19 Validation of Chlorine Dioxide Sterilization

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19.1 INTRODUCTION

Chlorine dioxide (CD) is a highly effective sterilizing agent that has many applications in the Life Science research, food facilities, health care, medical device, and pharmaceutical industries including the sterilization of components, environments, and medical devices. It has key benefits and characteristics that make it extremely effective and well suited for use in component and device sterilization, small chamber (isolator) decontamination, room/suite decontamination, and facility/building decontamination. It is a true gas at typical use temperatures and therefore can penetrate into hard-to-reach areas such as those found in needles, lumens, or devices with complex geometries. It is efficacious at ambient room temperatures, so it is excellent for temperature-sensitive materials and devices. It has a yellowish-green color, which allows its concentration to be precisely monitored and controlled by ultraviolet visible (UV-VIS) spectrophotometry. This ensures a repeatable and robust cycle by providing tight process control from beginning to end.

19.2 HISTORY/BACKGROUND

Chlorine dioxide was recognized for its disinfecting properties in the 1930s by Schaufler[1] (1933) and by Kovtunovitch and Chemaya[2] (1936). Chlorine dioxide was first prepared in 1802 by Chenevix[3] and later independently prepared and verified in 1811 by Sir Humphrey Davy.[4] Chlorine dioxide is primarily used in the pulp and paper industry, accounting for 95% of all chlorine dioxide produced. About 5% of large water-treatment facilities (serving more than 100,000 persons) in the United States use chlorine dioxide to treat drinking water. An estimated 12 million persons may be exposed in this way to chlorine dioxide and chlorite ions. It is also estimated that there were 743,015 pounds (337,026 kg) of chlorine dioxide released into the atmosphere from over 100 manufacturing, processing, and waste disposal facilities in 2000.[5]

The United States Food and Drug Administration (FDA) permits chlorine dioxide to be used to wash fruits and vegetables according to 21 CFR Part 173.300 “Chlorine Dioxide”.

DOI: 10.1201/9781003163138-19

Additionally, chlorine dioxide has several applications approved for generally recognized as safe (GRAS) along with several approved food contact notices (FCNs) or food contact substances (FCSs). Section 409 of the FD and C Act defines an FCS as any substance that is intended for use as a component of materials used in manufacturing, packing, packaging, transporting, or holding food if such use of the substance is not intended to have any technical effect in such food. Chlorine dioxide is also approved for use in organic food and organic food processing according to 7 CFR part 205, Subchapter M—"Organic Foods Production Act Provisions". Chlorine dioxide gas has also been shown to be more effective than chlorine dioxide dissolved in aqueous solution.[6,7] Studies have shown that with equal concentrations (3 mg/L in gas and 3 mg/L in liquid), chlorine dioxide in the gas phase had a 7.4 log reduction of *L. monocytogenes* on peppers compared with 3.6 log reduction with 3 mg/L of gas dissolved in solution.

Chlorine dioxide is a single-electron-transfer oxidizing agent with a chlorine-like odor. This odor is the only similarity between chlorine dioxide and chlorine. Chlorine dioxide is not sufficiently stable to be bottled and shipped, so it must be generated at the point of use. Chlorine dioxide can be generated in a variety of methods as a gas dissolved in liquid or as a dry gas. The dry gas method generates chlorine dioxide gas by passing chlorine gas through solid sodium chlorite, which generates a pure ClO_2 gas (>99%)[8] with no byproducts:

$$Cl_{2(g)} + 2NaClO_{2(S)} \rightarrow 2ClO_{2(g)} + 2NaCl_{(S)}$$

Chlorine Dioxide chemical properties can be found in Table 19.1.

Other generation methods involve using an acid mixed with a sodium chlorite solution to generate chlorine dioxide and other byproducts that can cause corrosion.

Sodium Chlorite—Hydrochloric Acid method:

$$5NaClO_2 + 4HCl \rightarrow 5NaCl + 4ClO_2 + 2H_2O$$

Chlorite—Sulfuric Acid method:

$$8ClO_2- + 4H_2SO_4 \rightarrow 4ClO_2 + 2HClO_3 + 4SO4_2- + 2H_2O + 2HCl$$

Sodium Hypochlorite method:

$$2NaClO_2 + 2HCl + NaOCl \rightarrow 2ClO_2 + 3\ NaCl + H_2O$$

Chlorine dioxide is stable in concentrations of <10% in air at atmospheric pressure. The gas can become unstable at high concentrations (>10%) by contact with substances that catalyze its decomposition such as organic materials, phosphorus, potassium hydroxide, sulfur, mercury, and carbon monoxide, as well as exposure to heat.[10] When this explosive decomposition occurs, the volume increase is small, such that the decomposition is referred to as "puffing".[11] When using chlorine dioxide gas for disinfection/decontamination/sterilization the concentrations are much lower, typically 0.04%–1.1% (1–30 mg/L). The typical concentration for large-volume decontamination is 1mg/L with isolators having a concentration of 5 mg/L and vacuum sterilization runs typically occurring at 30 mg/L. In more recent studies in 2009, it was confirmed that the lower limit for decomposition was 9.5% (262 mg/L), confirming that there is no puffing or explosion hazard at these conditions.[12]

TABLE 19.1
Chlorine Dioxide Properties

Chemical Formula:	**ClO_2**
Molecular Weight:	67.45 g/mole
Melting Point (°C):	−59
Boiling Point (°C) at 100% concentrations:	+11
Boiling Point (°C) at isolator use concentrations:	−22
Boiling Point (°C) at room use concentrations:	−40
Density:	2.4 times that of air

Chlorine dioxide's method of microbial inactivation is different from that of chlorine (oxidation vs. chlorination), thus it is gentler on materials and provides a highly controllable and reproducible process. It does not react with organic materials to form chlorinated species or with ammonia to form chloramine. Additionally, chlorine dioxide is well suited for sterilizing components and medical devices because it is compatible with the many materials found in those components such as stainless steel, aluminum, glass, and most plastics.

Chlorine dioxide's method of kill is by oxidation and as such it can oxidize materials. It has a measured oxidation potential of 0.95V. This corrosion potential is lower than that of other common decontaminating/sterilizing agents such as hydrogen peroxide (1.78V), ozone (2.07V), sodium hypochlorite (1.49V), and peracetic acid (1.81V).

Stainless steel and most plastics (Teflon, KYNAR (PVDF), PVC, PE, PP) and gasket materials (silicone, EPDM, Buna, Viton, neoprene) that are commonly used in facilities and chambers have good material compatibility with chlorine dioxide gas. Aluminum will show signs of

O — Cl • ?? O

FIGURE 19.1 Structure of chlorine dioxide.[9]

oxidation over time, but if anodized, it does not. Chlorine dioxide gas will cause corrosion on ferrous metals if they are left unpainted, untreated, or uncoated. Electronics are acceptable as shown by Girouard (2016).[13] In other decontaminations of whole facilities, installed lab and manufacturing equipment were shown to be functional after being exposed.[14,15,16,17,18,19,20,21,22]

The rapid sterilizing activity of chlorine dioxide is present at relatively low gas concentrations of 1 to 30 mg/L compared with those of Ethylene Oxide (EtO), which requires higher gas concentration at ambient temperatures. Chlorine dioxide gas has lower ambient temperatures than steam, and it does not raise the temperature of the chamber.

19.3 EFFECTIVENESS

Chlorine dioxide is an oxidizing agent and reacts with several cellular constituents. Its most widely reported mechanism is the oxidation of the cell surface membrane protein[23,24] and free fatty acids.[25] Studies have shown efficacious results as a gas and in solution against a wide variety of microorganisms including viruses, bacteria, fungi, spores, and protozoa. Chlorine dioxide studies have shown it is capable of inactivating rotavirus, polioviruses, enteroviruses, and human immunodeficiency virus (HIV).[26,27,28,29,30,31,32,33] Chlorine dioxide was proven effective on major bacterial pathogens responsible for outbreaks in the food industry such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica*[34] and also effective against various protozoal, fungal, and algal species, such as *Cryptosporidium parvum* oocysts, *Streptomyces griseus*, and yeasts[35,36,37,38]. Chlorine dioxide has also shown promising results against prions and prion-associated diseases.[39,40,41] The sterilant or sporicidal capabilities were initially demonstrated by Rosenblatt et al[42,43] and Jeng and Woodworth[44,45].

With *E. coli*, it was reported that chlorine dioxide causes surface damage and degradation followed by the damage of several inner cellular components.[46,47] Membrane damage has also been shown with bacterial spores including damage to the inner cell membrane, change in cell permeability, and interruption of germination of *B. subtilis* spores.[48] With *B. cereus*, similar damage was shown to cell walls noted as surface roughness and indentations.[49] With poliovirus, chlorine dioxide alters capsid proteins.[50] Chlorine dioxide does not act via chlorination and as such does not form trihalomethanes.[51] These reactions with organic matter have been studies by Gordon (1972),[52] Masschelein and Rice (1979)[53] and Aieta and Roberts (1985).[54]

Phenolic compounds are easily oxidized by chlorine dioxide[55], and it has been used to reduce the toxicity of chlorinated phenolic compounds.[56] Chlorine dioxide has also been shown to denature proteins[57,58] and has shown good results in inactivating beta-lactams from pharmaceutical production facilities.[59,60] This ability is significant because buildings that produce beta lactams cannot be used to produce other non-beta lactam products. The residues from beta lactams remain in a facility for long periods and can cause allergic reactions. Anaphylactic reactions to penicillin can be fatal. Beta lactam allergic reactions are the most common cause of adverse drug reactions mediated by specific immunological mechanisms.[61] The United States Centers for Disease Control and Prevention (CDC) states that 3%–10% of adults in the United States have had an allergic reaction to penicillin.[62] In other studies, chlorine dioxide has shown promising results in endotoxin reduction on various surfaces (304 and 316 stainless steel and titanium [anodized and un-anodized]).[63]

A series of square wave studies was performed in a two glove 23 ft^3 (0.65 m^3) flexible wall isolator to determine the effect of chlorine dioxide gas concentration on the inactivation rate of *Bacillus atrophaeus* spores. The D-value (the time at a specified chlorine dioxide gas concentration required to reduce the microbial population by 1 log or 90%) of *B. atrophaeus* spores on unwrapped paper carriers, when exposed to gas concentrations of 3 mg/L and 5mg/L, was determined using the Stumbo-Murphy-Cochran Method (Table 19.2 and Table 19.3). Each biological indicator (BI) was stored at 75% ±b2% relative humidity (RH) prior to entering the isolator and preconditioned in the isolator at 75% ± 2% RH for 30 ±1 minutes prior to exposure. The decline in %RH during the gas injection and exposure phases of the cycle was recorded for each of the D-value runs.

Data calculations for the 3 and 5 mg/L exposure concentrations utilizing the Stumbo-Murphy-Cochran are in Table 19.2 and 19.3. The results can be seen in Table 19.4.

TABLE 19.2
D-Value Determinations Using the Stumbo-Murphy-Cochran (SMC) Method (3 mg/L)

Gas Conc. (mg/L)	U	n	r	Nu	Log Nu	D-Value
3	21	10	0	N/D	N/D	N/D
3	24	10	1	2.30	0.362	3.92
3	27	10	1	2.30	0.362	4.41
3	30	10	2	1.61	0.207	4.78
3	33	10	6	0.51	–0.292	4.87
3	36	10	8	0.22	–0.651	5.05
3	39	10	9	0.11	–0.977	5.23
3	42	10	8	0.22	–0.651	5.89
3	45	10	10	N/D	N/D	N/D
					AVERAGE:	4.88 min

Note: When the number of sterile replicates (r) is 0 or 10 the D-value is not determined.
Stumbo-Murphy-Cochran formula: D-value = U/log No–log Nu
Key:
U = time in minutes
n = number of replicates tested
r = number of sterile replicates out of the number tested
Nu = natural log of *n/r* (ln *(n/r)*)
No = population of unexposed 81 (3.00×10^6 CFU/strip)

TABLE 19.3
D-Value Determinations Using the Stumbo-Murphy-Cochran (SMC) Method (5 mg/L)

Gas conc. (mg/L)	U	n	r	Nu	Log Nu	D-value
5	18	10	1	2.30	0.362	2.94
5	21	10	2	1.61	0.207	3.35
5	24	10	7	0.36	–0.448	3.46
5	27	10	9	0.11	–0.977	3.62
5	30	10	10	*N/D*	*N/D*	*N/D*
5	33	10	9	0.11	0.977	4.43
5	36	10	10	*N/D*	*N/D*	*N/D*
5	39	10	10	*N/D*	*N/D*	*N/D*
5	42	10	10	*N/D*	*N/D*	*N/D*
					AVERAGE:	3.56 min

Note: When the number of sterile replicates (r) is 0 or 10 the D-value is not determined.
Stumbo-Murphy-Cochran formula: D-value = U/log No–log Nu
Key:
U = time in minutes
n = number of replicates tested
r = number of sterile replicates out of the number tested
Nu = natural log of *n/r* (ln *(n/r)*)
No = population of unexposed 81 (3.00×10^6 CFU/strip)

TABLE 19.4
Results and Conclusion: D-Value vs. Chlorine Dioxide Concentration

Chlorine dioxide concentration (mg/L)	D-value (minutes)
3	4.88
5	3.56
10	0.75
20	0.27
30	0.12

Note: Spore strips were preconditioned for 30 minutes at 70% relative humidity and ambient temperatures.

19.4 SAFETY/TOXICITY

The Occupational Safety and Health Administration (OSHA) 8-hour Time-Weighted-Average (TWA) for chlorine dioxide is 0.1 ppm. The 15-minute Short-Time-Exposure-Limit (STEL) is 0.3 ppm. Chlorine dioxide is a respiratory/mucous membrane irritant. One of the important safety features of chlorine dioxide is that it has a 0.1 ppm odor threshold, which makes it self-alerting.[64] Most other sterilants are well over their STEL before they can be detected by the user's sense of smell. Because chlorine dioxide has such widespread usage in the water treatment and paper and pulp industries, there is a wide selection of environmental monitors and personnel safety badges available. Because of this broad usage, there have been numerous safety studies conducted both for environmental effects, inhalation, as well as ingestion. Because it is also used in the food industry for sanitization and disinfection, there are allowable limits from the US government for ingestion. The Environmental Protection Agency (EPA) has set the maximum concentration of chlorine dioxide in drinking water at 0.8 mg/L.[65]

Chlorine dioxide's special properties make it an ideal choice to meet the challenges of today's environmentally concerned world. Chlorine dioxide is an environmentally preferred alternative to EtO. The major concerns with EtO center on its flammability and high reactivity. Acute exposures to EtO gas may result in respiratory irritation and lung injury, headache, nausea, vomiting, diarrhea, shortness of breath, and cyanosis (OSHA Safety Fact Sheet).[66] Chronic exposure has been associated with the occurrence of cancer, reproductive effects, mutagenic changes, neurotoxicity, and sensitization (NIOSH 2019).[67]

Chlorine dioxide is used in large quantities in the water treatment and pulp and paper industries and can be used safely and effectively. Although chlorine dioxide is a sterilant and chemical disinfectant, it has not revealed clear evidence of other adverse health effects.[68] Chlorine dioxide is considered a mucous membrane irritant, and inhalation of excessive amounts can lead to pulmonary edema.[69]

Animal studies done with chlorine dioxide showed no significant association with any teratogenic potential effects of chlorine dioxide,[70,71,72] except for one study by Suh et al.[73] Data currently available support that chlorite, (the US-EPA considers chlorite and chlorine dioxide synonymous) is not considered carcinogenic,[74] and there is no published research related to chlorine dioxide carcinogenicity. Low concentration exposures (0.05 ppm and 0.1 ppm) of continuous exposure for 6 months showed no chlorine dioxide gas-related toxicity.[75] The rats showed no weight gain or changes in water or food consumption, and normal relative organ weight was observed. There were no changes in respiratory organs and no chlorine dioxide gas-related toxicity was observed.

Chlorine dioxide underwent a full review in 2006 in the Reregistration Eligibility Decision (RED) for Chlorine Dioxide and Sodium Chlorite (Case 4023). The US-EPA considers chlorine dioxide and sodium chlorite synonymous because both have the same toxicological end points. In the findings, the food quality protection safety factor was reduced from 10X to 1X. The food quality protection safety factor is for the protection of infants and children in relation to pesticide residues in food, drinking water, or residential exposures. This reduction was based upon a complete database for developmental and reproductive toxicity. Also in the RED, chlorine dioxide was designated with a toxic category of II for exposure by the oral route, a toxic category of III for skin toxicity, a toxic category of III for eye irritation, and a toxic category

of II for inhalation toxicity. Toxicity Category I is considered DANGER, Toxicity Category II is WARNING, Toxicity Category III requires CAUTION, and Toxicity Category IV is safe.

19.5 KNOWN INCOMPATIBILITIES

Chlorine dioxide reacts with carbohydrates, such as glucose, to oxidize the primary hydroxyl groups first to aldehydes and then to carboxyl acids.[76] Ketones are also oxidized to carboxyl acids.[77] Although chlorine dioxide has "Chlorine" in its name, its chemistry is very different from that of chlorine. When reacting with other substances, it is weaker and more selective. Chlorine dioxide, as with other oxidizers as well as water, causes oxidation to uncoated ferrous materials as well as other materials subject to oxidation. Control of moisture during the decontamination process mitigates the oxidation potential. Chlorine dioxide gas is soluble in water and does not dissociate in water to form hydrochloric acid. It is easy to confuse chlorine dioxide and chlorine, but they are different and do not affect materials the same way.

19.6 STABILITY OF THE GAS

Chlorine dioxide is not a stable gas that can be produced, bottled, and shipped; it is typically produced by a system at the point of use. Chlorine dioxide exposure to light can lead to decomposition. The photochemical reaction is a homolytic fission of the Chlorine-Oxygen bond to form ClO• and O•.[78] When light catalyzes the reaction mechanism for decomposition of dry gaseous chlorine dioxide, this is postulated as:

$$ClO_2 + h\nu \rightarrow ClO\bullet + O\bullet$$
$$ClO_2 + O\bullet \rightarrow ClO_3$$
$$2ClO\bullet \rightarrow Cl_2 + O_2$$

When gaseous chlorine dioxide is exposed to light in the presence of moisture, a visible mist may form. This mist does not contain chlorine but rather a complex mixture of hypochlorous and other acids.[79] The following mechanism has been proposed for the photolytic decomposition of chlorine dioxide in the presence of moisture:[80]

$$ClO_2 + h\nu \rightarrow ClO\bullet + O\bullet$$
$$ClO_2 + O\bullet \rightarrow ClO_3$$
$$2ClO_3 \rightarrow Cl_2O_6$$
$$ClO\bullet + ClO_2 \rightarrow Cl_2O_3$$
$$CL_2O_6 + H_2O \rightarrow HClO_3 + HClO_4$$
$$Cl_2O_3 + H_2O \rightarrow 2HClO_2$$
$$2HClO_2 \rightarrow HClO + HClO_3$$

Because chlorine dioxide is a true gas and will not condense, the stability of chlorine dioxide as the sterilizing agent is enhanced over methods using vapors (mixtures of a gas with a suspended liquid). Because concentration can be easily monitored and controlled, the concentration is precisely maintained throughout the cycle.

19.7 CYCLE DESCRIPTION

All sterilization or decontamination cycles are similar and have the same general steps. There is a sterilant injection phase, then an exposure phase followed by a removal or aeration phase. For dry gasses such as chlorine dioxide, the cycle is similar to the EtO cycle such that an additional moisture conditioning phase is required. The typical chlorine dioxide cycle can be carried out at pressures from negative pressure (2 KPa) to slightly above atmospheric. Figure 19.2 shows an example cycle of an ambient pressure chlorine dioxide cycle.

Even though the general steps for sterilization are similar, each process has specific steps for sterilization. The chlorine dioxide gas cycle steps are as follows:

- Pre-Condition
- Condition
- Charge
- Exposure
- Aeration

19.7.1 Pre-Condition

Pre-condition is the first step of the chlorine dioxide cycle. At this point, the chamber should be leak tested. When using any sterilant, it is good practice to perform a chamber leak test prior to each decontamination cycle to ensure chamber integrity. For a vacuum chamber, vacuum is pulled down to a desired level, and then the chamber is held static for a period of time. The pressure difference from the beginning of the dwell time to the end is noted. If the pressure rise is not within acceptable parameters, the chamber must be properly sealed and retested before any sterilant is injected into the chamber. If ambient pressure chambers are used, the leak test pressurizes the chamber then monitors for pressure decay to be acceptable before continuing the cycle. Once the chamber has been leak tested, the chamber can be brought to the RH set point (60%—75%). Humidity can be generated by a variety of methods such as steam, atomization, etc. Steam offers the quickest, cleanest, and most efficient way to raise the humidity.

19.7.2 Conditioning

Once the humidity is at the proper level (60%–75%), the cycle can advance to the next step. Conditioning allows the load to pick up moisture. During the conditioning time, typically 30 minutes, the RH is monitored. If the RH drops by any significant amount (>5%), additional moisture is added to raise the RH. Once the conditioning time is completed, gas is then introduced into the chamber in the Charge phase. Product can be pre-conditioned with higher humidity prior to starting the cycle, which will shorten overall process time.

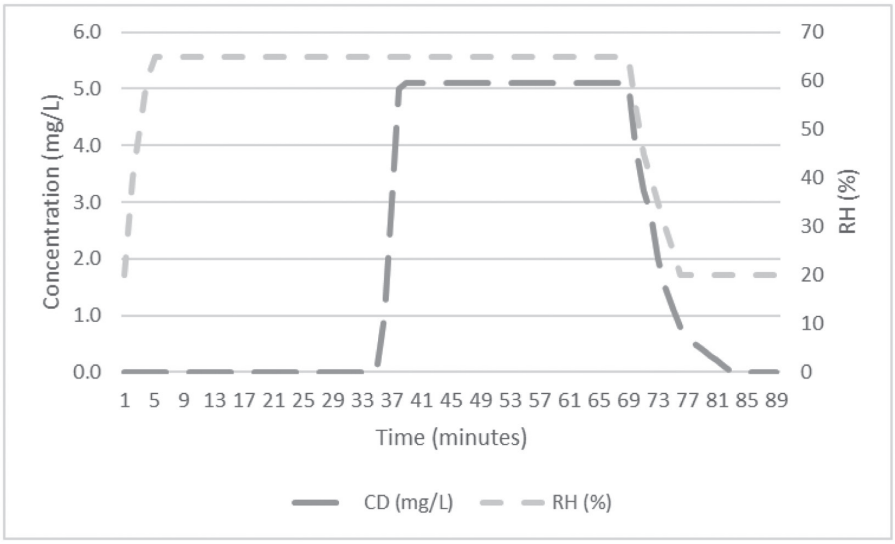

FIGURE 19.2 Cycle chart.

Note: CD = chlorine dioxide; RH = relative humidity.

19.7.3 Charge

During Charge or gas injection, chlorine dioxide gas is generated and introduced into the chamber to achieve the defined concentration. The target concentration is dependent on different factors: cycle time, cost, type of load, etc. If cycle time is extremely important, a higher concentration is selected to achieve a faster kill. At higher concentrations, the D-values are much shorter thereby shortening the overall cycle. If agent cost is the driving factor, then a lower concentration can be selected to preserve consumables, but the exposure time must be extended accordingly. Usually, a higher concentration is selected when using vacuum to ensure penetration into complex loads. Under vacuum conditions, the penetration of chlorine dioxide gas is quite rapid. Chlorine dioxide is a surface sterilant and does not have the penetrating abilities of EtQ. Chlorine dioxide does not penetrate into plastic polymers or through cardboard but it does reach confined areas (inside of syringes, bottles, tips and caps, lumens, stents, etc.). As chlorine dioxide does not penetrate into the polymers, there is an additional advantage of rapid aeration.

Because the chlorine dioxide concentration is easily measurable in real time, the target concentration can be repeatedly achieved, thus giving the assurance of a reproducible sterilization cycle. When gas concentration reaches the target concentration, the cycle proceeds to the Exposure step

19.7.4 Exposure

During Exposure, the concentration of chlorine dioxide gas is monitored and maintained to keep the concentration at the target concentration for the entire exposure time (typically 30–45 minutes). If the gas concentration drops during the cycle, additional gas is injected to ensure the gas concentration remains at the set point during the Exposure step.

19.7.5 Aeration

The Aeration step starts once the exposure step is completed. In this step, the chlorine dioxide gas is removed from the chamber. For vacuum chambers, this is accomplished by a series of vacuum pulls and filtered air or Nitrogen backfills.

Table 19.5 calculates the amount of chlorine dioxide used for a typical cycle.

Table 19.6 details the same cycle aeration curve. Aeration time is primarily dependent on the rate that the vacuum pump can evacuate the sterilization chamber. Aeration times of 15 minutes are usually attainable. This aeration brings the chamber environment to safe levels of 0.1 ppm or less.

Table 19.6: This table shows the aeration time from 5 mg/L down to 0.0002 mg/L. The aeration rate is one air exchange per minute. So, if the room is 1000 ft^3, the exhaust rate is 1000 CFM (cubic feet per minute).

19.8 CYCLE DEVELOPMENT

19.8.1 Moisture Conditioning

As mentioned previously, the presence of moisture in the load is critical to obtaining optimal lethal rates and effective sterilization with gaseous chlorine dioxide. Moisture is critical for the inactivation of spores regardless of the sterilizing agent.[81,82,83,84] Important points to consider when developing

TABLE 19.5
Quantity of Chlorine Dioxide for the Given Chamber

Chamber Volume (Cu Ft)	100 ≅ 28.32 cu meters
Target Concentration (mg/L)	5
Exhaust Rate (CFM)	100 ≅ 169.9 cu meters/hour
Amount of chlorine dioxide in the chamber	14.16 g

TABLE 19.6
Time and Amounts of Chlorine Dioxide during Aeration for above Chamber and Concentration. Aeration Is Down to OSHA Safe PEL Levels of 0.1 ppm

Air exchanges	mg/L	PPM	Time (min)
1	2.5000	905.00	1
2	1.2500	452.50	2
3	0.6250	226.25	3
4	0.3125	113.13	4
5	0.1563	56.56	5
6	0.0781	28.28	6
7	0.0391	14.14	7
8	0.0195	7.07	8
9	0.0098	3.54	9
10	0.0049	1.77	10
11	0.0024	0.88	11
12	0.0012	0.44	12
13	0.0006	0.22	13
14	0.0003	0.11	14
15	0.0002	0.06	15

Note: In each air exchange 1/2 of the chlorine dioxide present is removed

and optimizing the chlorine dioxide sterilization process are as follows:

- What moisture condition has the load been exposed to/stored at prior to sterilization?
- Can the moisture level be affected by seasonal RH variation?
- Are there components or packaging materials that may become desiccated during storage in a dry environment prior to sterilization?
- Could the density of the load or its physical geometry affect the penetration of moisture into the least accessible areas?

The choice of a moisture conditioning time in a traditional sterilizer-based application is a function of the prior concerns as well as the approach used to perform the moisture conditioning. Moisture conditioning can be accomplished either in an external chamber or within the sterilization chamber itself. Typical loads can attain the required moisture with 30 minutes of conditioning as part of the sterilization cycle. Appropriate validation studies are important to assure moisture penetration into the least accessible areas.

19.8.2 Exposure Time/Gas Concentration

Early studies with a traditional sterilizer-based application used a gas concentration of 30 mg/L. This concentration was chosen because of the density and composition of the sterilization load. Rapid inactivation of the BIs was observed with sterilization of 10^6 *B. atrophaeus* (formerly *subtilis* ATCC 9372) spores occurring in <15 minutes in almost all cases. In one application, testing was performed at a chlorine dioxide gas concentration of 3 mg/L with reproducible sterilization of 10^6 BIs. As would be expected, the required total gas exposure time was longer than that used at higher gas concentrations.

Based upon studies using a number of test systems, the following guidance can be given with respect to the choice of gas concentration and exposure time in process development studies:

In a chlorine dioxide sterilizer, the recommended gas concentrations for process development studies are 15–30 mg/L. A number of sterilization exposures have been performed using a chlorine dioxide gas concentration of 30 mg/L. In almost all cases, complete kill of 10^6 BIs was observed with 15 minutes of gas exposure. At a chlorine dioxide concentration of 5 mg/L, complete kill of 10^6 BIs should be observed with 30 minutes of exposure.[85] These results were obtained in sterilizers that were not densely loaded but did include a desiccated load. In the event of a dense, desiccated load where moisture penetration may be impeded, a longer conditioning and/or exposure time may be required for similar sterilization efficacy. Isolator studies have shown excellent penetration abilities with densely loaded transfer isolators at 5 mg/L for 30 minutes of exposure along with gas penetrating into half-suit armpits with sleeves down. These results were based upon a fixed concentration for a fixed time. In this work, the charge time was not considered and only the exposure concentration used. The cycle time can be shortened if both phase times (Charge and Exposure) are considered and combined to accrue a contact time or dosage. Dosage is the accumulation of concentration over time.

To calculate the dosage, the chlorine dioxide must be converted from mg/L to ppm. The calculations following can convert mg/L to ppm.

ppm calculation for 1 mg/L chlorine dioxide concentration

$$\text{ppm} = (\text{mg/m}^3)\,(24.45)/\text{molecular weight}$$
$$= (\text{mg/L})\,(1000)\,(24.45)/\text{molecular weight}$$
$$= (1\ \text{mg/L})\,(1000\ \text{L/m}^3)\,(24.45)/67.5$$

= 362.2

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

So the dosage for a 2-hour exposure at 1 mg/L concentration has a dosage or contact time of 724 ppm-hrs (362 ppm × 2 h = 724 ppm-h).

Dosage or contact time (CT) starts accumulating when the monitoring system starts reading actual gas concentrations. This has the effect of combining the charge time and exposure time, thereby shortening the overall cycle time.

Other studies have shown 5-log reduction of spores at 400 ppm-hrs[86] in a large animal hospital. In isolators and processing vessels, a 6-log reduction of spores has been demonstrated in several publications.[87,88,89,90] Czarneski showed reductions at a dosage of 900ppm-hrs, whereas Eylath demonstrated 6-log reductions at dosages of 540 ppm-hrs, 600 ppm-hrs, 900 ppm-hrs, and up to 1800 ppm-hrs. Leo demonstrated a 4-log reduction at a low dosage of 180 ppm-hrs.[91] In these same studies, Leo documented a predicted 16.65-log reduction based upon four quartile results accrued. To follow up these studies, tests were performed to see if the results were based upon the concentration or dosage and Lorcheim demonstrated a 6-log reduction of spores at the same dose (720 ppm-hrs) with varying concentrations (0.3, 0.5, 1, 5, 10, and 20 mg/L).[92] In these studies, the dose was held constant and the concentration varied thus indicating that dose is the critical factor.

TABLE 19.7
Examples of Gaseous Chlorine Dioxide Process Development Studies Using *B. atrophaeus* Spores

Preconditioning		Chlorine dioxide exposure		# Nonsterile/ # Tested	Comments
Min	% RH	mg/L	Min		
30	75	10	5	10/10	Spores on unwrapped paper spore strips stored at 23% RH prior to use. Duplicate series of runs on different days.
30	75	10	10	5/10	
30	75	10	15	0/10	
30	75	10	5	8/10	
30	75	10	10	1/10	
30	75	10	15	0/10	
30	70	5	30	0/20	Spores on paper spore strips in Tyvek envelopes.
30	75	10	15	0/10	Spores on paper strips, unwrapped
30	75	10	30	0/10	
30	75	10	15	10/10	Spores on paper strips in blue glassine envelopes
30	75	10	30	0/10	
30	75	10	15	0/10	Spores on paper strips in Tyvek envelopes
30	75	10	30	0/10	
30	75	10	15	0/10	Spores on glass fiber discs in Tyvek envelopes
30	75	10	30	0/10	

Abbreviation: RH = relative humidity.

19.8.3 Examples of Chlorine Dioxide Process Development

Table 19.7 presents examples of gaseous chlorine dioxide process development studies in sterilizers using 10^6 *B. atrophaeus* (formerly *subtilis*) spores as the BI. This work evaluated different substrates.

19.8.4 Biological Indicators

Historical data has shown *Bacillus atrophaeus* (ATCC 9372) spores as the appropriate BI for chemical sterilants such as chlorine dioxide. To confirm the applicability for gaseous chlorine dioxide, tests were done with *B. atrophaeus* as well as other commonly used Bls.

Four spore-forming organisms were initially selected; *Geobacillus stearothermophilus* (ATCC 12980), traditionally used in steam sterilization activities, *Bacillus pumilus* (ATCC 27142), most often used in irradiation studies, *G. stearothermophilus* (ATCC 7953), used for hydrogen peroxide systems, as well as *B. atrophaeus* (ATCC 9372). A study was developed to expose each type of BI to a fixed chlorine dioxide cycle. In each of three runs, 15 Bls of each type were exposed to the chlorine dioxide cycle, removed from the chamber, and aseptically transferred to nutrient media. Microbial growth, as indicated by media turbidity, was recorded as a positive result. This testing was performed in triplicate.

The results are shown in Table 19.8. As Table 19.8 shows, *B. atrophaeus* spores were more resistant (highest number of Bls remaining non-sterile) than either of the G. *stearothermophilus* strains or *B. pumilus*. Based on this data, the use of spores of *B. atrophaeus* as the BI for gaseous chlorine dioxide is a good choice. Additionally, these results demonstrate that *G. stearothermophilus* is also a good choice. Either BI could be used because both *B. atrophaeus* and *G. stearothermophilus* show high levels of resistance to chlorine dioxide. G. *stearothermophilus* has the benefit of a higher incubation temperature, thereby reducing the potential for false positives because not many organisms grow at the elevated temperature of 55°C –60°C. When using chlorine dioxide gas and spore strips, the indictor strip should be wrapped in a Tyvek/Tyvek or Tyvek/mylar envelop. Tyvek does not impede the penetration abilities of chlorine dioxide gas. Glassine envelopes (blue envelopes) do impede the penetration of the gas and will require a slightly longer exposure time. It is also recommended to use spores inoculated on paper strips. Spores inoculated on rigid carriers such as stainless steel can have clumping issues depending upon the inoculation procedures. The size of the stainless-steel carrier can also have an effect on the spore loading. If the spores are inoculated on

TABLE 19.8
Biological Indicator Resistance Study Results

Biological Indicator	Run 1 # nonsterile/ total tested	Run 2 # nonsterile/ total tested	Run 3 # nonsterile/ total tested	Total # nonsterile/ total tested
B. atrophaeus (formerly *subtilis*) ATCC 9372	10/15	13/15	15/15	38/45
B. pumilus ATCC 27142	0/15	2/15	1/15	3/45
G. stearothermophilus ATCC 12980	1/15	2/15	2/15	5/45
G. stearothermophilus ATCC7953	9/15	9/15	8/15	26/45

Note: Cycle parameters for above studies: 30 mg/L gas concentration, 90% relative humidity pre-humidification, 6-minute exposure time

paper carriers, the same issue does not arise because of the paper creating a wicking effect causing the spores to spread throughout the carrier. Paper carriers also can be considered a porous load and this further tests the sterilization process for penetration into porous loads. Studies at ClorDiSys Solutions have been done to show chlorine dioxide does not absorb into the paper strip and cause residual kill.[93,94] These studies were performed using guidance from the EPA neutralization of disinfectants and used exposure at two different concentrations, 1 mg/L (final dosage of 729 ppm-hrs) and 5 mg/L (final dosage of 1960 ppm-hrs). The BIs were pulled from the isolator immediately after exposure (pre aeration), after aeration was completed, 30 minutes post aeration, and 60 minutes post aeration. Aeration was completed when concentrations in the isolator were at or below 0.1 ppm. Post-exposure/pre-aeration BIs should contain the most amount of chlorine dioxide absorbed into the spore strips. These samples were not capable of providing residual kill on the microorganisms added to the test tubes prior to incubation. All other samples removed after increasing lengths of aeration contained far less chlorine dioxide gas absorbed into the spore strips, and as a result, were also unable to provide any residual kill during incubation.

19.8.5 Validation

In determining sterilization parameters, the cycles utilized are based upon the "overkill concept" and are intended to provide a 12-log reduction of a resistant biological indicator. To provide an additional margin of safety, the sterilization parameters utilized during validation studies should represent "worst-case" conditions when compared with those provided for routine sterilization.

To determine final cycle parameters, reductions could be made in the following areas: Conditioning—the target humidity could be reduced (from the typical set point range of 60%–75% RH). The Charge/Exposure—the gas concentration and Exposure dwell time can be reduced (from the typical 45 min). They can be reduced by utilizing the contact time or dosage. In doing this, the charging phase and exposure phase can be combined to reduce the overall cycle time. Additionally, the condition time could be combined with the exposure phase to further reduce overall cycle time.

Additionally, load patterns can affect the efficacy of the sterilization. The maximum load type should be used because this will typically be the most difficult sterilization. Each load type needs to be tested in three consecutive microbial challenge studies to achieve a validated sterilization cycle.

19.8.6 Measurement/Quantification

A UV/VIS spectrophotometer should be integrated into the sterilization system. It precisely monitors and controls the chlorine dioxide concentration during Charge and Exposure, and the Aeration steps until it reaches approximately 0.1 mg/L. Chlorine dioxide gas is green colored and can be measured by photometric methods. In these methods, light is passed through a continuous sample of the gas brought to the photometer by a sample pump from a location within the chamber. The monitoring location is typically chosen by the user in the most difficult location. Typically, the most difficult location would be buried in the load, but using a sample pump in this location would actually improve penetration by drawing gas to this location, so the user should rely on the BI to confirm the decontamination. Typically, the gas sample tubing is placed in corners or behind objects in the chamber. The gas will absorb light, and this absorption can be measured and quantified. The adsorption is converted to concentration, thereby providing an accurate and, more importantly, repeatable concentration measurement. Additionally, as chlorine dioxide is a true gas it does not condense, making aeration quick and repeatable. This repeatability allows for aeration to be validated, ensuring that safe conditions are attained for repeat applications. There are also devices such as Draegar tubes or other sensors, which can verify that safe levels are attained prior to opening the chamber.

19.9 IN-PROCESS CONTROLS

Ease of process control is one of the greatest strengths of the chlorine dioxide technology and is superior to that of most other methods of sterilization. Chlorine dioxide is a gas at use concentrations (boiling point of −40°C at 0.04% and −22°C at 0.18%) that distributes rapidly and evenly throughout the chamber. Because it is a true gas, issues with temperature gradients, cold spots, heat sinks because of the materials of construction, and other issues that can affect the condensation of vapor decontaminating agents such as hydrogen peroxide and peracetic acid do not affect the decontamination effectiveness of chlorine dioxide. Also because of its gaseous properties, it can easily penetrate down long lumens and effectively sterilize complex components sealed in Tyvek bags.

An RH/Temperature probe monitors the RH and temperature conditions inside the chamber. A pressure transmitter monitors the chamber pressure.

The tight process control and accurate concentration monitoring, along with a detailed run record, can lead to parametric release when used for product sterilization as well as expedite validation efforts for all applications.

19.10 DELIVERY SYSTEMS

The ClorDiSys Solutions, Inc. Steridox-VP sterilizer and Cloridox-GMP Sterilization System (Figure 19.3) can be used for component or device sterilization. The Steridox-VP is a stand-alone sterilizer. The Cloridox-GMP is a portable chlorine dioxide gas generator system designed for interfacing with an existing steam or EtO sterilization chamber. Additionally, the Cloridox-GMP can be used to decontaminate isolators, clean rooms, processing vessels, pass-throughs, bio-safety cabinets, or other sealed chambers. Chlorine dioxide sterilizers provide a rapid and highly effective method for component or device sterilization. These systems feature a sophisticated sterilant concentration monitoring system to ensure a tightly controlled process. All instrumentation, including the photometer for concentration monitoring, is easily calibrated to traceable standards. The Human-Machine Interface (HMI) system features a password protected, recipe management system with historical and real-time trending. The process is easy to validate because of the repeatable cycle, tight process control, and highly accurate sterilant monitoring system. A run record is produced that contains the date, cycle time, cycle steps, as well as the critical operating parameters of relative humidity, temperature, pressure, and chlorine dioxide concentration.

19.11 MEDICAL DEVICE STERILIZATION

There have been few advancements in the medical device sterilization industry compared to most other industries. EtO has been facing increasing pressure due to potential environmental health hazards. Even with the issues, its market share has not decreased and has held steady since the mid-1990s. This is due to the industry resistance to change and as such innovation has taken a back seat. Recently, the first commercially available medical device has been sterilized with CD gas and has hit both the US and European markets.

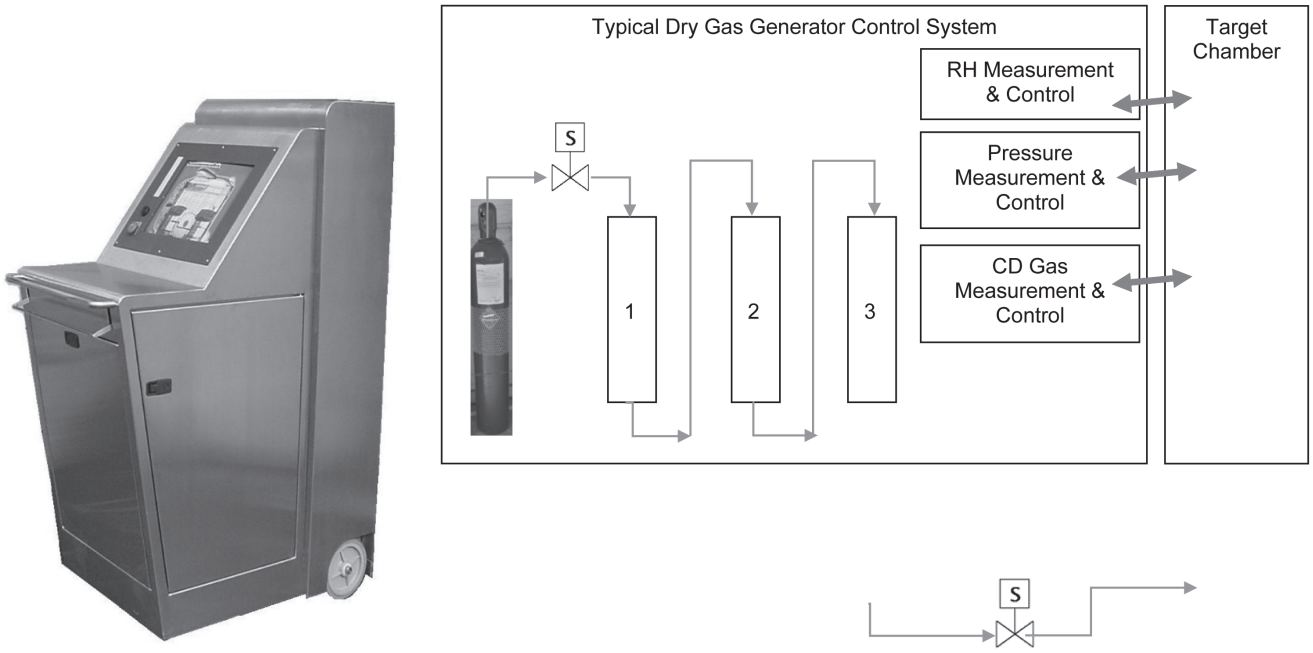

FIGURE 19.3 Cloridox-GMP.

In 2006, the EPA released a draft of its review of EtO and determined it is a human carcinogen, after reviewing studies by the National Institute of Occupational Safety and Health (NIOSH). In 2018, the EPA released its latest National Air Toxics Assessment based on industry supplied emissions data from 2014. The report showed that 109 of the 73,057 census tracts within the US faced cancer risks due to exceeding emissions levels on EPA guidelines. One of the areas with very high EtO levels was in Illinois near an EtO contract sterilization facility. In February 2019, the Governor decided to ban the use of EtO at the plant, but a legal settlement eventually allowed the company to resume operations after installing equipment to reduce EtO emissions.

In the late 2020, the FDA approved a contract sterilization facility for medical devices solely utilizing CD gas. This facility recently processed the first commercially sold medical device sterilized with CD gas that has both US and EU approval. A draft reference file for the use of CD gas has been created mimicking ISO and AAMI EtO guidelines to aid in submission packages to both the FDA and EU regulatory agencies. This provides a regulatory roadmap for validating the sterilization process outside of the typical EtO, gamma irradiation, E-beam / X-ray, steam, and dry-heat methods.

19.11.1 Recent Updates

CD gas medical device sterilization validation studies challenged the process with External Process Control Devices placed outside the load and Process Control Devices placed inside the product load. The studies were done at much lower concentrations (3 mg/L) compared to early sterilization studies (30 mg/L) with CD gas. CD gas was also found to penetrate the display box and did not require any external aeration. After exposures, the product was tested for endotoxin residuals. Endotoxins were tested using the LAL gel-clot test technique and <0.03 EU/mL or <l. 2 EU/device was found. This result is well under the maximum allowable limit of 20.0 EU/device for medical devices and under the 2.15 EU/device limit for cerebrospinal contact devices. CD gas residuals are typically not detected on surfaces, since they are aerated and removed as part of the sterilization process and does not condense onto materials like other methods can. Residual testing for chlorine dioxide gas examines its byproducts that can be chlorite, chlorate, and chloride. Chlorite has a maximum acceptable level of 6.83 mg/device, and testing was unable to detect any (minimum detection level 3.78 µg/device). Chlorate has no maximum acceptable level set, and testing showed an average of 216.5 µg/device over 3 runs. Chloride has a maximum acceptable level of 70.65 mg/device with none detected from the testing (minimum detection level 1.89 µg/device).

AuQ56

Other studies with elastomeric closures showed no clear detrimental effect of CD gas sterilization on the functional properties, even when a 20-fold overexposure was used. There was minor increase in the number of fragments and piercing force observed for styrene-butadiene compounds, although the difference was too small to be able to assign this as an effect of the sterilization process. The effect of CD gas sterilization on the chemical and functional properties of styrene-butadiene compounds and coated and uncoated bromobutyl compounds was studied. The coating was a fluorinated polymer coating. The compounds were investigated and tested according to the tests as described in the USP. In general, it was found that sterilization with CD gas can be competitive with the classical sterilization techniques. The stoppers perform equally well or even slightly better after being subjected to CD gas compared to gamma radiation or EtO.

19.12 OTHER NON-STERILIZATION APPLICATIONS

Chlorine dioxide has a long history of use, and it has been used longest in the potable water industry, and the pulp in paper industry is the largest user. Chlorine dioxide gas, as an oxidizing agent, has been shown to oxidize many things including chemical weapons. Gordon at Public Health Agency in Canada demonstrated chlorine dioxide gas as an effective method to inactivate anthrax toxins.[95] Chlorine dioxide gas has also been shown to be effective (>99%) against the chemical nerve agent VX, but has been shown to be ineffective or partially effective for soman and sarin.[96] Chlorine dioxide has been used for years for odor control in residential and industrial markets. As an example, NosGuard SG (Fort Lauderdale, FL) is used to eliminate odors caused by mold, mildew, pets, food, smoke, and more. There are many other companies with products and services based around odor control. Chlorine dioxide gas has also been shown to be effective again insects. Lowe showed the gas was able to kill bedbugs (*Cimex lectularius* and *C. hemipterus*) with a 100% mortality rate after exposure to various concentrations (1, 2, and 3 mg/L) at various dosages (519, 1029, 1132, and 3024 ppm-hrs).[97] Czarra has shown chlorine dioxide gas was effective again pinworm eggs.[98] Pinworm infections are considered nonpathogenic but with certain research mice they can have adverse effects on behavior, growth, intestinal physiology, and immunology that may affect research.

19.13 CONCLUSIONS

Chlorine dioxide is an oxidizing agent and as such it has shown good biocidal efficacy against viruses, bacteria, fungi, and spores. It is registered as a sterilant, which means it kills all forms of life. It has been shown effective at inactivating other substances such as proteins, endotoxins, beta lactams, chemical weapons, smoke odors, and odors of various kinds. It is used as a gas and a gas dissolved in solution as a sanitizing, broad-spectrum disinfectant and sterilant. It is used in many industries from food to life science research to the pharmaceutical, medical device, and biologics industries. Although it is an oxidizer, it has shown good material compatibility with most materials found in the pharmaceutical industries including electronics. This chapter documents many of the applications of chlorine dioxide gas in its true form, as a gas. As a gas it shows excellent penetration and distribution properties, which is critical in any decontamination. It

is easily monitored with a photometric device, which makes for good repeatability, which makes for a good and thorough decontamination/sterilization.

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