

Memorandum

To: Laura, Darrel
CC:
From: Chip Bettle
Date: 3/25/2007
Re: Progress Report #2 – Experiment 4

On March 13/14, 2007, a series of experiments (Experiment 4) were run in the Orlando Shop to determine whether or not the Vapex fog could kill MRSA-inoculated stainless steel coupons.

Previous experiments with direct impingement of Vapex fog on similarly inoculated SS plates showed that at least one hour of exposure was needed and that fog condensate was an ineffective kill agent. We also showed that a high ozone, medium droplet fog took an hour to achieve kill.

In Experiment 4, we showed that:

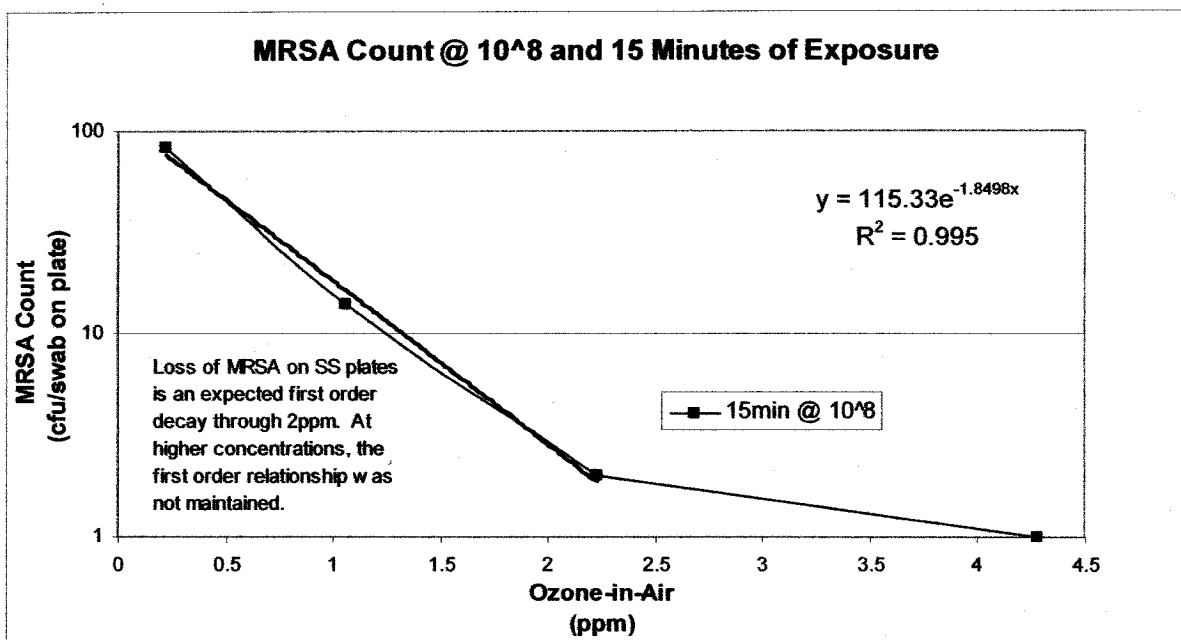
1. Condensate-free fog killed MRSA in 15 minutes
2. The best conditions for kill appear to be 1ppm ozone-in-air for 30 minutes @1ppm plus the natural decay.
3. There is an unexpected observation after the fog is turned off.
 - a. Ozone-in-air drops exponentially, and then stabilizes; relative humidity takes a step function drop then increases when the ozone stabilizes. Thereafter, both decline as expected.
 - b. There is likely a common cause for this since it happened at both 1ppm and 2ppm.
4. 1ppm ozone-in-air is a more stable process than ozone-without-fog and more stable than higher ozone-in-fog set points as illustrated by the table showing the standard deviation of the observations.
5. The attitude of the plates (up, down sideways) had no impact on the results.

The first table shows all the micro results. The shaded area is samples where no counts were observed. [TNTC is short hand for To Numerous To Count.] One can see by observation that results improve at lower inoculation doses.

Vapex Experiment 4 Day One
 Experiment Date: March 14, 2007
 Results Date: March 17, 2007

Time at Set Point	MRSA Concentration	Treatment		0.2 ppm		1.0 ppm		2.0 ppm		4.0 ppm		Ozone Only 2.0 ppm	
		A	B	A	B	A	B	A	B	A	B	A	B
15 Minutes	1.68 X 10 ⁸ cfu/ml	TNTC	TNTC	25	58	9	5	2	<1	<1	1	4	37
30 Minutes	1.68 X 10 ⁸ cfu/ml	TNTC	TNTC	3	15	<1	<1	3	4	<1	<1	<1	9
45 Minutes	1.68 X 10 ⁸ cfu/ml	TNTC	TNTC	8	2	4	<1	<1	<1	<1	<1	6	6
60 Minutes	1.68 X 10 ⁸ cfu/ml	TNTC	TNTC	28	11	<1	<1	<1	<1	<1	<1	1	<1
15 Minutes	1.68 X 10 ⁶ cfu/ml	184	88	5	3	<1	<1	1	<1	<1	<1	<1	<1
30 Minutes	1.68 X 10 ⁶ cfu/ml	139	73	4	6	<1	<1	<1	1	<1	<1	3	<1
45 Minutes	1.68 X 10 ⁶ cfu/ml	137	112	<1	<1	<1	<1	<1	<1	<1	<1	<1	2
60 Minutes	1.68 X 10 ⁶ cfu/ml	141	76	1	1	<1	<1	<1	<1	<1	<1	<1	<1
15 Minutes	1.68 X 10 ⁴ cfu/ml	4	3	1	17	<1	<1	<1	<1	<1	<1	<1	<1
30 Minutes	1.68 X 10 ⁴ cfu/ml	2	1	1	<1	<1	<1	<1	<1	1	<1	<1	<1
45 Minutes	1.68 X 10 ⁴ cfu/ml	1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
60 Minutes	1.68 X 10 ⁴ cfu/ml	3	3	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
15 Minutes	1.68 X 10 ² cfu/ml	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
30 Minutes	1.68 X 10 ² cfu/ml	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
45 Minutes	1.68 X 10 ² cfu/ml	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
60 Minutes	1.68 X 10 ² cfu/ml	3	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1

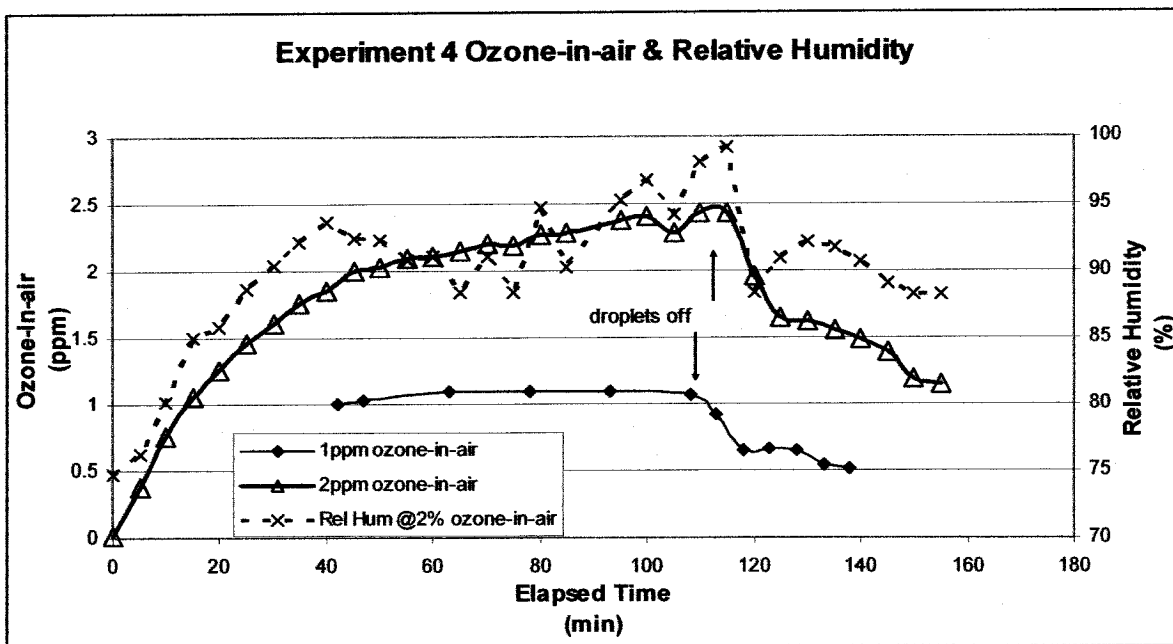
The 10⁸ data set were analyzed quantitatively. The counts showed a first order decay (expected) at lower doses. At 4ppm, this relationship did not continue.



An unexpected observation was made as the fog dissipated once turned off.

Referring to the embedded chart, relative humidity increased once the fogging started (2ppm test). Ozone-in-air increased slightly during the 60minute steady state test period and relative humidity increased. This is an expected result. The fogging was stopped at the end of the test period. Ozone-in-air declined exponentially (also expected) and then plateaued for 10 minutes (1ppm) before declining slowly.

When this plateau-effect occurred, the relative humidity jumped up. This does not appear to be a random event and needs explanation.



The data was analyzed during the steady state phase of the experiment. 1ppm was reasonably stable. At higher doses, there was more variation (look at the index number). For example, ozone-only had 5½ times more variation than 1ppm.

Warehouse Operating Results

Set Point	.2ppm	1.0ppm	2.0ppm	4.0ppm	ozone only
Actual Average	0.23	1.06	2.23	4.27	2.07
st dev	0.02	0.04	0.12	0.26	0.24
index to 1.0ppm	38	100	265	604	548

At 2ppm ozone-in-air, there was similar kill on plates facing up, down and sideways. The attitude of the surface does not appear to be a variable in MRSA kill on stainless steel surfaces.

Vapex Experiment 4 Day Two
 Experiment Date: March 15, 2007
 Results Date: March 18, 2007

Time at Set Point	MRSA Concentration	Plate Orientation					
		Up (2.0 ppm)		Down (2.0 ppm)		Sideways (2.0 ppm)	
		A	B	A	B	A	B
30 Minutes	1.38×10^8 cfu/ml	1	<1	<1	1	<1	<1
	1.38×10^6 cfu/ml	<1	<1	<1	<1	<1	1
	1.38×10^4 cfu/ml	<1	<1	<1	<1	<1	<1
	1.38×10^2 cfu/ml	<1	<1	<1	<1	<1	<1
60 Minutes	1.38×10^8 cfu/ml	1	1	2	<1	<1	<1
	1.38×10^6 cfu/ml	<1	<1	<1	<1	<1	<1
	1.38×10^4 cfu/ml	<1	<1	<1	<1	1	<1
	1.38×10^2 cfu/ml	<1	<1	<1	<1	<1	<1

Note: All data reported as cfu/swab

We need to determine the size of the fog droplets for the direct impinging test, the "box" experiment and the warehouse fog test. It may be that only the smallest droplets are active. This would be an important finding. We also need to replicate the humidity/ozone-in-air data to determine whether or not these data are real or an artifact. If they are real, we need to know what is happening.

Anecdotally, there was considerable condensate on the warehouse floor. It would be important to know whether a smaller nozzle(s) (e.g. 1cfm nozzle) would be as effective as a bigger nozzle and solve the condensate issue.