



“The Chlorine Dioxide People”

Application Note:

Low RH Chlorine Dioxide Gas Treatment of *Salmonella*

Bacteria, and *Salmonella* in particular, cannot grow in low RH environments, but can survive extremely well. This is a particular problem for contamination control in any type of dry powder facility as most sterilization agents require a humidification step. Without humidity most of these agents are not well suited to provide an acceptable level of kill. Since ClorDiSys’ proprietary chlorine dioxide gas is a dry fumigation method normally requiring humidity in the range of 65-75%, ClorDiSys investigated whether or not the gas can be efficacious at extremely low RH (25%) levels.

To test this, ClorDiSys performed tests on inoculated glass slides held at 25% RH and processing them at different dosages (720, 1000, 3000, 5000, and 10,000 ppm-hrs).

Objectives: Calculate the log reduction of *Salmonella typhimurium* (ATCC# 14028) at different dosages and low RH.

Method: Inoculated glass slides were used to test the efficacy of chlorine dioxide gas at different dosages. Chlorine dioxide gas dosages will range from 720 ppm-hrs up to 10,000 ppm-hrs. Glass slides will be taken out at different time intervals corresponding to the dosages.

Salmonella preparation: A *Salmonella* suspension of 4×10^7 cfu/m was first prepared. Glass slides were prepared in triplicate for testing by inoculating each with 100 μ L of the prepared *Salmonella*. Upon gas completion, each slide was placed into a separate 100 mL bottle of sterile phosphate buffer solution. 10 mL portions from each bottle/interval were filtered through a membrane, placed on TSA w/ 5% Sheep’s Blood agar and incubated at 35°C. After incubation, the plates were examined for growth of *Salmonella*, and any colonies present were counted.

Results: Chlorine dioxide gas was shown to be effective at delivering a 6-log reduction of salmonella at 25% RH at all dosages tested. See Table 1 for results.

Target ppm-hrs	Condition % RH	Positive Controls	Biological Indicator Results (positive / total)
720	25	3.2×10^7	<10 CFU*
1,000	25	4.3×10^7	<10 CFU
3,000	25	4.0×10^7	<10 CFU
5,000	25	3.3×10^7	<10 CFU
10,000	25	2.0×10^7	<10 CFU

Table 1: Experimental Results (*No CFU’s were detected.)

Conclusion: Chlorine dioxide gas is efficacious against *Salmonella typhimurium* (ATCC# 14028) in a dry environment (RH level of 25%) over a wide range of dosages.

Outcomes: Applying these findings to applications would allow for log reductions of *Salmonella typhimurium* (ATCC# 14028) without the introduction of humidity as long as the RH was at least 25%.

Equipment Used

- 1 Minidox-M CD Gas Generator with Control by PPM-Hours
- 17 ft³ (0.5m³) Isolator
- Glass slides inoculated with *Salmonella typhimurium* (ATCC 14028)
- BSC Scrubber (remove CD gas)

Background Dosage / PPM-Hour Explanation

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

$$\begin{aligned} & \text{PPM calculation for 1mg/L} \\ & \text{PPM} = (\text{mg/M}^3) (24.45) / \text{Molecular Weight} \\ & \text{PPM} = (\text{mg/L}) (1000) (24.45) / \text{Molecular Weight} \\ & \text{CD ppm} = (1.0\text{mg/L}) (1000\text{L/M}^3) (24.45) / 67.5 \\ & \text{CD ppm} = 362.2 \end{aligned}$$

Exposure Contact Time (CT)

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

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

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

Discussion

During large facility decontamination, especially in dry powder facilities, the addition of humidity is not allowed or strongly discouraged due to the possibility of bacterial growth. When a situation like this comes up a cycle targeting 6 log reduction on bacteria such as Salmonella can still be accomplished at these low RH levels.

Experiments performed at various chlorine dioxide gas concentrations (720, 1000, 3000, 5000, 10000 ppm-hours) of inoculated glass slides with Salmonella typhimurium (ATCC# 14028) all exhibited complete decontamination. A dosage of 720 was sufficient to get a 6 log kill at all tested dosages at an RH level of 25%.