

## Application Note #29

### Is RH necessary when using Chlorine Dioxide Gas for sporicidal kill?

Does Relative Humidity (RH) need to be added when a decontamination cycle occurs? With dry gas decontaminations (chlorine dioxide, formaldehyde and ethylene oxide) RH is typically added prior to the sterilant injection. RH causes the spores to soften and grow allowing the sterilant to easily enter the spore and kill it. Based upon this idea, what RH level is necessary? Is more RH better? Is less RH better? Based upon studies performed by ClorDiSys lower RH values required increased exposure times. Increased RH values, decrease the exposure times.

**Objectives:** Verify the relationship between RH and exposure time for achieving 6-log sporicidal kill with chlorine dioxide (CD) gas.

**Method:** 6-log biological indicators were used to test the efficacy of chlorine dioxide gas with varying RH levels to determine what overall dosage is required to achieve complete kill. Chlorine dioxide gas concentrations will remain constant at 1mg/L and the dosage will increase until all BI's are killed.

**Results:** Data has shown that exposure times increase as RH levels are decreased. When RH levels are 65% or greater the dosage of 720 ppm-hrs was adequate to achieve total kill. A dosage of 1000 ppm-hrs was required for 55% RH and a dosage off 1550ppm-hrs was required for 45%. See tables 1, 2 & 3 for results.

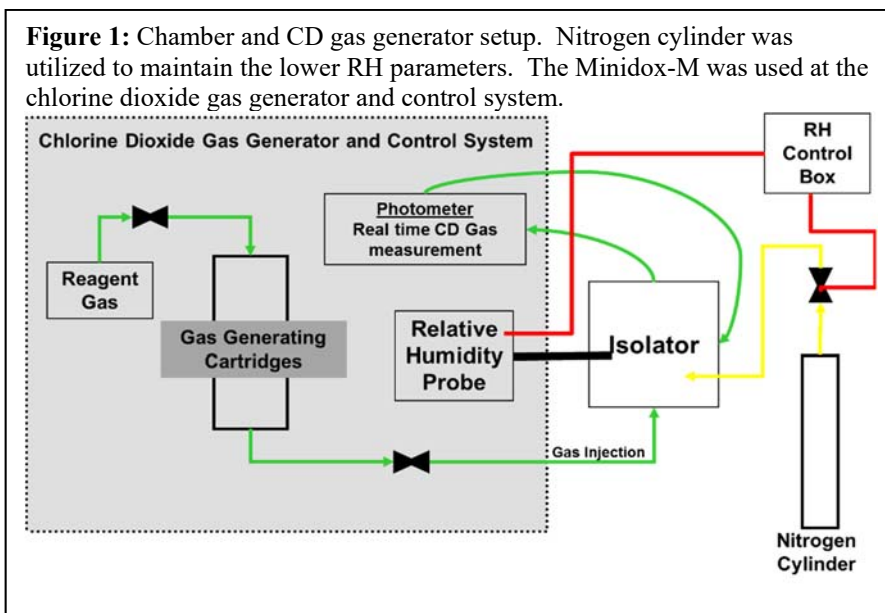
**Conclusion:** Chlorine dioxide gas achieves kill at various RH levels. The lower the RH the longer the exposure or dosage.

**Outcomes:** Applying these findings to applications, would allow for lower RH cycles when necessary due to certain materials requiring lower RH values or difficulty in humidifying an area.

#### Equipment Used (see figure 1 for setup)

- 1 Minidox-M CD Gas Generator
- 17 cu ft Isolator (2 glove)
- SGM Spore Strips ACD/6, *bacillus atrophaeus* (ATCC 9372)
- SGM Releasat® fast readout culturing media
- BI Challenge Fixture, test fixture to mimic small gaps (0.185” [4.7mm]) see figure 2.
- Incubation at 37 Deg. C
- BSC Scrubber (remove CD gas)
- RH Controller
- Using dry nitrogen to control isolator (45% & 55%)

All BI's were stored at 45% RH prior to decontamination runs.



**Figure 2:** BI Challenge Fixture. This is a test fixture to mimic small gaps. BI is placed into the small opening or slot to test the penetration of CD gas into small gaps or openings. Simulates a 5mm gap.



**Background Dosage / PPM-Hr Explanation**

Dosage is described as an exposure at a concentration multiplied by an amount of time, typically hours (Hrs). For CD gas this is referred to as PPM-Hrs. To determine the PPM-Hrs the concentration in PPM is accumulated every minute. This accumulation then accrues PPM-Hrs.

**NOTE:** For quick charging chambers, such as small isolators, a 30 minute condition time is required.

**Discussion**

Is RH necessary during a decontamination? Should the RH be lowered or raised before gassing? To get faster results or shorter cycle 65% RH should be used since the dosage was the lowest at 720 ppm-hrs. If it is difficult to raise the RH or some items in the space do not like high RH then the 55% RH cycle will work. If 55% RH is chosen then a dosage of 1000 ppm-hrs. is required. If 45% RH is chosen then a dosage of 1550 ppm-hrs. is necessary to achieve a 6-log reduction of spores. See tables 1, 2 & 3 for results. Figure 3 below shows the cycle data for one cycles during the 45% RH runs.

Standard sporidical cycle parameters are: RH - 65%<sup>1</sup> with 5 minutes of condition time, CD Concentration - 1mg/L and CD Exposure time – 2 hrs.

PPM calculation for 1mg/L

$$PPM = (mg/M3) (24.45) / \text{Molecular Weight}$$

$$PPM = (mg/L) (1000) (24.45) / \text{Molecular Weight}$$

$$CD \text{ ppm} = (1.0mg/L) (1000L/M3) (24.45) / 67.5$$

$$CD \text{ ppm} = 362.2$$

Exposure Contact Time (CT)

$$\text{Exposure CT} = 362ppm * 2 \text{ hrs}$$

$$\text{Exposure CT} = 724 \text{ ppm-hrs}$$

24.45 = volume (liters) of a mole (gram molecular weight) of a gas at 1 atmosphere and at 25°C.

**Table 1:** The following table show the runs at 65% with increasing dosages until all BI's are killed. The results in ( ) are the BI placed into the BI Challenge fixture.

ppm-hrs	mg/L	RH	Condition Time	Results
450	1	65	5	0/3, 3/3, 3/3, 2/3 (N/A)
550	1	65	5	0/3, 1/3, 0/3, 2/3, 0/3, 0/3 (0/1, 0/1, 0/1, 1/1, 1/1, 0/1)
600	1	65	5	1/3, 0/3 (0/1, 0/1)
720	1	65	5	0/3, 0/3, 0/3 (0/1, 0/1, 0/1)

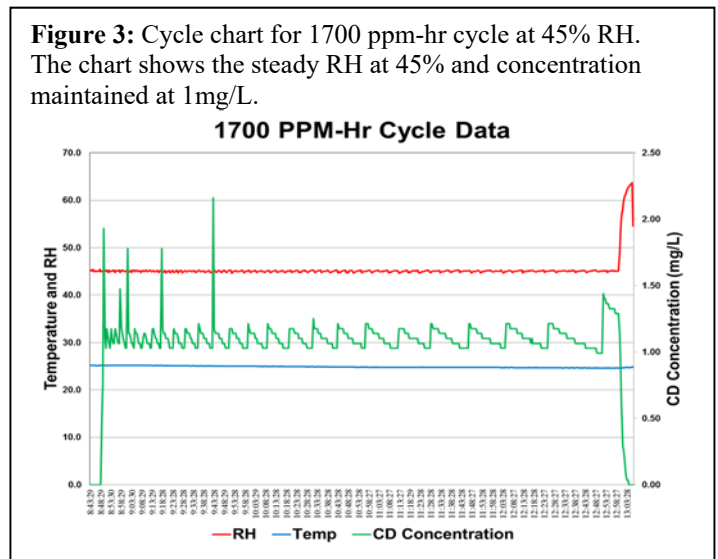
**Table 2:** The following table show the runs at 55% with increasing dosages until all BI's are killed. The results in ( ) are the BI placed into the BI Challenge fixture.

ppm-hrs	mg/L	RH	Condition Time	Results
550	5	55	30	3/3 (1/1)
850	1	55	5	2/3 (0/1)
925	1	55	5	0/3, 1/3, 1/3, 0/3, 0/3, 1/3, 0/3, 0/3 (0/1, 0/1, 0/1, 0/1, 0/1, 0/1, 0/1, 0/1)
1000	1	55	5	0/3, 0/3, 0/3 (0/1, 0/1, 0/1)
1400	1	55	5	0/3 (N/A)
2100	1	55	5	0/3 (0/1)

**Table 3:** The following table show the runs at 45% with increasing dosages until all BI's are killed. The results in ( ) are the BI placed into the BI Challenge fixture.

ppm-hrs	mg/L	RH	Condition Time	Results
1400	1	45	5	0/3, 1/3, 1/3, 1/4 (0/1, 0/1, 0/1, N/A)
1550	1	45	5	0/3, 0/3, 0/3, 0/3 (0/1, 0/1, 0/1, 0/1)
1700	1	45	5	0/3, 0/3, 0/3, 0/3 (0/1, 0/1, 0/1, 0/1)
2100	1	45	5	0/3 (0/1)

**Figure 3:** Cycle chart for 1700 ppm-hr cycle at 45% RH. The chart shows the steady RH at 45% and concentration maintained at 1mg/L.



Data for these studies (Table 1, 2, & 3 and Figure 3) were presented at 54th annual ABSA conference, 2011, "Effects of Relative Humidity, Concentration, and Exposure Time on the Efficacy of Chlorine Dioxide Gas Decontamination", Mark A. Czarneski.