, healthy, adult males and normal, healthy, adult male G6PD individuals to daily 12-week ingestion of 500 mL of chlorine dioxide at a concentration of 5 mg/L.[111,112]

AQ7

Michael et al[113] studied the population of a village that converted to chlorine dioxide as a potable water disinfectant during the summer months to avoid taste and odor problems. One person, identified as being G6PD deficient, displayed a reduction of hemoglobin concentration, hematocrit, and red blood cell count. No other significant differences were observed in any subjects.

In 1982, Tuthill et al[114] published a retrospective study of neonates from Chicopee, Massachusetts, in the time frame of 1945-1950, when the town used chlorine dioxide (about 0.5 ppm) in water posttreatment. The data were compared, for the same time, with neonates from Holyoke, Massachusetts (a community with similar demographics but using chlorination). The rates of jaundice, birth defects, and fetal and neonatal mortality did not differ significantly between the communities. A statistically significant positive association was found between prematurity and the chlorine dioxide–treated water. Of the roughly 1000 births examined in each community, Chicopee showed 7.8% premature births, and Holyoke experienced a 5.8% rate. This finding was not statistically significant when the age of the mother was controlled.[115]

In 2004, Ouhoummane et al[116] compared thyroid function of newborns from 11 municipalities where chlorine dioxide disinfection was used with 15 municipalities that used chlorine disinfection. They found no evidence of suppressed thyroid function in newborns exposed to drinking water treated with chlorine dioxide. There was no statistical difference in thyroid-stimulating hormone (TSH) or prevalence of congenital hypothyroidism between those exposed to chlorine dioxide disinfection and those exposed to other disinfection. These results were compiled from 32 978 newborns over the period 1993-1999 in Quebec, Canada, for neonatal screening for congenital hypothyroidism.

Akamatsu et al[117] showed low levels of exposure (0.05 and 0.1 ppm of gas) to rats for 24 hours a day, 7 days a week, for a total time of 6 months and no chlorine dioxide gas–related toxicity was found. No weight gain, food or water consumption, or relative organ weight was observed. No biochemistry and hematology examinations changes occurred, and in the respiratory organs, no chlorine dioxide gas–related toxicity was observed.

Chlorine dioxide and sodium chlorite have gone through an EPA's Reregistration Eligibility Decision (RED) for Chlorine Dioxide and Sodium Chlorite (Case 4023) in 2006. Sodium chlorite is used as a precursor in the generation of chlorine dioxide, so the EPA combined the results of both because they have the same toxicologic end points. The RED compiled data and papers and summed up the safety aspects for chlorine dioxide. The Food Quality Protection Act (FQPA) Safety Factor (as required by FQPA) is intended to provide a 10-fold safety factor (10X), for the protection of infants and children in relation to pesticide residues in food, drinking water, or residential exposures. After the RED was completed, the FQPA Safety Factor has been removed (ie, reduced to 1X). This was based on a complete database for developmental and reproductive toxicity; the risk assessment does not underestimate the potential exposure for infants and children, and the end point selected for assessment of risk from dietary and nondietary exposure to chlorine dioxide is protective of potentially susceptible populations including children.

Also in the RED, the acute toxicity of chlorine dioxide was found to be moderate by the oral route and was assigned a toxicity category II. Toxicity category I is considered DANGER, toxicity category II is WARNING, toxicity category III requires CAUTION, and toxicity category IV is safe. For skin, the acute toxicity of chlorine dioxide using sodium chlorite as the test material is considered minimal with a toxicity category of III and for inhalation using sodium chlorite as the test material, chlorine dioxide was

moderately toxic category II. For eye irritation, chlorine dioxide was a mild irritant with a toxicity category of III. For primary dermal irritation, sodium chlorite was a primary irritant with a toxicity category of II. The EPA considers chlorine dioxide and sodium chlorite as essentially the same.

APPLICATIONS

The list of applications and potential applications for chlorine dioxide continues to develop. A search of the recent patent literature disclosed hundreds of filings related to chlorine dioxide. Several of these are quite novel and involve applications employing small amounts of chlorine dioxide generated in situ for disinfection or deodorizing. An example of one such application is a novel plastic packaging film that releases chlorine dioxide over time.[118]

Potable Water

Chlorination has, for many years, been the standard for water disinfection in the United States. There are, however, problems associated with its use in this application. Some raw water supplies contain phenols, other organic compounds, and certain heavy metal ions, which, after chlorination, result in undesirable tastes and odors in the treated water. Furthermore, chlorination of certain waters results in the production of a variety of chlorinated species, including trihalomethanes and dioxins, in the finished water. The presence of these compounds with their associated toxicity raised serious concerns and led to the search for alternatives to chlorine.

The water industry was introduced to chlorine dioxide in 1940 when the Mathieson Chemical Company made available the first commercial quantities of sodium chlorite. This dry, flaked or powdered material dissolved readily in water, where it reacted with chlorine to liberate chlorine dioxide. The potential of generating chlorine dioxide from a water solution of this compound by simply passing chlorine through it had great appeal, especially because early studies indicated that chlorine dioxide was capable of treating water without producing a disagreeable odor or taste.

In 1944, a water-treatment plant in Niagara Falls, New York, was the first in the United States to adopt chlorine dioxide to their water purification process.[89] Used primarily for the control of odors and taste, it eliminated phenolic odors and the unpleasant taste caused by chlorination of dissolved ferrous ion. Cyanides, sulfides, aldehydes, and mercaptans also were oxidized, and pesticides and herbicides, particularly aldrin, methoxychlor, paraquat, and diquat, were reported to be "removed." Because of the higher cost of chlorine dioxide, its use was initially restricted to specialty applications.

In Europe, chlorine dioxide was used more widely in various water-treatment applications. As early as 1940, chlorine dioxide was, and still is, used in various stages of water processing. It is used in raw, presettled water or prior to filtration for disinfection, taste, and odor control where it is applied at levels of 0.1 to 5 mg/L. Today in Europe, several thousand utilities use chlorine dioxide in their water distribution systems.

In 1977, 103 facilities in the United States were identified to be using chlorine dioxide. Estimates place the number of US utilities with chlorine dioxide treatment equipment at 300 to 400, and rapid growth is projected for the next decade.[15]

Today, sodium chlorite remains the preferred raw material for the generation of chlorine dioxide in water treatment and disinfection applications.[14] Although in situ generation processes in which aqueous sodium chlorite is treated with chlorine or acid (HCl or H_2SO_4) and sodium hypochlorite remain the major methodology for water treatment, concerns have arisen about chlorite and chlorate residuals in the finished water. A promising alternative method involves the direct injection of gaseous chlorine dioxide produced from dry sodium chlorite and chlorine gas in a separate reactor into the water stream, thereby eliminating the problem of the contribution of the unreacted chlorite and side product chlorate.[119]

Antibiotic Inactivation

Many people have an allergy to β-lactams, an important class of antibiotics. Some of these allergies can be severe, and as such, pharmaceutical manufacturers must take precautions to avoid cross-contamination. The issue can be severe, and as such, there are regulations surrounding this issue (eg, in the United States, under 21 CFR 211.176):

> If a reasonable possibility exists that a non-penicillin drug product has been exposed to cross-contamination with penicillin, the non-penicillin drug product shall be tested for the presence of penicillin. Such drug product shall not be marketed if detectable levels are found when tested according to procedures specified in "Procedures for Detecting and Measuring Penicillin Contamination in Drugs."

Chlorine dioxide gas is an oxidizer, and as such, it was thought that this reaction will inactivate β-lactams. In one study, β-lactams from the penicillin, cephalosporin, and carbapenem groups were tested.[44] Specifically, penicillin G, penicillin V, ampicillin, and amoxicillin from the penicillin group; cefadroxil, cefazolin, and cephalexin from the cephalosporin group; and imipenem from the carbapenem group. A cocktail of these β-lactams were inoculated on three different carriers (stainless steel, aluminum, and Lexan) and tested at various concentrations

and dosages. It was found that a dosage of 7240 ppm-h achieved a 3 log reduction of all the β-lactams. Based on this study, many old penicillin production facilities have been cleaned successfully with data submitted to US Food and Drug Administration (FDA) and other worldwide regulatory bodies.[120]

Chemical Weapon Decontamination

Chlorine dioxide gas, as an oxidizing agent, will oxidize items that it encounters, including many chemical agents. Gordon et al[121] at Public Health Agency in Canada showed gaseous chlorine dioxide as effective in inactivating anthrax toxins lethal factor and protective antigen within a short amount of time. When Snyder[122] performed studies for the EPA, chlorine dioxide fumigation achieved >99% inactivation of chemical nerve agent VX. But chlorine dioxide was found to be ineffective or only partially effective for thickened soman and sarin, which may be related to lack of penetration by the gas to the chemical tested.

Food Industry

Chlorine dioxide has found many applications in the food industry; it has been used for disinfecting both equipment and materials and raw and finished products. In solution, it is used to disinfect fruits and vegetables and, in poultry, chiller and process water to control contamination.[123] Chlorine dioxide is also used to bleach and mature flour.[11] At levels of 10 to 40 ppm, it oxidizes and decolorizes the carotenoid pigments and accelerates aging to produce flour that will make an elastic dough. It is used to bleach certain fats and fatty oils by oxidation of pigments to colorless forms.[11] Other applications include disinfection of spices, removal of the medicinal odor from cooked shrimp, and extension of the shelf life of tomatoes.[11] Reina et al[124] found that the cooling water for pickling cucumbers reached relatively high populations of bacteria during a typical day's operation, and these could be optimally controlled by the addition of 1.3 ppm chlorine dioxide.

Chlorine dioxide is legally permitted in at least the United States and China for sanitizing fruits and vegetables in water.[125,126] In the United States, the application of chlorine dioxide in the food industry is regulated by the FDA and the EPA. The FDA states the specific conditions in using chlorine dioxide as a food additive for human consumption and allows the use of chlorine dioxide as a disinfectant agent in water at a concentration not exceeding 3 mg/L residual to wash whole fruits and vegetables. Treatment of produce with chlorine dioxide is followed by a potable water rinse or by blanching, cooking, or canning.[125] However, the use of liquid chlorine dioxide to disinfect fresh-cut fruits and vegetables and the direct application of chlorine dioxide gas on fresh fruits and vegetables cannot be established in the regulations. Of note, there are over 20 Food Contact Notifications (FCNs) issued by the FDA with the use of aqueous and gaseous chlorine dioxide (FCN 644, 645, 1400, 1421, 1804 to specify a few). Food contact notices or substances can be defined as "any substance intended for use as a component of materials used in manufacturing, packing, packaging, transporting, or holding food if such use is not intended to have a technical effect in such food" based on the Food, Drug, and Cosmetic Act. Chlorine dioxide is also allowed to be used in the production of organic foods according to 7 CFR §205.601, which specifies synthetic substances allowed for use in organic crop production. In the European Union (EU), there are no regulations concerning the use of chlorine dioxide for surface washing of fresh produce.

Numerous studies have proven that chlorine dioxide is effective in reducing some major foodborne pathogens in foods, such as *E coli* O157:H7, *Salmonella* species, *L monocytogenes*, as well as food spoilage microorganisms.[51] Overall, the disinfection level achieved through gaseous chlorine dioxide treatment is higher than those with aqueous washing, and a greater than 8 log reduction has been reported. But results can be greatly dependent on several factors, such as experimental design, sample type, and treatment scale.

Han et al[127] determined the reductions of *L monocytogenes* on injured and noninjured green pepper surfaces by both aqueous and gaseous chlorine dioxide. Gaseous chlorine dioxide showed significantly higher log reduction than aqueous chlorine dioxide treatment for both injured and uninjured food surfaces. The main advantage of gaseous chlorine dioxide over aqueous form is that gas has more powerful ability to penetrate. Gaseous chlorine dioxide could reach microorganisms present in foods, which are protected by surface irregularities, biofilms, and so on. The efficacy of gaseous chlorine dioxide in reducing *E coli* O157:H7 and *L monocytogenes* on strawberries was also determined using batch and continuous flow chlorine dioxide gas treatment systems.[128] A batch treatment of strawberries with 4 mg/L gaseous chlorine dioxide for 30 minutes and continuous treatment with 3 mg/L gaseous chlorine dioxide for 10 minutes achieved greater than a 5 log reduction for both *E coli* O157:H7 and *L monocytogenes*.

Due to the high populations of natural microflora in foods, microorganisms should be carefully controlled especially during storage because foods may contain some weakened or damaged points that can become niches for microbial contamination. It has been widely documented that gaseous chlorine dioxide is effective against natural microflora in foods. Gómez-López et al[129] found that after gaseous chlorine dioxide treatment, the disinfection levels of mesophilic aerobic bacteria, psychrotrophs, and yeasts in grated carrots achieved were 1.88, 1.71, 2.60, and 0.66 log CFUs/g, respectively. A lag phase of at least 2 days was observed for mesophilic aerobic bacteria, psychrotrophs, and lactic acid bacteria in treated samples, whereas

mesophilic aerobic bacteria and psychrotrophs increased in parallel. Mahmoud and Linton[130] found lower levels of psychrotrophic bacteria, yeasts, and molds on shredded lettuce after gaseous chlorine dioxide treatment, which stayed lower compared to the untreated samples during storage at 4°C for 7 days. Apart from laboratory-scale tests, pilot studies have also been previously conducted. In a pilot study of Popa et al,[131] five separate half-pint plastic clamshell containers each containing 100 g of blueberries were placed on a metal rack inside a sealed 20-L bucket and exposed to gaseous chlorine dioxide. Significant reductions of 2.33, 1.47, 1.63, and 0.48 log CFUs/g were observed for mesophilic aerobic bacteria, coliforms, yeasts, and molds, respectively.

Aqueous chlorine dioxide has also been proved efficient to eliminate pathogens in foods, although no complete elimination has been observed. The early study of Zhang and Farber[132] showed only a 1.1 log reduction of *L monocytogenes*, when 5 mg/L aqueous chlorine dioxide was applied to shredded lettuce or cabbage for 10 minutes. Reductions as high as 5 log CFUs/mL have been lately reported by other authors. Rodgers et al[133] reported that 5 mg/L aqueous chlorine dioxide could achieve over 5 log reductions of *L monocytogenes* and *E coli* O157:H7 on apples, lettuce, and cantaloupe. The data obtained in the study of Pao et al[134] revealed significant reductions (5 log CFUs/cm^2) of *S enterica* and *Erwinia carotovora* after 20 mg/L chlorine dioxide treatment on tomato only when the fruit was freshly inoculated. When antimicrobial ice preparations containing chlorine dioxide was applied to mackerel skin for 120 minutes, total reductions of *E coli* O157:H7, *Salmonella* ser Typhimurium, and *L monocytogenes* were 4.8, 2.6, and 3.3 log, respectively.[135]

Studies on the disinfection of aqueous chlorine dioxide on the natural microflora in foods have indicated various results, ranging from no significant inactivation to higher reductions. An early study by Costilow et al[136] revealed that washing cucumbers in water with even high concentrations 100 mg/L chlorine dioxide failed to significantly reduce populations of yeasts, molds, and lactic acid bacteria. In contrast, some studies on the effect of aqueous chlorine dioxide on the spoilage microbiota of foods have yielded generally satisfactory results. Aqueous chlorine dioxide has been reported to significantly reduce the levels of aerobic mesophilic bacteria, aerobic psychrotrophic bacteria, yeasts and molds, and lactic acid bacteria in fresh-cut asparagus, lettuce, and mulberries.[137,138] In the study of Hong et al,[139] 100 mg/L aqueous chlorine dioxide treatment reduced the populations of total aerobic bacteria, yeast and mold, and coliforms in chicken legs by 0.93, 1.15, and 0.94 log CFUs/g, respectively. Researchers have also developed methods that implemented aqueous chlorine dioxide as one additional hurdle combined with other interventions to achieve effective control of spoilage microorganisms. Chen and Zhu[140] effectively inactivated aerobic mesophilic bacteria, aerobic psychrotrophic bacteria, yeasts and molds, and lactic acid bacteria in plums using a combination of aqueous chlorine dioxide and ultrasound.

The effects of chlorine dioxide treatment in both its liquid and gaseous forms on the shelf life of foods have been thoroughly studied. On fruits and vegetables, chlorine dioxide is effective in controlling postharvest spoilage. Gómez-López et al[129] prolonged the shelf life of grated carrots stored at 7°C by 1 day using gaseous chlorine dioxide treatment. The treatment with 100 mg/L aqueous chlorine dioxide for 20 minutes could prolong the shelf life of fresh-cut asparagus lettuce stored at 4°C to 14 days, compared to 4 days for the control.[137] Chen et al[138] reported that aqueous chlorine dioxide treatment of 60 mg/L for 15 minutes with and stored at −1°C prolonged the shelf life of mulberries to 14 days compared to 8 days for the control. Treatment of beef trimmings before grinding with 200 mg/L aqueous chlorine dioxide for 3 minutes could also extend the patty shelf life at 2°C.[141]

Chemical residues after both aqueous and gaseous chlorine dioxide treatments have been investigated. With respect to chlorine dioxide in the gaseous phase, Tsai et al[142] could not detect any residues of chlorine dioxide, chlorite, or chlorate in potatoes stored in an atmosphere with gaseous chlorine dioxide. As for Roma tomatoes, a 10 mg/L aqueous chlorine dioxide treatment for 3 minutes left residues of chlorine dioxide, chlorate, and chlorite but not chloride, although they were not detectable after 1 day.[143] Trinetta et al[144] evaluated the residues of chlorine dioxide, chlorite, chlorate, and chloride on tomatoes, oranges, apples, strawberries, lettuce, alfalfa sprouts, and cantaloupes after treatment with 5 mg/L gaseous chlorine dioxide for 10 minutes followed by a subsequent water rinse. The treatment may leave detectable residual levels on lettuce and sprouts, whereas treated tomatoes, oranges, apples, strawberries, and cantaloupes were all found to have very low residuals compared to the EPA acceptable levels for drinking water. The application of aqueous chlorine dioxide to mulberries and plums did not leave residues of chlorine dioxide, chlorite, or chlorate.[138,140] This could be attributed to the fact that the treatment was followed by a water rinse according to the FDA,[124] which was designed to remove chemical residues.

Food Facility Treatment

Gaseous or liquid chlorine dioxide is also used for the treatment of the food production facilities rooms and equipment. *E coli*, *Salmonella*, *Listeria* are a few organisms that are known pathogen concerns for facilities. These contaminations have been known to survive for years in facilities and are difficult to clean.[145] They last for years because many areas of a production facility are hard to or impossible to clean. Many facilities are old and were not designed with current cleaning practices in mind.

This can lead to ineffective decontamination, cleaning, and disinfection processes. The use of chlorine dioxide in its gas form has allowed these facilities to reduce these risks. Chlorine dioxide as a gas has a greater opportunity to get to all areas and allow for greater disinfection assurance because the gas molecule is small and can reach hard to reach areas. In the past, it was incumbent on the user to get the disinfecting agent, typically liquids, to all surfaces. When using chlorine dioxide in its true form (as a gas), it can reach all surfaces to provide a thorough kill independent of the users' actions.

Odor Control

Chlorine dioxide has been used for years in odor control in both the residential and industrial markets. In the industrial market, chlorine dioxide has been used in scrubbing systems to prevent emission of noxious odors. Chlorine dioxide scrubbers have been used to eliminate the odors associated with sulfur compounds as well as the difficult-to-control odors produced by fat-rendering works and fish reduction plants.[8,11]

In the residential market, there are hundreds of products that can produce small amounts of chlorine dioxide for use in deodorizing everything from refrigerators to garbage cans. As an example, NosGUARD SG (Fort Lauderdale, Florida) is used to eliminate foul odors caused by mold, mildew, pets, food, smoke, and more. Other companies have services based around odor control using chlorine dioxide solutions and gas.

Sanitization and Disinfection

In addition to the applications mentioned previously, solutions containing chlorine have been used for an additional variety of sanitization and disinfection applications. In these applications, the in situ generation of chlorine dioxide is most often used. The products used in these applications are advertised as "stabilized" chlorine dioxide and contain chlorite along with other proprietary ingredients. Chlorine dioxide is generated by the addition of an "activator," which may be provided by combination with or addition of another chemical or electrolytically. Gutman and Marzouk[146] disclosed solid compositions that, when dissolved in water, release chlorine dioxide quickly and almost quantitatively. These can be used in a variety of applications ranging from industrial and water-treatment installations to antiseptic and sanitizing preparations. Aseptrol (Engelhard Corp, Iselin, New Jersey) dissolvable capsules[147] are commercially advertised for application as hard-surface sanitizers. Aseptrol is solid sodium chlorite and a proprietary activator contained in a capsule. When placed in water, a chlorine dioxide solution results.

Yi et al[148] demonstrated the effective use of chlorine dioxide gas to disinfect gastrointestinal endoscopes. Treatment with 4 mg/L chlorine dioxide gas for 30 minutes and 75% RH resulted in complete inactivation of *Bacillus atrophaeus* spores and demonstrated the use of chlorine dioxide gas as an effective sterilant.

Chlorine dioxide generating preparations with sporicidal activity have been applied with hand sprayers to disinfect isolators used for the containment of gnotobiotic animals.[149] Novartis (formerly the Sandoz Corp) validated an isolated filling line that uses chlorine dioxide solution for sanitization,[149] although no data on sporicidal efficacy were published.

The Alcide Corp markets a line of products based on a technology in which chlorine dioxide is generated in situ when sodium chlorite and lactic acid are mixed. These products are intended for several different applications. One such application is the control of infectious diseases in aquaculture and for the treatment of surfaces, media, or animals directly to remove bacterial, viral, fungal, and protozoan parasites.[150] Two additional applications propose the treatment of blood to reduce the infectivity of HIV in blood and blood products and to reduce the risk of disease transmission through transplantation of optical sclera and corneas.[151,152]

Another patent addresses a method for inactivating viruses in blood using an unspecified two component method for chlorine dioxide generation and a process involving a closed, controlled-communication container.[153] Allergan Inc has patented some technologies for the use of stabilized or electrolytically prepared chlorine dioxide as a contact lenses disinfectant.[154-156]

Tristel Solutions Ltd produces a series of high-level and sporicidal liquid disinfectant products that harness the biocidal power of chlorine dioxide. The Tristel products based on the liquid formulation technologies contribute significantly to the prevention of hospital-acquired infections (HAIs) and can play an important role in managing cross-contamination and eliminating outbreaks.

Insect and Pest Fumigation

Chlorine dioxide has primary been shown as an antimicrobial agent. Czarra et al[156] have shown that chlorine dioxide gas can be effective against insects. In life science research, mice and rats have been widely used. When working with rodents, pinworms are typically nonpathogenic, but with immunocompromised rodents, they can have adverse effects on behavior, growth, intestinal physiology, and immunology, which may affect research. Rodents are typically treated with a special diet to eliminate the worms, but the environment still contains pinworm eggs, which typically leads to reinfection. In the lab, chlorine dioxide gas has been shown to completely render pinworm eggs (*Syphacia ova*) nonviable with a 1440 ppm-h cycle. Cole and Czarneski[157] demonstrated the complete pinworm egg inactivation in the field in a medical research

animal facility in Australia and completely eradicated the infestation and remained pinworm free 18 months later.

Bedbugs (*Cimex lectularius* and *Cimex hemipterus*) have had a resurgence in recent years. These insects have proven themselves as hardy organisms with the capability of surviving for several months without an available food source. As such, they can be difficult to remove from the environment. Typically, the manual cleaning process is used to eliminate them. Gibbs et al[158] have shown that gaseous chlorine dioxide can achieve a 100% mortality rate after exposure to various concentrations (1, 2, and 3 mg/L) at various dosages (519, 1029, 1132, and 3024 ppm-h).

Gas Disinfection and Sterilization Applications

As noted previously, although the potent antimicrobial activity of chlorine dioxide in solution was known since 1947, it was not until 1985 that its sporicidal activity in the gas phase was recognized.[68,69] Subsequent development of disinfection and sterilization processes has opened new areas of application for this versatile agent and has been used in varied applications.

Chlorine dioxide gas has been shown to be effective at large-scale facility decontamination when new facilities were disinfected prior to the start of research. RIKEN, Japan's largest comprehensive research institution, disinfected a new facility, which houses approximately 20 000 mice and 3000 rats.[160] Czarneski[161] reported the use of chlorine dioxide gas to decontaminate a new 180 000 ft^3 (5097 m^3), 65-room facility, which included some new and used equipment. The whole facility disinfection process allowed the equipment to be treated in place, which reduced the probability of cross-contamination from old equipment to new equipment. Luftman et al[162] also demonstrated the successful disinfection of a *Salmonella*-contaminated facility at very low concentrations of 0.3 mg/L over time. In addition to entire facility disinfection, Girouard and Czarneski[163] demonstrated the disinfection of a three-room suite (approximately 4077 ft^3 [115 m^3]) in 3.5 hours total cycle time including aeration at low concentrations of 1 mg/L. Sensitive equipment such as computers and other lab equipment were disinfected in place during multiple validation exposures to demonstrate the relative good material compatibility of the gas phase. One computer underwent 35 exposures and continued to function normally. Likewise, Leo et al[164] showed the repeated disinfection of a pharmaceutical Blow-Fill-Seal machine at 1 mg/L chlorine dioxide concentrations and predicted a $\log_{10}$ spore reduction of a predicted minimum of 16.65 based on three different disinfection cycles with a summary of four 24-minute quarterly results. Sherman et al[165] showed good material compatibility by disinfecting a cryoelectron microscope with minimal impact on the equipment. Lowe et al[166-168] validated hospital rooms, ambulances, and suites or rooms with various organisms and locations also showing material compatibility. Chlorine dioxide has also been validated in isolators from various manufacturers,[169–171] processing vessels and tanks,[172,173] and biological safety cabinets.[174,175] Isolator cycle times were as short as 83 minutes for small transfer isolator and 115 minutes for a train of isolators at 5 mg/L concentrations.[171] Eylath et al[169] observed kill under half suit armpits with the arms in the down position at 5 and 7.5 mg/L concentrations. They also showed no residuals after gas exposure by rinsing 304 stainless steel coupons with water for injection (WFI) and measured no residual as measured using a high-performance liquid chromatography (HPLC) method for detection of chloride.

In a study of the sterilization of overwrapped foil suture packages, Kowalski[176] demonstrated the practical utility of gas-phase chlorine dioxide sterilization. They found that excellent kill of spores could be achieved routinely at concentrations of as low as 10 mg/L of chlorine dioxide. Jeng and Woodworth[72] showed that blood oxygenators, devices that present a particularly rigorous challenge, could be sterilized with chlorine dioxide gas.

In 2001, ClorDiSys Solutions, Inc, acquired the rights to gaseous chlorine dioxide sterilization technology from Johnson & Johnson, the original developer of an automated dry gas technology. Since this acquisition, efforts directed toward further developing commercial markets (life science, health care, pharmaceutical, research, and food) for this process, and various types of generators have been developed for different markets. The life science market uses chlorine dioxide in research areas, where clean environments are needed in barrier facilities to keep research clean or to protect research animals or people. In research labs, such as biosafety level 3 or 4 labs, disinfection is critical because users in these labs regularly work with deadly infectious agents. In the health care industry, hospitals are becoming more important as more information is becoming known of HAI and the risks of environmental contamination in-patient isolation rooms or wings. In the pharmaceutical industry, isolators and clean rooms have routinely been disinfected with chlorine dioxide gas and in an increasing number of biologic facilities. These facilities are critical because any contamination can cause entire batches to be disposed of, often because of a small-level biological contamination. In recent years, the FDA Food Safety Modernization Act (FSMA) of 2011 has required facilities to be proactive instead of reactive. This has led to chlorine dioxide gas to be used on significantly large facilities (over 1 million ft^3 [28 000 m^3]) in the food industry, particularly in the dairy, dry processing and wet processing facilities.

A schematic diagram of a typical chlorine dioxide generator is shown in Figure 27.3.

The availability of small, production-scale chlorine dioxide disinfecting units or sterilizers has allowed detailed investigation of the applicability of chlorine dioxide sterilization to selected products. One such study involved

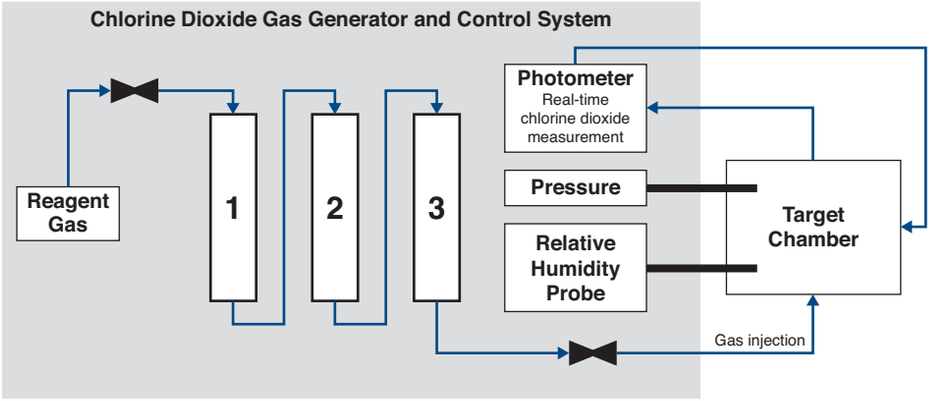

FIGURE 27.3 Typical dry gas chlorine dioxide generator. The reagent gas (2% chlorine/98% nitrogen) flows through sodium chlorite and generates >99% pure chlorine dioxide gas + sodium chloride. The chlorine dioxide/nitrogen mixture is delivered to the target chamber and the sodium chloride is left in the consumable cartridges (1-2-3). The relative humidity is measured, and the control system raises it to the typical target set point of 65%. The control system then injects and controls the concentration to reach a target set point of 0.5-20 mg/L depending on the application. The pressure is measured in some applications to ensure high pressure is released to protect the chamber.

polymethylmethacrylate intraocular lenses and was reported by Kowalski.[176] In this instance, humidification of product to be sterilized was conducted in the sterilization chamber itself. A representative process diagram for this study is shown in Figure 27.4.

The results of the intraocular lens study, as well as others, indicated the potential of chlorine dioxide as an alternative to ethylene oxide for selected applications. Chlorine dioxide offers some significant advantages over ethylene oxide for selected sterilization applications.[176] It is effective

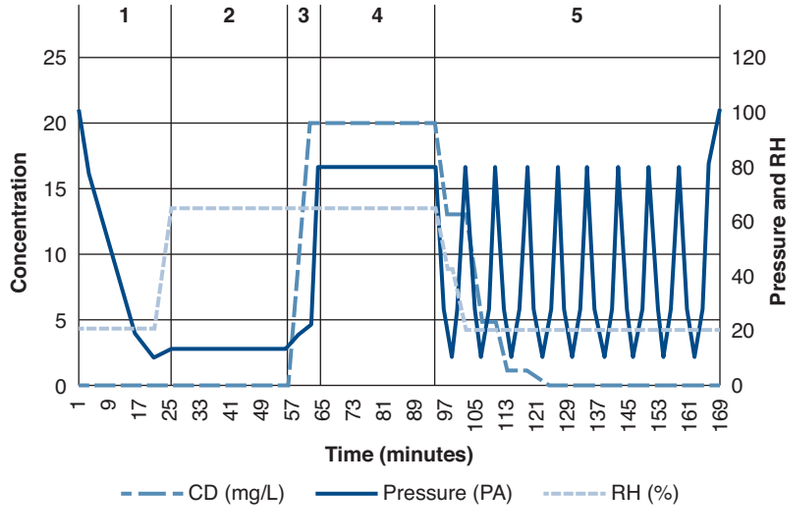

FIGURE 27.4 Typical vacuum sterilization cycle. Step 1—precondition: This step lowers the vacuum to set point (10 kPa) and adds relative humidity to set point (65%). Step 2—condition: This step holds the relative humidity at set point for typically 30 minutes. Step 3—charge: This step raises the chlorine dioxide gas concentration to set point (20 mg/L) and then backfills the chamber with filtered air to 80 kPa. Step 4—exposure: This step holds the chlorine dioxide gas concentration at 20 mg/L for 30 minutes. If the concentration drops for any reason, the gas is returned to the target set point. Step 5—aeration: This step removes the chlorine dioxide gas from the chamber by pulling vacuum and breaking with filtered air. Typically, eight vacuum/break cycles are required to remove the gas to safe levels. Abbreviations: CD, ; PA, ; RH,

in comparatively low concentrations at atmospheric pressure or below. At the concentrations used, it is not explosive and does not require expensive, damaging limiting construction. Additionally, the light-absorbing properties of chlorine dioxide allow for convenient, real-time, spectrophotometric measurement of gas concentration during sterilization. Coupled with complete process control, the sterilization process is amenable to the validation of parametric release of sterilized product. Additionally, chlorine dioxide does not possess the "solvent-like" properties of ethylene oxide, and consequently, residual chlorine dioxide remaining in sterilized products is low and does not require extensive aeration.[176]

The demonstrated efficacy of chlorine dioxide at low concentrations and atmospheric pressure has established many applications for this agent. Figure 27.5 shows a typical isolator or small chamber cycle. Small isolators can have total cycle times under 90 minutes including the aeration time. The cycle consists of five phases or steps. The first step is precondition, which raises the relative humidity from ambient to 65%. This 65% is then maintained for 30 minutes in the condition step. Once the conditioning is completed, the cycle advances to the charge step, which injects chlorine dioxide gas to the target concentration. Once the concentration is reached and verified by photometric measurement, the cycle advances to the exposure step, which typically is 30 minutes at 5 mg/L for small chambers. When this time has elapsed, the final step of aeration is started. In this step, fresh air is brought into the chamber and the gas is exhausted to the outside.

Figure 27.6 shows a typical room disinfection cycle. These cycles have times typically under 4 hours. Some large rooms or suites of rooms can have cycles under 8-hour total cycle time. Large chambers (20 000 ft^3 [566 m^3]) typically use lower concentrations of 0.5 mg/L and hold for 240 minutes. Room cycles are very similar to small chamber or isolator runs such that the steps are identical, but the times and concentrations vary. Because of this and the longer cycle times, some steps were combined. For rooms or large chambers, the condition time is shortened. Because the chambers are large, the charge step is long and the conditioning time can be combined with the charge step. This allows the cycle times to be optimized.

To further shorten cycle times, the charge time and exposure time can be combined. This is done by using a dosage counter compared to an exposure timer. To do this, the concentration is measured as soon as it starts injecting and the ppm/h are accumulated. The value of 720 ppm/h is based on current published knowledge; for example, a minimum of 5 $\log_{10}$ reduction of spores was demonstrated at 400 ppm/h by Luftman et al[162] inside a large animal hospital disinfection cycle, and a 6 $\log_{10}$ reduction was demonstrated by Czarneski and Lorcheim[171] in an isolator at 900 ppm/h. Eylath et al[169] showed several cycles in isolators and processing vessels applications

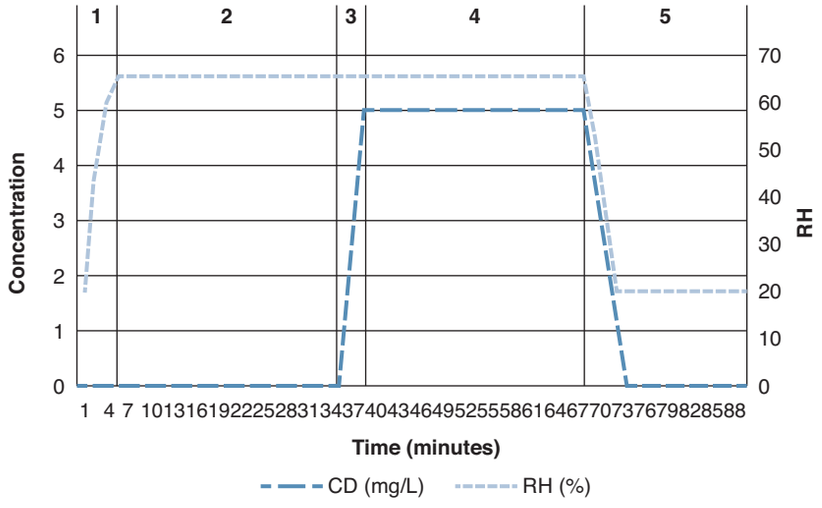

FIGURE 27.5 Typical ambient isolator decontamination cycle. Step 1—precondition: This step raises the relative humidity to set point (65%). Step 2—condition: This step holds the relative humidity at set point for typically 30 minutes. Step 3—charge: This step raises the chlorine dioxide gas concentration to set point (5 mg/L). Step 4—exposure: This step holds the chlorine dioxide gas concentration at 5 mg/L for 30 minutes. If the concentration drops for any reason, the gas is returned to the target set point. Step 5—aeration: This step removes the chlorine dioxide gas from the chamber by using house exhaust to remove the gas. Typically, 12-15 air exchanges are required to remove the gas to safe levels. Abbreviations: CD, ; RH, .

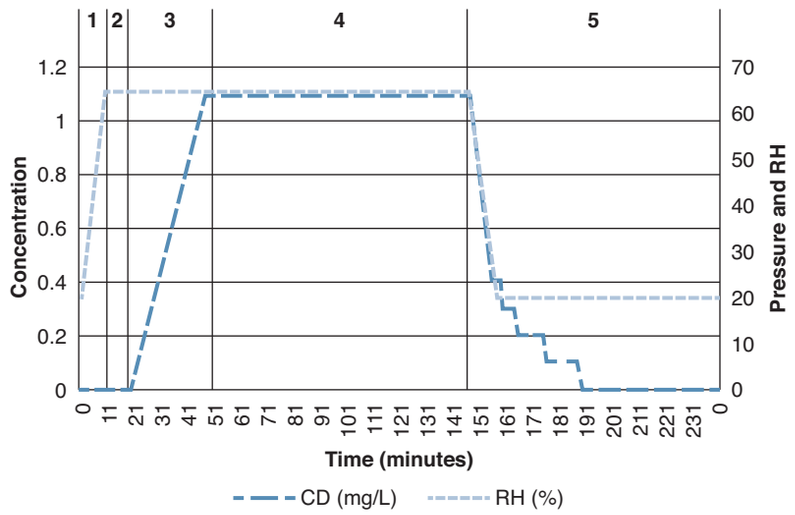

FIGURE 27.6 Typical ambient room decontamination cycle. Step 1—precondition: This step raises the relative humidity to set point (65%). Step 2—condition: This step holds the relative humidity at set point for typically 10 minutes. Step 3—charge: This step raises the chlorine dioxide gas concentration to set point (1 mg/L). Step 4—exposure: This step holds the chlorine dioxide gas concentration at 1 mg/L for 120 minutes. If the concentration drops for any reason, the gas is returned to the target set point. Step 5—aeration: This step removes the chlorine dioxide gas from the chamber by using house exhaust to remove the gas. Typically, 12-15 air exchanges are required to remove the gas to safe levels.

that demonstrated 6 $\log_{10}$ reduction of spores. The tests were run at various contact time ranging from 540 to 600 ppm/h as well as 900 ppm/h. In isolators, Eylath et al[173] demonstrated 6 $\log_{10}$ reductions in exposures of 675 to 1800 ppm/h. A 4 $\log_{10}$ reduction was demonstrated by Leo et al[164] at a low exposure of 180 ppm/h (1 mg/L for 30 minutes) and Czarneski[161] demonstrated a 6 $\log_{10}$ reduction at 820 ppm/h.

To follow up on all the various doses, Lorcheim and Melgaard[177] performed studies with a constant dosage (720 ppm/h) and varied the concentrations (0.3, 0.5, 1, 5, 10, and 20 mg/L) and found that the dosage of 720 ppm/h provided a 6 $\log_{10}$ reduction of spores regardless of concentration. This indicates that dosage or contact time is the more critical parameter compared to exposure time. The dosage that is required for good cycles typically is 720 ppm/h. This dosage is the accumulation of concentration over time or labeled as ppm/h.

To calculate chlorine dioxide ppm from mg/L, the below calculations can be used:

ppm calculation for 1 mg/L chlorine dioxide concentration
ppm = (mg/m^3) (24.45) / molecular weight = (mg/L) (1000) (24.45) / molecular weight
Chlorine dioxide ppm = (1 mg/L) (1000 L/m^3) (24.45) / 67.5 = 362.2
Exposure contact time = 362 ppm × 2 h = 724 ppm/h

The number 24.45 in the equations is the volume (liters) of a mole (gram molecular weight) of a gas at 1 atmosphere and at 25°C.

So, the overall dosage or contact time starts when gas is being injected and the accumulation starts. This has the effect of combining the exposure time and charge time and shortening the overall cycle time.

CONCLUSIONS

Chlorine dioxide has a broad-spectrum biocidal activity against a variety of bacteria, viruses, yeasts, mycobacteria, and bacterial spores. Because chlorine dioxide is an oxidizing agent, the primary mode of action is via electron exchange within the microbial molecular structure. Chlorine dioxide is favored over nonoxidizing disinfectants due to its greater efficacy. Although all oxidizing agents are capable, at suitable concentrations, of providing biocidal disinfection, they have several drawbacks when compared to chlorine dioxide. Unlike chlorine, chlorine dioxide does not readily form halogenated by-products due to the nature of the molecular bonding. Chlorine dioxide is known to be compatible with most of the materials commonly found in medical and pharmaceutical equipment and environments and has an excellent health and safety record when used at recommended levels.

Chlorine dioxide has been widely applied in medical and pharmaceutical environments. It has also been used in various applications such as drinking water treatment and the washing and treatment of foods. As with all choices among alternative approaches that provide solutions to a given problem, an informed choice based on current evaluation and understanding of pertinent existing data is the best one that can be made. It is the authors' intent that this overview of chlorine dioxide should aid in making an informed choice regarding its application.

REFERENCES

1. Health and Safety Executive. *Chlorine Dioxide: Risk Assessment Document EH72/14*. Sudbury, United Kingdom: HSE Books; 2000.
2. Clayton G, Clayton F. *Patty's Industrial Hygiene and Toxicology*. New York, NY: John Wiley & Sons; 1994.
3. Landner L, Grimvall A, Håkansson H, et al. *Chlorine and Chlorinated Compounds in Sweden. Survey on Fluxes to and in the Environment, Pools in the Environment and Health and Environmental Risks*. Solna, Sweden: Swedish National Chemicals Inspectorate; 1995. KEMI report 5/95.
4. Sidgwick NV. Halogen oxides. In: *The Chemical Elements and Their Compounds*. Vol 2. Oxford, United Kingdom: Oxford University Press; 1951:1202-1218.
5. Davy H. On a combination of oxymuriatic gas and oxygen gas. *Philos Trans*. 1811;101:155-161.
6. Taylor NW, Lewis GN. The paramagnetism of "odd molecules." *Proc Natl Acad Sci U S A*. 1925;11:456-457.
7. Gordon G, Keiffer RG, Rosenblatt DH. The chemistry of chlorine dioxide. In: Lippaer SJ, ed. *Progress in Inorganic Chemistry*. Vol 15. New York, NY: John Wiley & Sons; 1972:201-287.
8. Vaida V, Simone JD. The photoreactivity of chlorine dioxide. *Science*. 1995;268:1443-1448.
9. Leonesi D, Piantoni G. Dissociation constant and spectrum of chlorous acid. *Annali di Chimica (Rome)*. 1965;55:668-675.
10. Nielson AH, Woltz PJH. The infrared spectrum of chlorine dioxide. *J Phys Chem*. 1952;20:1879-1883.
11. Masschelein WJ, Rice RG. *Chlorine Dioxide: Chemistry and Environmental Impact of Oxychlorine Compounds*. Ann Arbor, MI: Ann Arbor Science; 1979:111-145.
12. Bigorgne M. A new rapid method for the determination of the formula of certain hydrates. *C R Hebd Seances Acad Sci*. 1953;236:1966-1968.
13. Williamson HV, Hample CA, inventors; Cardox Corp, assignee. Nonexplosive chlorine dioxide hydrate composition and process for producing same. US patent 2,683,651. July 13, 1954.
14. Kaczur JJ, Cawfield DW. Chlorine oxygen acids and salts. In: *Kirk-Othmer Encyclopedia of Chemical Technology*. Vol 5. 4th ed. New York, NY: John Wiley & Sons; 1991:968-997.
15. Aieta EM, Berg JD. A review of chlorine dioxide in drinking water treatment. *J Am Water Works Assoc*. 1986;78:62-72.
16. Hong CC, Rapson WH. Kinetics of disproportionation of chlorous acid. *Can J Chem*. 1968;46:2053-2060.
17. Kieffer RG, Gordon G. Disproportionation of chlorous acid II. Kinetics. *Inorg Chem*. 1968;7:235-239.
18. Windholz M, ed. *Merck Index*. 10th ed. Rahway, NJ: Merck; 1983.
19. Cheesman GH. Parachor of chlorine dioxide. *J Chem Soc*. 1930;35-37.
20. Young CL. Sulfur dioxide, chlorine, fluorine, and chlorine oxides. In: Young CL, ed. *IUPAC Solubility Data Series*. Vol 12. Oxford, United Kingdom: Pergamon Press; 1988:xi-xvii, 454-456.
21. Cowley G. Safety in the design of chlorine dioxide plants. *Loss Prev*. 1993;113:1-12.
22. Jin R, Hu S, Zhang Y, et al. Concentration-dependence of the explosion characteristics of chlorine dioxide gas. *J Hazard Mater*. 2009;166:842-847.
23. Ward WJ, Gasper L, Gasper KE, inventors; Olin Corp, assignee. Chlorine dioxide generation. US patent 4,013,761. March 22, 1977.
24. Derby RI, Hutchinson WS. Chlorine (IV) oxide. In: *Inorganic Synthesis*. Vol 4. New York, NY: McGraw-Hill; 1953:152-156.
25. Grubitsch H, Suppan E. Zur kenntnis der dampfdruckkurve von chlordioxid. *Monatsh Chem*. 1962;93:246-251.
26. Hutchinson WS, Derby RI. Improved flour bleaching process using chlorine dioxide produced by an electric arc method. *Cereal Chem*. 1947;24:372-376.
27. Aieta EM, Roberts PV. The chemistry of oxy-chlorine compounds relevant to chlorine dioxide generation. In: Jolley RL, ed. *Water Chlorination: Environmental Impact and Health Effects*. Vol 5. Chelsea, MI: Lewis; 1985:783-794.
28. McHale ET, von Elbe G. The explosive decomposition of chlorine dioxide. *J Phys Chem*. 1968;72:1849-1856.
29. Spinks JWT, Porter JM. Photodecomposition of chlorine dioxide. *J Am Chem Soc*. 1934;56:264-270.
30. Crawford RA, DeWitt B. Decomposition rate studies in the gaseous chlorine dioxide-water system. *Tappi J*. 1968;50:226-230.
31. Gray P, Ip JK. Spontaneous ignition supported by chlorine dioxide. *Combust Flame*. 1972;18:361-371.
32. Haller JF, Northgraves WW. Chlorine dioxide and safety. *Tappi J*. 1955;38:199-202.
33. Flis IE. The oxidation potentials of chlorite and chlorine dioxide solutions. *Zhurnal Fiziko Khimic*. 1958;32:573-579.
34. Noss CI, Hauchman FS, Olivieri VP. Chlorine dioxide reactivity with proteins. *Water Res*. 1986;20:351-356.
35. Hoigné J, Bader H. Kinetics of typical reactions of chlorine dioxide with substances in water. *Vom Wasser*. 1982;59:253-267.
36. Neta P, Huie RE, Ross AB, Alberta B. Rate constants for reactions of inorganic radicals in solution. *J Phys Chem Ref Data*. 1988;17:1027.
37. Rosenblatt DH, Hayes AJ Jr, Harrison BR, Streaty RA, Moore KA. The reaction of chlorine dioxide with triethylamine in aqueous solution. *J Org Chem*. 1963;28:2790-2794.
38. Rosenblatt DH, Hull LA, DeLuca DC, Davis GT. Oxidations with amines. II. Substituent effects in chlorine dioxide oxidations. *J Am Chem Soc*. 1967;89:1158-1163.
39. Hull LA, Davis GT, Rosenblatt DH. Oxidation of amines. 9. Correlation of rate constants for reversible one-electron transfer in amine oxidation with reactant potentials. *J Am Chem Soc*. 1969;91:6247-6250.
40. Hull LA, Davis GT, Rosenblatt DH, et al. Oxidation of amines III, duality of mechanism in the reaction of amines with chlorine dioxide. *J Am Chem Soc*. 1967;89:1163-1170.
41. Noss CI, Dennis WH, Olivieri VP. Reactivity of chlorine dioxide with nucleic acids and proteins. In: Jolley RL, ed. *Water Chlorination: Chemistry, Environmental Impact and Health Effects*. Vol 5. Chelsea, MI: Lewis; 1985:1077-1086.
42. Jolley RL, Jones G, Pitt WW, et al. Chlorination of organics in cooling water and process effluents. In: Jolley RL, ed. *Water Chlorination: Environmental Impact and Health Effects*. Vol 1. Ann Arbor, MI: Ann Arbor Science; 1977:105-138.
43. Ogata N. Denaturation of protein by chlorine dioxide: oxidative modification of tryptophan and tyrosine residues. *Biochemistry*. 2007;46:4898-4911.
44. Lorcheim K. Chlorine dioxide gas inactivation of beta-lactams. *Appl Biosaf*. 2011;16:34-43.
45. Ooi BG, Branning SA. Correlation of conformational changes and protein degradation with loss of lysozyme activity due to chlorine dioxide treatment. *Appl Biochem Biotechnol*. 2017;182:782-791.
46. Grimley E, Gordon G. The kinetics and mechanism of the reaction between chlorine dioxide and phenol in aqueous solution. *J Inorg Nucl Chem*. 1973;35:2383-2392.
47. Ghanbari HA, Wheeler WB, Kirk JR. Reactions of aqueous chlorine and chlorine dioxide with lipids: chlorine incorporation. *J Food Sci*. 1982;147:482-485.
48. Schaufler C. Antiseptic effect of chlorine solutions from interactions of potassium chlorate and hydrochloric acid. *Zentralbl Chir*. 1933;60:2497-2500.

49. Kovtunovitch GP, Chemaya LA. Bactericidal effects of chlorine solutions from interaction of potassium chlorate and hydrochloric acid. *Sovetskaya Khirurgiya*. 1936;2:214-221.
50. Ridenour GM, Ingols RS. Bactericidal properties of chlorine dioxide. *J Am Water Works Assoc*. 1947;39:561-567.
51. Gómez-López VM, Rajkovic A, Ragaert P, Smigic N, Devlieghere F. Chlorine dioxide for minimally processed produce preservation: a review. *Trends Food Sci Technol*. 2009;20:17-26.
52. Alvarez ME, O'Brien RT. Mechanisms of inactivation of poliovirus by chlorine dioxide and iodine. *Appl Environ Microbiol*. 1982;44: 1064-1071.
53. Sansebastiano G, Mori G, Tanzi ML, et al. Chlorine dioxide: methods of analysis and kinetics of inactivation of poliovirus type 1. *Ig Mod*. 1983;79:61-91.
54. Berman D, Hoff JC. Inactivation of simian rotavirus SA 11 by chlorine, chlorine dioxide, and monochloramine. *Appl Environ Microbiol*. 1984;48:317-323.
55. Harakeh MS, Butler M. Inactivation of human rotavirus, SA11 and other enteric viruses in effluent by disinfectants. 1984. *J Hyg*. 1984;93: 157-163.
56. Olivieri VP, Hauchman FS, Noss CI, et al. Mode of action of chlorine dioxide on selected viruses. In: Jolley RL, ed. *Water Chlorination: Chemistry Environmental Impact and Health Effects*. Vol 5. Chelsea, MI: Lewis; 1985:619-634.
57. Sansebastiano G, Cesari C, Bellelli E. Further investigations on water disinfection by chlorine dioxide. *Ig Mod*. 1986;85:358-380.
58. Chen YS, Vaughn JM. Inactivation of human and simian rotaviruses by chlorine dioxide. *Appl Environ Microbiol*. 1990;56:1363-1366.
59. Farr RW, Walton C. Inactivation of human immunodeficiency virus by a medical waste disposal process using chlorine dioxide. *Infect Control Hosp Epidemiol*. 1993;14:527-529.
60. Korich DG, Mead JR, Madore MS, Sinclair NA, Sterling CR. Effects of ozone, chlorine dioxide, chlorine, and monochloramine on *Cryptosporidium parvum* oocyst viability. *Appl Environ Microbiol*. 1990;56: 1423-1428.
61. Whitmore TN, Denny S. The effect of disinfectants on a geosmin-producing strain of *Streptomyces griseus*. *J Appl Bacteriol*. 1992;72:160-165.
62. Wang Y, Jiang Z. Studies on bactericidal and algaecidal ability of chlorine dioxide. *Water Treat*. 1995;10:347-352.
63. Bundgaard-Nielsen K, Nielsen PV. Fungicidal effect of 15 disinfectants against 25 fungal contaminants commonly found in bread and cheese manufacturing. *J Food Prot*. 1996;59:268-275.
64. Brown P, Rohrer RG, Moreau-Dubois MC, Green EM, Gajdusek DC. Use of the golden Syrian hamster in the study of scrapie virus. *Adv Exp Med Biol*. 1981;134:365-373.
65. Brown P, Rohrer RG, Green EM, Gajdusek DC. Effect of chemicals, heat and histopathologic processing on the high-infectivity hamster-adapted scrapie virus. *J Infect Dis*. 1982;145:683-687.
66. Brown P, Gibbs CJ Jr, Amyx HL, et al. Chemical disinfection of Creutzfeld-Jakob disease virus. *N Engl J Med*. 1982;306:1279-1282.
67. Ryu JH, Beuchat LR. Biofilm formation and sporulation by *Bacillus cereus* on a stainless steel surface and subsequent resistance of vegetative cells and spores to chlorine, chlorine dioxide, and a peroxyacetic acid-based sanitizer. *J Food Prot*. 2005;68:2614-2622.
68. Rosenblatt DH, Rosenblatt AA, Knapp JE, inventors; Johnson and Johnson, assignee. Use of chlorine dioxide gas as a chemosterilizing agent. US patent 4,504,442. March 12, 1985.
69. Rosenblatt DH, Rosenblatt AA, Knapp JE, inventors; Johnson and Johnson, assignee. Use of chlorine dioxide gas as a chemosterilizing agent. US patent 4,681,739. July 21, 1987.
70. Rosenblatt AA, Knapp JE. Chlorine dioxide gas sterilization. In: *Proceedings from HIMA Conference*; 1988;.
71. Jeng DK, Woodworth AG. Chlorine dioxide gas sterilization under square-wave conditions. *Appl Environ Microbiol*. 1990;56:514-519.
72. Jeng DK, Woodworth AG. Chlorine dioxide gas sterilization of oxygenators in an industrial scale sterilizer: a successful model. *Artif Organs*. 1990;14:361-368.
73. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin Microbiol Rev*. 2002;15:167-193.
74. Lindsay D, Brözel VS, Mostert JF, von Holy A. Differential efficacy of a chlorine dioxide-containing sanitizer against single species and binary biofilms of a dairy-associated *Bacillus cereus* and a *Pseudomonas fluorescens* isolate. *J Appl Microbiol*. 2002;92:352-361.
75. Vaid R, Linton RH, Morgan MT. Comparison of inactivation of *Listeria monocytogenes* within a biofilm matrix using chlorine dioxide gas, aqueous chlorine dioxide and sodium hypochlorite treatments. *Food Microbiol*. 2010;27:979-984.
76. Martin DJ, Wesgate RL, Denyer SP, McDonell G, Maillard Y. *Bacillus subtilis* vegetative isolate surviving chlorine dioxide exposure: an elusive mechanism of resistance. *J Appl Microbiol*. 2015;119:1541-1551.
77. Ayyildiz O, Ileri B, Sanik S. Impacts of water organic load on chlorine dioxide disinfection efficacy. *J Hazard Mater*. 2009;168:1092-1097.
78. US Environmental Protection Agency. *Alternative Disinfectants and Oxidants Guidance Manual*. Washington, DC: US Environmental Protection Agency; 1999.
79. Roller SD, Olivieri VP, Kawata K. Mode of bacterial inactivation by chlorine dioxide. *Water Res*. 1980;14:635-642.
80. Fukayama MY, Tan H, Wheeler WB, Wei CL. Reactions of aqueous chlorine and chlorine dioxide with model food compounds. *Environ Health Perspect*. 1986;69:267-274.
81. Berg JD, Roberts PV, Matin A. Effect of chlorine dioxide on selected membrane functions of *Escherichia coli*. *J Appl Bacteriol*. 1986;60: 213-220.
82. Cho M, Kim J, Kim JY, Yoon J, Kim JH. Mechanisms of *Escherichia coli* inactivation by several disinfectants. *Water Res*. 2010;44:3410-3418.
83. Young SB, Setlow P. Mechanisms of killing of *Bacillus subtilis* spores by hypochlorite and chlorine dioxide. *J Appl Microbiol*. 2003;95:54-67.
84. Peta ME, Lindsay D, Brözel VS, Von Holy A. Susceptibility of food spoilage *Bacillus* species to chlorine dioxide and other sanitizers. *S Afr J Sci*. 2003;99:375-380.
85. Li JW, Xin ZT, Wang XW, Zheng JL, Chao FH. Mechanisms of inactivation of hepatitis A virus in water by chlorine dioxide. *Water Res*. 2004;38:1514-1519.
86. Zhu C, Chen Z, Yu G. Fungicidal mechanism of chlorine dioxide on *Saccharomyces cerevisiae*. *Ann Microbiol*. 2013;63:495-502.
87. Smith RP, Willhite CC. Chlorine dioxide and hemodialysis. *Regul Toxicol Pharmacol*. 1990;11:42-62.
88. American Conference of Governmental Industrial Hygienists. *Industrial Hygienic Guide*. Cincinnati, OH: American Conference of Governmental Industrial Hygienists; 1990.
89. White GC. *Handbook of Chlorination*. New York, NY: Reinhold; 1972.
90. Meggs W, Elsheikh T, Metzger W, et al. Nasal pathology and ultrastructure in persistent rhinitis with chemical sensitivity after chlorine dioxide (ClO_2) exposure. *J Toxicol Clin Toxicol*. 1995;33:525.
91. Meggs WJ, Elsheikh T, Metzger WJ, et al. Nasal pathology of persistent rhinitis after chlorine dioxide exposure. *J Allergy Clin Immunol*. 1995;95:260.
92. Robinson GM, Bull RJ, Schamer M, Long RE. Epidermal hyperplasia of mouse skin following treatment with alternative drinking water disinfectants. *Environ Health Perspect*. 1986;69:293-300.
93. Abdel-Rahman MS, Skowronski GA, Turkall RM, Gerges SE, Kadry AR, Abu-Hadeed AH. Subchronic dermal toxicity studies of alcide allay gel and liquid in rabbits. *J Appl Toxicol*. 1987;7:327-333.
94. Abdel-Rahman MS, Couri D, Bull RJ. Metabolism and pharmacokinetics of alternate drinking water disinfectants. *Environ Health Perspect*. 1982;46:19-23.
95. Scatina J, Abdel-Rahman MS, Gerges SE, Alliger H. Pharmacokinetics of Alcide®, a germicidal compound in rats. *J Appl Toxicol*. 1983;3:150-153.
96. Moore GS, Calabrese EJ. Toxicological effects of chlorite in the mouse. *Environ Health Perspect*. 1982;46:31-37.
97. Abdel-Rahman MS, Couri D, Bull RJ. Toxicity of chlorine dioxide in drinking water. *Int J Toxicol*. 1984;3:277-284.
98. Abdel-Rahman MS, Couri D, Bull RJ. The kinetics of chlorite and chlorate in rats. *J Environ Pathol Toxicol Oncol*. 1985;6:97-103.

99. Harrington RM, Romano RR, Gates D, Ridgway P. Subchronic toxicity of sodium chlorite in the rat. *J Am Coll Toxicol*. 1995;14:21-33.
100. Orme J, Taylor DH, Laurie RD, Bull RJ. Effects of chlorine dioxide on thyroid function in neonatal rats. *J Toxicol Environ Health*. 1985;15:315-322.
101. Bercz JP, Jones L, Gamer L, Murray D, Ludwig DA, Boston J. Subchronic toxicity of chlorine dioxide and related compounds in drinking water in the nonhuman primate. *Environ Health Perspect*. 1982;46: 47-55.
102. Revis NM, McCauley P, Bull R, Holdsworth G. Relationship of drinking water disinfectants to plasma cholesterol and thyroid hormones levels in experimental studies. *Proc Natl Acad Sci U S A*. 1986;83:1485-1489.
103. Couri D, Miller CH Jr, Bull RJ, Delphia JM, Ammar EM. Assessment of maternal toxicity, embryotoxicity and teratogenic potential of sodium chlorite in Sprague-Dawley rats. *Environ Health Perspect*. 1982;46:25-29.
104. Carlton BD, Basaran AH, Mezza LE, George EL, Smith MK. Reproductive effects in Long-Evans rats exposed to chlorine dioxide. *Environ Res*. 1991;56:170-177.
105. Skowronski GA, Abdel-Rahman MS, Gerges SE, Klein KM. Teratologic evaluation of Alcide liquid in rats and mice. I. *J Appl Toxicol*. 1985;5:97-103.
106. Suh DH, Abdel-Rahman MS, Bull RJ. Effect of chlorine dioxide and its metabolites in drinking water on fetal development in rats. *J Appl Toxicol*. 1983;3:75-79.
107. Ishidate M Jr, Sofuni T, Yoshikawa K, et al. Primary mutagenicity screening of food additives currently used in Japan. *Food Chem Toxicol*. 1984;22:623-636.
108. Hayashi M, Kishi M, Sofuni T, Ishidate M Jr. Micronucleus tests in mice on 39 food additives and eight miscellaneous chemicals. *Food Chem Toxicol*. 1988;26:487-500.
109. Bianchine JR, Lubbers JR, Chauhan S, Miller J. *Study of Chlorine Dioxide and Its Metabolites in Man*. Cincinnati, OH: US Environmental Protection Agency Health Effects Laboratory; 1981. EPA-600/1-81-068 (PB82-109356).
110. Lubbers JR, Chauhan S, Bianchine JR. Controlled clinical evaluations of chlorine dioxide, chlorite and chlorate in man. *Environ Health Perspect*. 1982;46:57-62.
111. Lubbers JR, Chauhan S, Miller JK, Bianchine JR. The effects of chronic administration of chlorine dioxide, chlorite and chlorate to normal healthy adult male volunteers. *J Environ Pathol Toxicol Oncol*. 1984;5:229-238.
112. Lubbers JR, Chauhan S, Miller JK, Bianchine JR. The effects of chronic administration of chlorite to glucose-6-phosphate dehydrogenase deficient healthy adult male volunteers. *J Environ Pathol Toxicol Oncol*. 1984;5:239-242.
113. Michael GE, Miday RK, Bercz JP, et al. Chlorine dioxide water disinfection: a prospective epidemiology study. *Arch Environ Health*. 1981;36:19-27.
114. Tuthill RW, Giusti RA, Moore GS, Calabrese EJ. Health effects among newborns after prenatal exposure to ClO_2-disinfected drinking water. *Environ Health Perspect*. 1982;46:39-45.
115. Condie LW. Toxicological problems associated with chlorine dioxide. *J Am Water Works Assoc*. 1986;78:73-78.
116. Ouhoummane N, Levallois P, Gingras S. Thyroid function of newborns and exposure to chlorine dioxide by-products. *Arch Environ Health*. 2004;59:582-587.
117. Akamatsu A, Lee C, Morino H, Miura T, Ogata N, Shibata T. Six-month low level chlorine dioxide gas inhalation toxicity study with two-week recovery period in rats. *J Occup Med Toxicol*. 2012;7:2.
118. Wellinghoff ST, inventor; Southwest Research Institute, assignee. Chlorine dioxide generating polymer packaging films. US patent 5,360,609. November 1, 1994.
119. Gordon G, Rosenblatt AA. Gaseous, chlorine-free chlorine dioxide for drinking water. Paper presented at: American Water Works Association Water Quality Technology Conference; November 12-15, 1995; New Orleans, LA.
120. Cole B. *Site Remediation of a Penicillin Production Facility Using Chlorine Dioxide Gas as Sterilant*. Beaumaris, Australia: Association of Biosafety for Australia & New Zealand; 2016.
121. Gordon D, Krishnan J, Theriault S. *Inactivation Studies of Lipopolysaccharide and Anthrax Toxins Using Gaseous Decontamination Methods*. 2011.
122. Snyder E. *Systematic Decontamination of Chemical Warfare Agents and Toxic Industrial Chemicals: Report on the 2008 Workshop on Decontamination Research and Associated Issues for Sites Contaminated with Chemical, Biological, or Radiological Materials*. Washington, DC: US Environmental Protection Agency; 2009. EPA/600/R-09/035.
123. Engelhard Corp. *Chlorine Dioxide from Engelhard*. Iselin, NJ: Engelhard Corp; 1997. Product bulletin EC7097.
124. Reina LD, Fleming HP, Humphries EG. Microbiological control of cucumber hydrocooling water with chlorine dioxide. *J Food Prot*. 1995;58:541-546.
125. US Food and Drug Administration. *Code of Federal Regulations 21 CFR 173.300: Secondary Direct Food Additives Permitted in Food for Human Consumption: Chlorine Dioxide*. Silver Spring, MD: US Food and Drug Administration; 2017.
126. National Health and Family Planning Commission of the People's Republic of China. *Hygienic Standards for Use of Food Additives*. Beijing, China: National Health and Family Planning Commission of the People's Republic of China; 2017.
127. Han Y, Linton RH, Nielsen SS, Nelson PE. Reduction of *Listeria monocytogenes* on green peppers (*Capsicum annuum* L.) by gaseous and aqueous chlorine dioxide and water washing and its growth at 7 degrees C. *J Food Prot*. 2001;64:1730-1738.
128. Han Y, Selby T, Schultze K, Nelson P, Linton H. Decontamination of strawberries using batch and continuous chlorine dioxide gas treatments. *J Food Prot*. 2004;67:2450-2455.
129. Gómez-López VM, Devlieghere F, Ragaert P, Debevere J. Shelf-life extension of minimally processed carrots by gaseous chlorine dioxide. *Int J Food Microbiol*. 2007;116:221-227.
130. Mahmoud BSM, Linton RH. Inactivation kinetics of inoculated *Escherichia coli* O157:H7 and *Salmonella enterica* on lettuce by chlorine dioxide gas. *Food Microbiol*. 2008;25:244-252.
131. Popa I, Hanson EJ, Todd EC, Schilder AC, Ryser ET. Efficacy of chlorine dioxide gas sachets for enhancing the microbiological quality and safety of blueberries. *J Food Prot*. 2007;70:2084-2088.
132. Zhang S, Farber JM. The effects of various disinfectants against *Listeria monocytogenes* on fresh-cut vegetables. *Food Microbiol*. 1996;13:311-321.
133. Rodgers SL, Cash JN, Siddiq M, Ryser E. A comparison of different chemical sanitizers for inactivating *Escherichia coli* O157:H7 and *Listeria monocytogenes* in solution and on apples, lettuce, strawberries, and cantaloupe. *J Food Prot*. 2004;67:721-731.
134. Pao S, Kelsey DF, Khalid MF, Ettinger MR. Using aqueous chlorine dioxide to prevent contamination of tomatoes with *Salmonella enterica* and *Erwinia carotovora* during fruit washing. *J Food Prot*. 2007;70:629-634.
135. Shin JH, Chang S, Kang DH. Application of antimicrobial ice for reduction of foodborne pathogens (*Escherichia coli* O157: H7, *Salmonella* Typhimurium, *Listeria monocytogenes*) on the surface of fish. *J Appl Microbiol*. 2004;97:916-922.
136. Costilow RN, Uebersax MA, Ward PJ. Use of chlorine dioxide for controlling microorganisms during the handling and storage of fresh cucumbers. *J Food Sci*. 1984;49:396-401.
137. Chen Z, Zhu C, Zhang Y, Niu D, Du J. Effects of aqueous chlorine dioxide treatment on enzymatic browning and shelf-life of fresh-cut asparagus lettuce (*Lactuca sativa* L.). *Postharvest Biol Technol*. 2010;58:232-238.
138. Chen Z, Zhu C, Han Z. Effects of aqueous chlorine dioxide treatment on nutritional components and shelf-life of mulberry fruit *(Morus alba* L.). *J Biosci Bioeng*. 2011;111:675-681.
139. Hong YH, Ku GJ, Kim MK, et al. Inactivation of *Listeria monocytogenes* and *Campylobacter jejuni* in chicken by aqueous chlorine dioxide treatment. *J Food Sci Nutr*. 2007;12:279-283.

140. Chen Z, Zhu C. Combined effects of aqueous chlorine dioxide and ultrasonic treatments on postharvest storage quality of plum fruit (*Prunus salicina* L.). *Postharvest Biol Technol*. 2011;61:117-123.
141. Jimenez-Villarreal JR, Pohlman FW, Johnson ZB, Brown AH Jr. Effects of chlorine dioxide, cetylpyridinium chloride, lactic acid and trisodium phosphate on physical, chemical and sensory properties of ground beef. *Meat Sci*. 2003;65:1055-1062.
142. Tsai LS, Huxsoll CC, Robertson G. Prevention of potato spoilage during storage by chlorine dioxide. *J Food Sci*. 2001;66:472-477.
143. Trinetta V, Morgan M, Linton R. Use of high-concentration-short-time chlorine dioxide treatments for the inactivation of *Salmonella enterica* spp. inoculated onto Roma tomatoes. *Food Microbiol*. 2010;27:1009-1015.
144. Trinetta V, Vaidya N, Linton R, Morgan M. Evaluation of chlorine dioxide gas residues on selected food produce. *J Food Sci*. 2011;76:T11-T15.
145. Carpentier B, Cerf O. Review—persistence of *Listeria monocytogenes* in food industry equipment and premises. *Int J Food Microbiol*. 2011;145:1-8.
146. Gutman Y, Marzouk Y, inventors; Marzouk Y, Abic Ltd, assignee. Solid composition capable of releasing chlorine dioxide in water. US patent 5,399,288. March 21, 1995.
147. Engelhard Corp. *Controlled Sustained Release*. Iselin, NJ: Engelhard Corp; 1997. Product bulletin EC7098.
148. Yi Y, Hao L, Ma S, et al. A pilot study on using chlorine dioxide gas for disinfection of gastrointestinal endoscopes. *J Zhejiang Univ Sci B*. 2016;17:526-536.
149. Rickloff JR, Edwards LM. Modern trends in isolator sterilization. In: Wagner CM, Akers JM, eds. *Isolator Technology Applications in the Pharmaceutical and Biotechnology Industries*. Buffalo Grove, IL: Interpharm Press; 1995:151-152.
150. Kross AD, inventor; Treatment or prevention of microbial infections in mammals. PCT patent WO 9,523,511. 1995.
151. Rubenstein AI, inventor. Novel method to treat blood. US patent 4,944,920. July 31, 1990.
152. Rubenstein AI, inventor; International Medical Technologies Corp, assignee. Novel method for disinfecting red blood cells, blood platelets, blood plasma, and optical corneas and sclerae. US patent 4,971,760. November, 20, 1990.
153. Carmen R, Chong C, inventors; Miles Inc, assignee. Method for inactivating viruses in blood using chlorine dioxide. US patent 5,240,829. August 31, 1993.
154. Dziabo AJ, Ripley PS, inventors; Allergan Inc, assignee. Disinfecting contact lenses by passing current to chlorine dioxide from a precursor. US patent 5,320,806. June 14, 1994.
155. Dziabo AJ, Ripley PS, inventors; Allergan Inc, assignee. Aqueous ophthalmic composition especially for cleaning contact lenses containing chlorine dioxide as sole preservative. US patent 5,424,078. June 13, 1995.
156. Czarra JA, Adams JK, Carter CL, Hill WA, Coan PN. Exposure to chlorine dioxide gas for 4 hours renders *Syphacia* ova nonviable. *J Am Assoc Lab Anim Sci*. 2014;53:364-367.
157. Cole B, Czarneski MA. A multiple-staged site remediation of a medical research animal facility affected by a rodent pinworm infestation. Paper presented at: Association of Biosafety for Australia & New Zealand 5th Annual Conference; November 9-13, 2015; Canberra, Australia.
158. Gibbs SG, Lowe JJ, Smith PW, Hewlwtt AL. Gaseous chlorine dioxide as an alternative for bedbug control. *Infect Control Hosp Epidemiol*. 2012;33:495-499.
159. Park JY, Ripley PS, Dziabo AJ, inventors; Contact lens disinfection with liquid medium comprising stabilized chlorine dioxide and activator to release free chlorine dioxide. US patent 5,336,434. August 9, 1994.
160. Takahashi E, Czarneski MA, Sugiura A. Japan's RIKEN BSI: whole facility chlorine dioxide gas decontamination approach for a barrier facility—a case study. *Appl Biosaf*. 2014;19:201-210.
161. Czarneski MA. Microbial decontamination of a 65-room new pharmaceutical research facility. *Appl Biosaf*. 2009;14:81-88.
162. Luftman HS, Michael A, Regits MA, et al. Chlorine dioxide gas decontamination of large animal hospital intensive and neonatal care units. *Appl Biosaf*. 2006;11:144-154.
163. Girouard DJ Jr, Czarneski MA. Room, suite scale, class III biological safety cabinet, and sensitive equipment decontamination and validation using gaseous chlorine dioxide. *App Biosaf*. 2016;21:34-44.
164. Leo F, Poisson P, Sinclair CS, Tallentire A. Design, development and qualification of a microbiological challenge facility to assess the effectiveness of BFS aseptic processing. *PDA J Pharm Sci Technol*. 2005;59:33-48.
165. Sherman MB, Trujilloe J, Leahy I, et al. Construction and organization of a BSL-3 cryo-electron microscopy laboratory at UTMB. *J Struct Biol*. 2013;181:223-233.
166. Lowe JJ, Gibbs SG, Iwen PC, Smith PW. A case study on decontamination of a biosafety level-3 laboratory and associated ductwork within an operational building using gaseous chlorine dioxide. *J Occup Environ Hyg*. 2012;9:D196-D205.
167. Lowe JJ, Gibbs SG, Iwen PC, Smith PW, Hewlett AL. Impact of chlorine dioxide gas sterilization on nosocomial organism viability in a hospital room. *Int J Environ Res Public Health*. 2013;10:2596-2605.
168. Lowe JJ, Hewlett AL, Iwen PC, Smith PW, Gibbs SG. Evaluation of ambulance decontamination using gaseous chlorine dioxide. *Prehosp Emerg Care*. 2013;17:401-408.
169. Eylath AS, Wilson D, Thatcher D, Pankau A. Successful sterilization using chlorine dioxide gas: part one-sanitizing an aseptic fill isolator. *BioProcess Int*. 2003;7:52-56.
170. Barbu N, Zwick R. Isolators selection, design, decontamination, and validation. *Pharm Eng*. 2014;6-14.
171. Czarneski MA, Lorcheim P. Isolator decontamination using chlorine dioxide gas. *Pharma Technol*. 2005;4:124-133.
172. Han Y, Guentert AM, Smith RS, Linton RH. Efficacy of chlorine dioxide gas as a sanitizer for tanks used for aseptic juice storage. *Food Microbiol*. 1999;16:53-61.
173. Eylath AS, Madhogarhia ER, Lorcheim P, Czarneski M. Successful sterilization using chlorine dioxide gas: part two-cleaning process vessels. *BioProcess Int*. 2003;8:54-56.
174. Luftman HS, Regits MA, Lorcheim P, et al. Validation study for the use of chlorine dioxide gas as a decontaminant for biological safety cabinets. *Appl Biosaf*. 2008;13:199-212.
175. National Science Foundation. *Biosafety Cabinetry: Design, Construction, Performance, and Field Certification*. Alexandria, VA: National Science Foundation; 2008.
176. Kowalski JB. Sterilization of medical devices, pharmaceutical components, and barrier isolation systems with gaseous chlorine dioxide. In: Morrissey RF, Kowalski JB, eds. *Sterilization of Medical Products*. Vol 7. Champlain, NY: Polyscience; 1998:313-323.
177. Lorcheim K, Melgaard E. Linearity of the relationship between concentration and contact time for sterilization with chlorine dioxide gas. Paper presented at: American Biological Safety Association 58th Annual Biological Safety Conference; October 9-14, 2015; Providence, RI.