

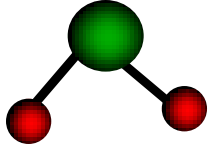


**ABSA**  
54th Annual  
Biological Safety Conference  
Anaheim Marriott Hotel  
Anaheim, California  
October 27 - November 2, 2011  
[www.absa.org](http://www.absa.org)

# Effects of Relative Humidity, Concentration, and Exposure Time on the Efficacy of Chlorine Dioxide Gas Decontamination

Mark A. Czarneski  
Director of Technology

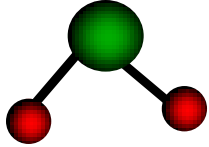




# Overview

---

- 1. Define Experiment and Chlorine Dioxide gas**
- 2. Define Chlorine Dioxide Decontamination Parameters**
- 3. Results**
- 4. Other Recent Data**
- 5. Conclusions**



# *Experiment*

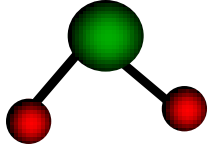
---

## ➤ Questions

- Does RH affect spore log reductions with CD Gas??
- Does CD Gas concentration / time affect spore log reductions??

## ➤ How to Do

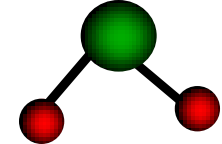
- Expose 6-log biological indicators (BI's) at various RH (45%, 55% & 65%) for different exposure times
- Minimum of 3 BI's per run
- Some runs had BI's placed in BI Challenge Fixture to simulate small gaps (0.185" [4.7mm])
- All BI's stored at 45% RH prior to decontamination cycle



# *Chlorine Dioxide Fumigation*

---

- **True Gas (boiling point 11°C)**
- **Short Contact times (0.5-2 hours)**
- **Fast overall cycle times (fast aeration)**
- **Non - carcinogenic**
- **Water Soluble**
- **Non - Flammable**
- **No post exposure cleanup required**
  - **Direct vented or scrubbed at end of exposure**
- **Low concentrations (360-1800 ppm)**
- **Scaleable by adding a generator for every 30,000 cubic feet (70,000 cu-ft can be done with a 6.6-hour exposure)**
- **EPA Registered as a Sterilant**
- **NSF approved for BSC Decontamination**

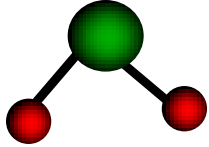


# *Chlorine Dioxide Generation Technology*

---



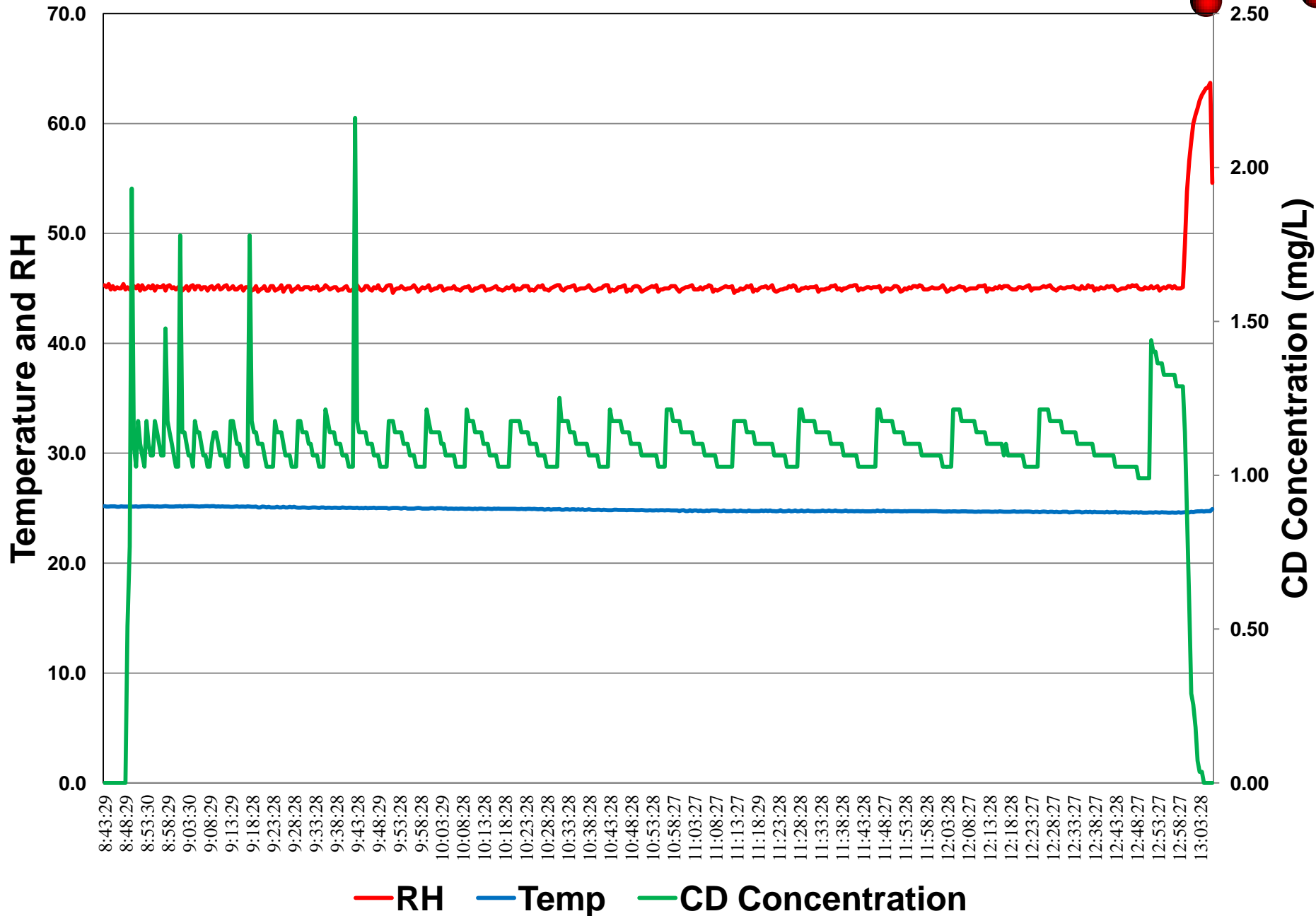
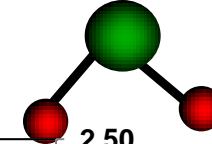
- **Performed in solid phase**
- **Gas generated on demand**
- **Self-Contained reagents**
- **Simple to replace consumables**
- **Only pure gas is delivered to the chamber, the salt solid remains in the CD Cartridge**



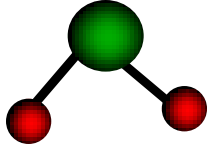
# *The Chlorine Dioxide Decontamination Process*

---

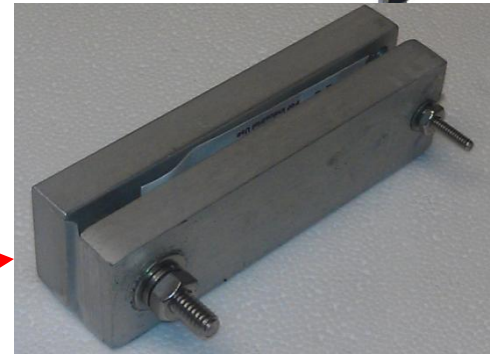
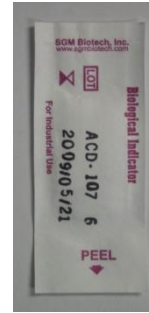
- **Pre-Conditioning**
  - Raise RH 45%, 55% or 65%
- **Conditioning**
  - Dwell time at RH SP (5 min)
- **Charge**
  - Raise CD Concentration 1 mg/L
- **Exposure**
  - Dwell time at CD SP (to achieve x ppm-hrs)
- **Aeration**
  - Remove CD Gas 12-15 air exchanges



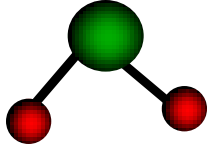
# Equipment Used



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

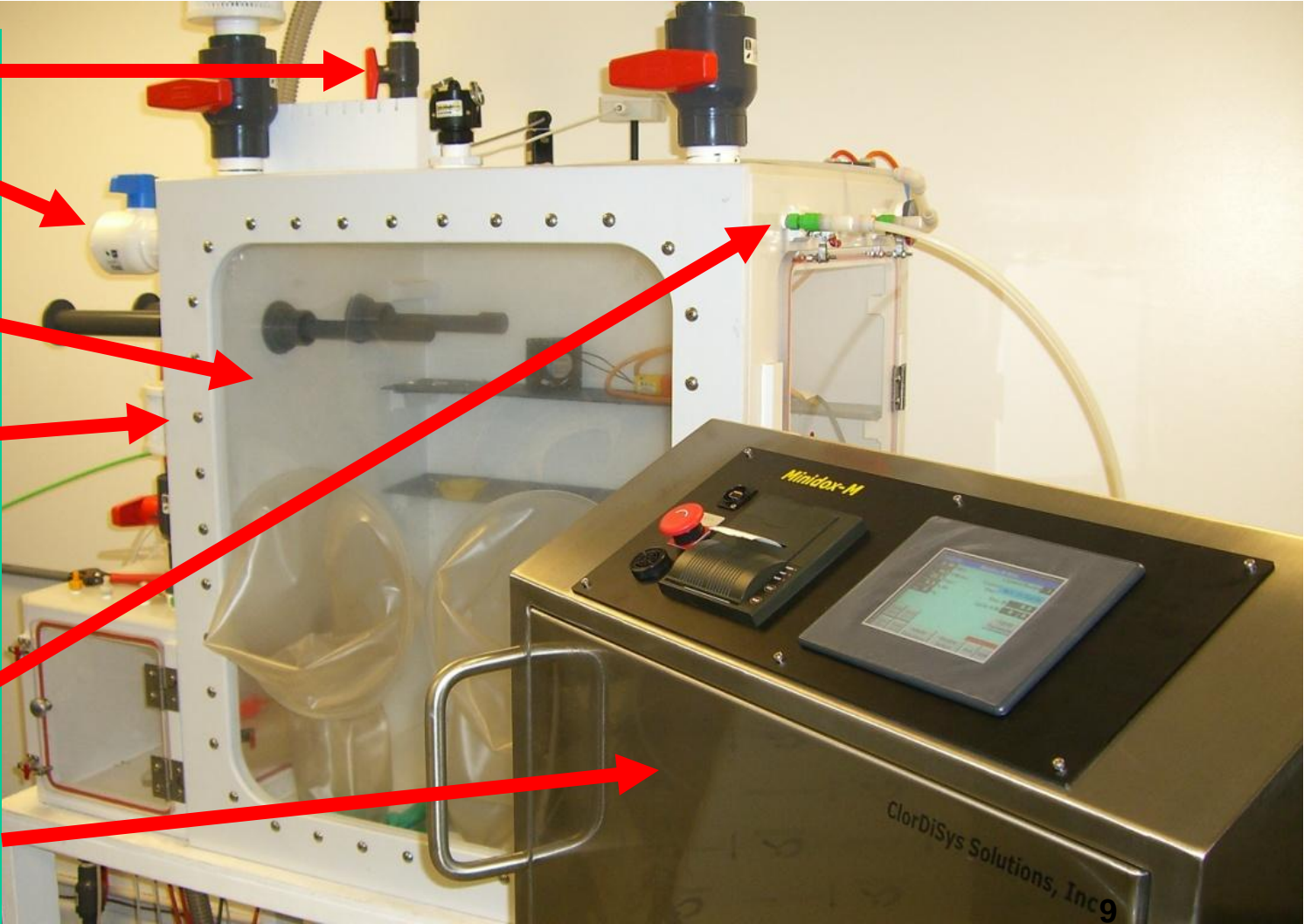


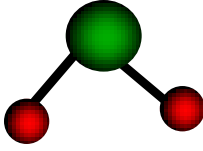




# Equipment Used

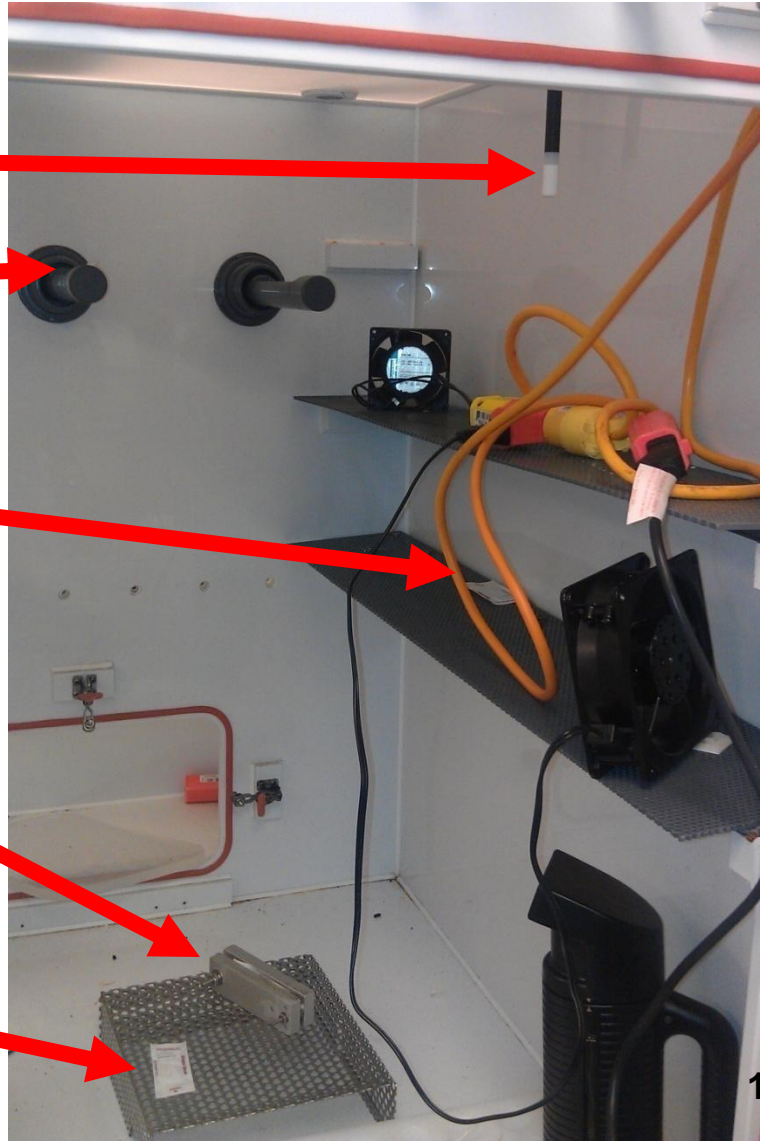
- Exhaust
- Aeration air supply
- CD Gas Sample
- 2 Glove Isolator
- CD Gas Inject
- Minidox-M CD Gas Generator

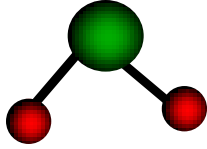




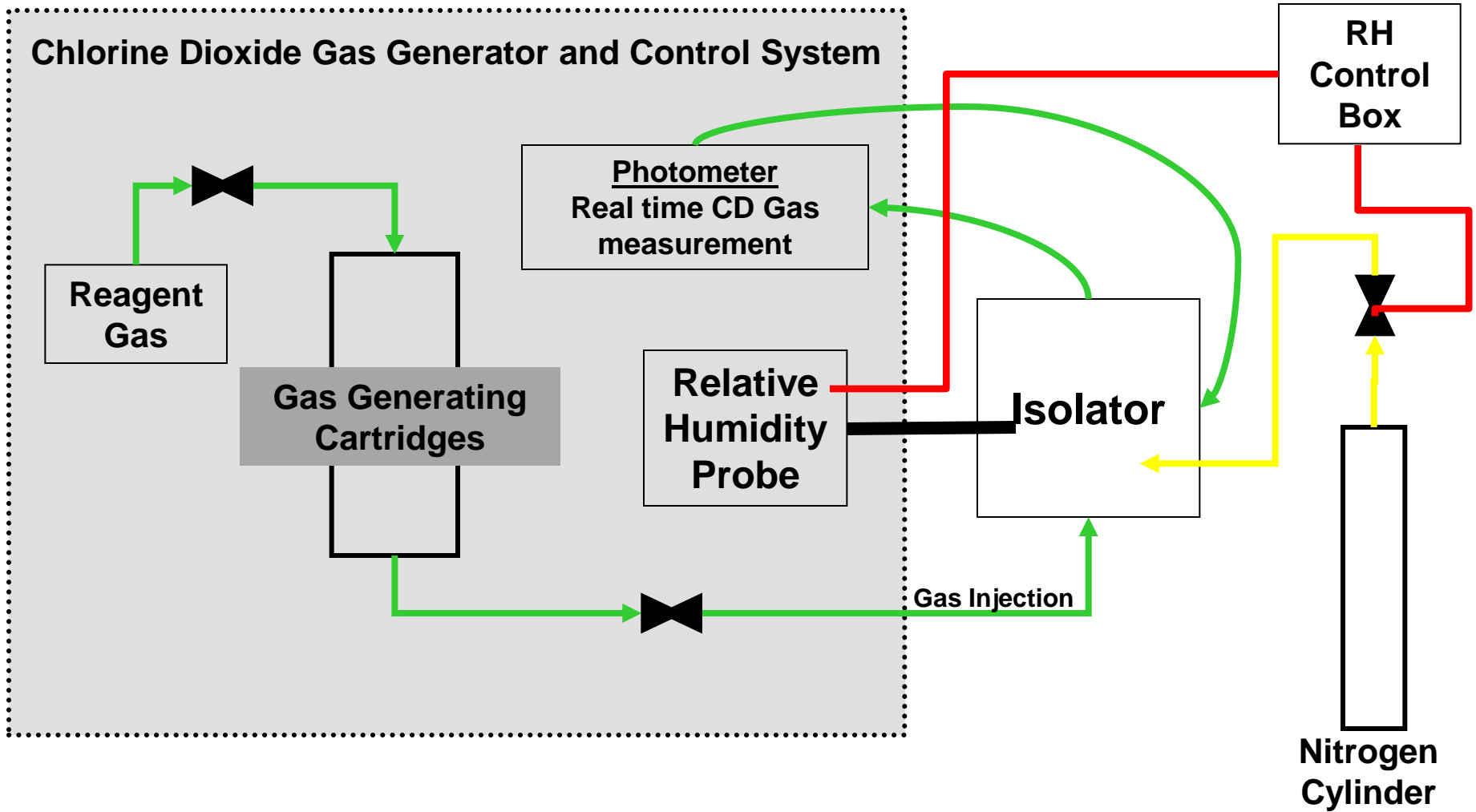
# BI Placement

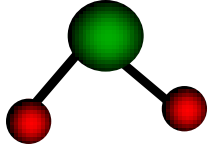
**RH Probe**  
**BI 1 (in sampler)**  
**Up High**  
**BI 2 (on shelf)**  
**Middle**  
**BI in BI Challenge**  
**Fixture**  
**BI 3 (on floor)**  
**Low**





# Chlorine Dioxide Generator Configuration





# *PPM-Hrs Explanation*

## Standard CD Cycle is

RH - 65% with 5 minutes of condition time

CD Concentration - 1mg/L

CD Exposure time – 2 hrs

## PPM calculation for 1mg/L

PPM = (mg/M<sup>3</sup>) (24.45) / Molecular Weight

PPM = (mg/L) (1000) (24.45) / Molecular Weight

CD ppm = (1.0mg/L) (1000L/M<sup>3</sup>) (24.45) / 67.5

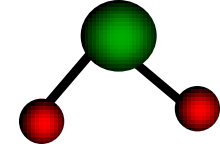
CD ppm = 362.2

## Exposure Contact Time (CT)

Exposure CT = 362ppm \* 2 hrs

Exposure CT = 724 ppm-hrs

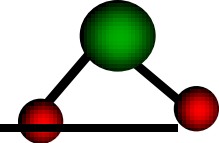
24.45 = volume (liters) of a mole (gram molecular weight) of a gas at 1 atmosphere and at 25 C.



# *Standard Runs – 65 % RH, 720 ppm-hrs*

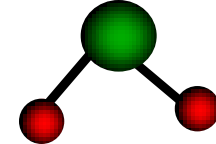
| ppm-hrs | mg/L | RH | Condition Time | Results                          |
|---------|------|----|----------------|----------------------------------|
| 720     | 1    | 65 | 5              | 0/3, 0/3, 0/3<br>(0/1, 0/1, 0/1) |
| 720     | 5    | 65 | 30             | 0/3, 0/3, 0/3<br>(0/1, 0/1, 0/1) |
| 720     | 10   | 65 | 30             | 0/3, 0/3, 1/3<br>(0/1, 0/1, 0,1) |
| 720     | 20   | 65 | 30             | 0/3, 0/3, 0/3<br>(0/1, 0/1, 0/1) |

***NOTE: 720 ppm-hrs is the standard CD cycle regardless of concentration  
Results in parentheses are from BI Challenge Fixture***



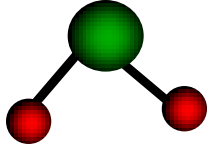
| ppm-hrs | mg/L | RH | Condition Time | Results  |
|---------|------|----|----------------|--|
| 450     | 1    | 65 | 5              | 0/3, 3/3, 3/3, 2/3 (N/A)                                       |
| 550     | 1    | 65 | 5              | 0/3, 1/3, 0/3, 2/3, 0/3, 0/3<br>(0/1, 0/1, 0/1, 1/1, 1/1, 0/1) |
| 600     | 1    | 65 | 5              | 1/3, 0/3 (0/1, 0/1)  |
| 550     | 1    | 65 | 30             | 0/3, 0/3, 1/3, 3/3, 3/3<br>(0/1, 0/1, 1/1, 1/1, 0/1)           |
| 600     | 1    | 65 | 30             | 1/3, 0/3, 0/3, 1/3<br>(0/1, 1/1, 0/1, 1/1)                     |
| 720     | 1    | 65 | 5              | 0/3, 0/3, 0/3 (0/1, 0/1, 0/1)                                  |

**NOTE: 720 ppm-hrs is the lower level for 6 SLR at 65% RH**  
**Results in parentheses are from 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)   |

**NOTE:** 1000 ppm-hrs is the lower level for 6 SLR at 55% RH  
 Results in parentheses are from BI Challenge Fixture

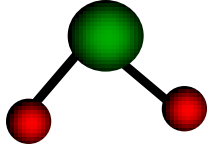


# Results - 45 % RH

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

**NOTE: 1550 ppm-hrs is the lower level for 6 SLR at 45% RH**  
**Results in parentheses are from BI Challenge Fixture**





# ***MRSA and VRE Data***

## **Standard Sporicidal Cycle Rooms**

**RH - 65% with 5 minutes of condition time**

**CD gas concentration of 1.0 mg/L**

**Exposure - 120 minutes**

**Overall exposure - 720 ppm-hrs**

**demonstrates a 6 log reduction**

## ***Staphylococcus aureus (MRSA) and Enterococcus faecium (VRE)***

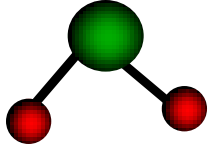
**RH - 65% with 30 minutes of condition time**

**CD gas concentration of 0.2 mg/L**

**Exposure - 45 minutes**

**Overall exposure - 54 ppm-hrs**

**demonstrates a 5 log reduction**



# *Salmonella Data*

## *Standard Sporocidal Cycle Rooms*

**RH - 65% with 5 minutes of condition time**

**CD gas concentration of 1.0 mg/L**

**Exposure - 120 minutes**

**Overall exposure - 720 ppm-hrs**

**demonstrates a 6 log reduction**

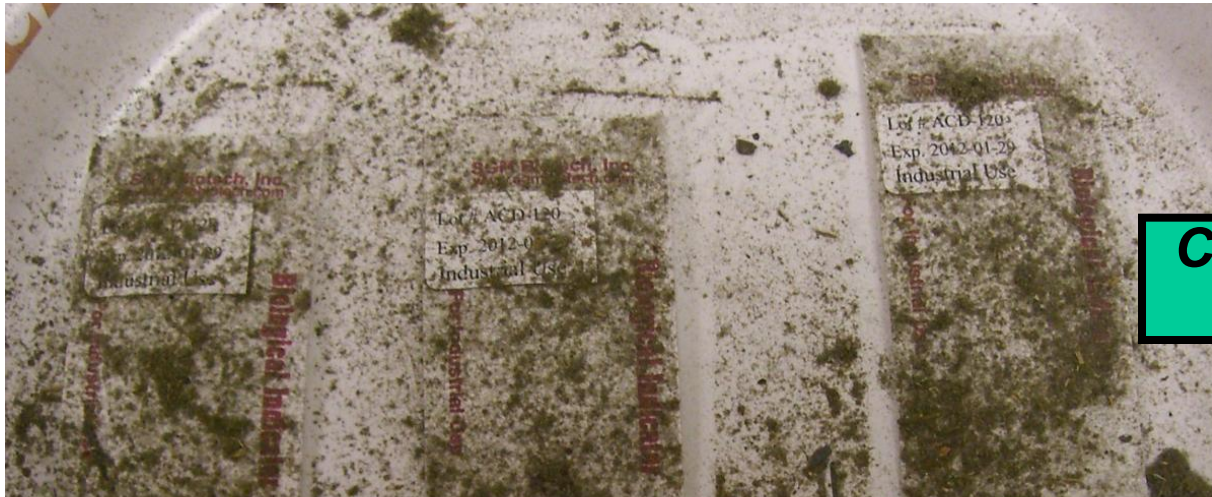
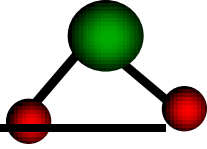
## *Salmonella typhimurium (ATCC 14028)*

**RH - 65% with 30 minutes of condition time**

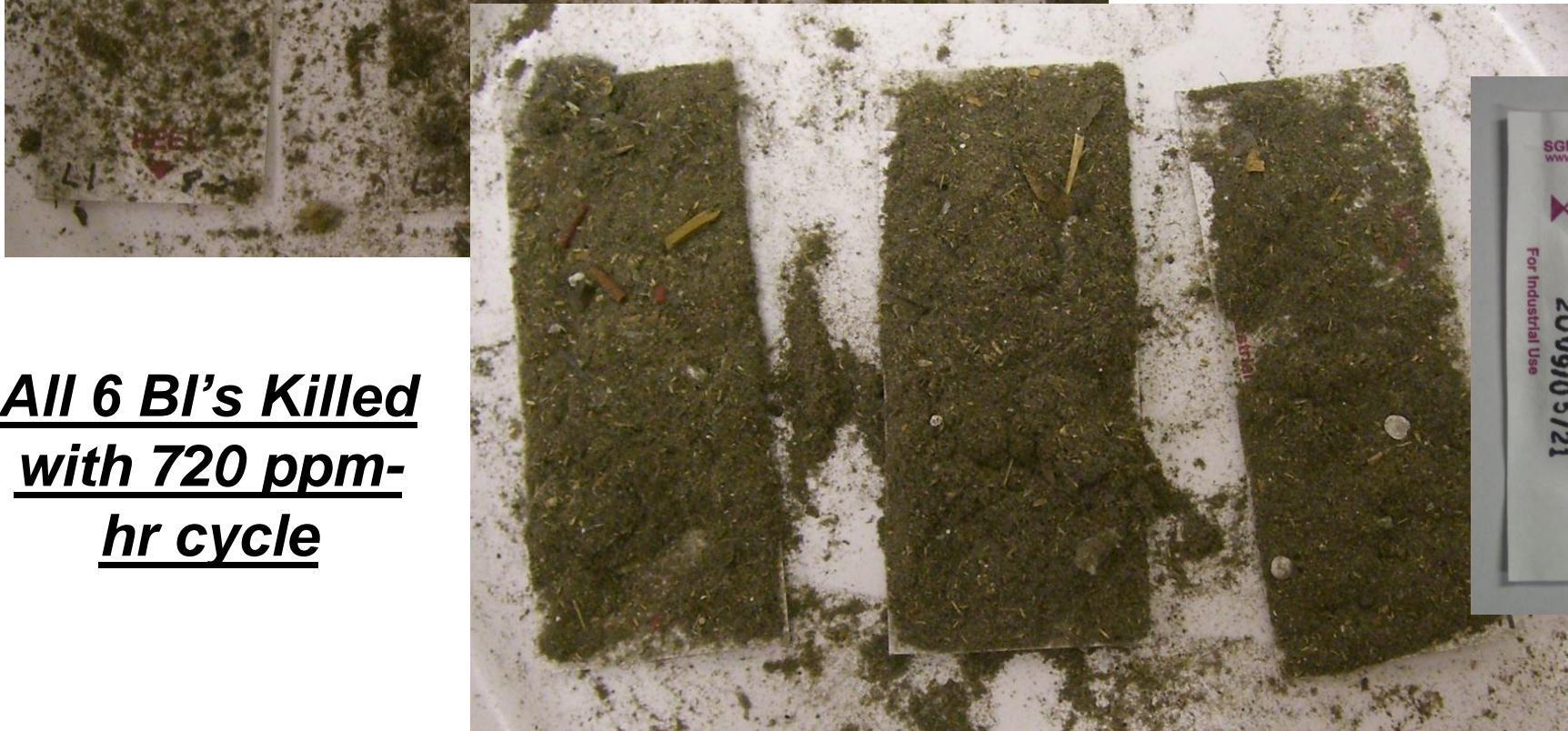
**CD gas concentration of 0.3 mg/L**

**100 ppm-hrs had a 6.999 log reduction**

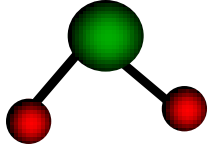
**200 ppm-hrs had a 7.01 log reduction**



**CD Gas penetration into Organic Load**



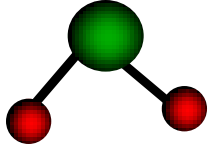
**All 6 BI's Killed**  
**with 720 ppm-**  
**hr cycle**



# *Conclusions*

---

- **Does RH affect spore log reductions (SLR)??**
  - **YES** (previous references Agalloco, 2008; Westphal, 2003; Whitney, 2003)
- **Does concentration-time affect spore log reductions??**
  - **YES**
  - **720 PPM-Hrs is good for 6 SLR at 65% RH**
  - **1000 PPM-Hrs is good for SLR at 55% RH**
  - **1550 PPM-Hrs is good for 6 SLR at 45% RH**



---

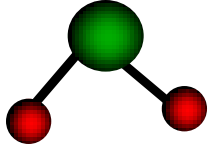
**Prepared by:**

**Mark A. Czarneski  
Director of Technology  
ClorDiSys Solutions, Inc  
[www.clordisys.com](http://www.clordisys.com)**

**PO Box 549  
Lebanon, NJ 08833  
Phone: 908-236-4100  
Fax: 908-236-2222**

**e-mail: [markczarneski@cloridsys.com](mailto:markczarneski@cloridsys.com)**

---



## References:

- Agalloco, J., Carleton, P., & Frederick, J. (2008). *Validation of pharmaceutical processes* (3rd ed., p. 283). New York: Informa Healthcare USA Inc.
- Westphal, A. J., Price, P. B., Leighton, T. J., & Wheeler, K. E. (2003). Kinetics of size changes of individual *Bacillus thuringiensis* spores in response to changes in relative humidity. *Proceedings of the National Academy of Sciences*, 100(6), 3461-3466.
- Whitney, E. A. S., Beatty, M. E., Taylor, Jr., T. H., Weyant, R., Sobel, J., Arduino, M. J., et al. (2003). Inactivation of *Bacillus anthracis* spores. *Emerging Infectious Diseases*, 9(6), 623-627.

## Acknowledgements:

**Chris Ballard ClorDiSys Solutions, Inc.**  
**Dan Paznek, ClorDiSys Solutions, Inc.**  
**Tyler Mattson, ClorDiSys Solutions, Inc.**