Confidential Research Report

Fungicidal Efficacy of SCO Technologies' Ozone Generation System Against *Stachybotrys chartarum*

For

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Purpose

To determine the fungicidal efficacy of Medallion's proprietary ozone generation system against viable *Stachybotrys chartarum* and associated mycotoxins.

Materials

1. *Stachybotrys chartarum* (UAMH 6417)
2. Potato Dextrose Agar (PDA), Kalmar Laboratories
3. Butterfield's Buffer (BFB), Kalmar Laboratories
4. Methanol
5. Test Equipment
    a. ARTI HHPC6 Particle Counter
    b. Ozone meter, provided by Medallion Healthy Homes
    c. SCO Technologies' Ozone Generator System, provided by Medallion Healthy Homes
    d. Humidifier - specifications
6. Dry wall sections, 3 x 1.5 x 0.5"
7. Incubator, 25°C
8. Microbial Test Chamber: Custom built chamber (11 x 9.5 x 8 ') constructed from non-porous, non-reactive construction materials and equipped with negative displacement ventilation and HEPA filtration.

Methods

The challenge organism was cultivated on Potato Dextrose Agar (PDA) at 25°C until confluent growth was obtained (Figure 1). Subsequently, the organism was harvested with a moistened sterile swab and inoculated on to the drywall test section. The inoculated drywall was placed into a sealable plastic bin on top of moistened paper towels and incubated at room temperature until confluent growth was observed (Figures 2 and 3).

On the day of the experiment, the microbial test chamber was placed under negative pressure and purged with HEPA filtered air to ensure no residual particulates were present. The inoculated drywall was positioned vertically inside the chamber with the side containing fungal growth facing outward. Representative 100 cm^2 areas of the drywall were sampled for viable fungi and mycotoxins using a sterile swab pre-moistened with Butterfield's Buffer and methanol, respectively (Figures 4 and 5). Medallion Healthy Homes personnel treated the chamber with ozone for 24 hours. Test chamber conditions were monitored for particulates, humidity, and temperature during the 24 hour treatment interval using ARTI HHPC6 Particle Counter. Once the ozone application was complete and safe ozone concentrations within the chamber were confirmed, the chamber was opened and representative 100 cm^2 areas of the drywall section were sampled in the same manner as the "Pre-Ozone" samples.

All samples were tested for viable fungi and mycotoxins. Swabs that were tested for fungal growth were cultured via the Spiral Biotech Autoplater and incubated for 7 days at 25°C. All analytical procedures met criteria set forth in Aerotech's Standard Operating Procedure for the Preparation of Swab, Bulk, CarpetChek, and Water Samples for Viable

Fungal Analysis, 20-0096.00. Mycotoxin surface samples were analyzed via High Performance Liquid Chromatography (HPLC) with Diode Array Detector (DAD) and Fluorescence Detector (FLD). All analytical procedures met criteria set forth in Aerotech's Standard Operating Procedure for the Determination of Mycotoxins using HPLC with DAD and FLD Detectors, 05-012.00.

Results and Discussion

The results of the test chamber conditions during treatment are shown in the graphs in Appendix A. The average relative humidity was 56%; average temperature was 92° Fahrenheit. Four ozone measurements were collected over the 24 hour period, the average ozone concentration was 14.65 parts per million (ppm).

As shown in Table I, the inoculated drywall section contained 2,700 viable *Stachybotrys chartarum* per square centimeter prior to ozone treatment. Following ozone treatment, the *Stachybotrys chartarum* was non-detectable at a detection limit of 18 colony forming units per square centimeter, indicating a 150 fold reduction (Figure 6).

A phenylspirodrimane mycotoxin was detected in the pre-treatment sample at an estimated concentration of 0.015 micrograms per square centimeter. This mycotoxin was non-detectable at an estimated detection limit of 0.001 micrograms per square centimeter following ozone treatment. Due to the discrete nature of the samples collected and the complex nature of mycotoxins production, it is not possible to definitively ascertain from this data set whether the absence of the mycotoxins in the treated sample is attributable to the ozone or is coincidental. Further testing is indicated.

Wendy Aber
Vice President of Technical Services

Janice Jones
Vice President of Laboratory Operations

References

1. Aerotech Standard Operating procedure for Microbial Test Chamber Operation and Maintenance, 35-003.01
2. Aerotech Standard Operating Procedure for the Determination of Mycotoxins using HPLC With DAD and FLD Detectors, 05-012.00
3. Aerotech Standard Operating Procedure for the Preparation of Swab, Bulk, CarpetChek, and Water Samples for Viable Fungal Analysis, 20-0096.00
4. Aerotech Standard Operating Procedure for the Enumeration and Identification of Fungi in Contact Plates, Swab, Bulk, CarpetChek, and Water Samples, 20-097.02

Table I

Results Showing Effect of Ozone Treatment on *Stachybotrys chartarum* and Associated Mycotoxins

Parameter	Pre Ozone	Post Ozone	Units
Viable Fungi	**2,700**	**<18**	CFU/cm^2
Aflatoxin B1	< 0.0010	< 0.0010	µg/cm^2
Aflatoxin G1	< 0.0010	< 0.0010	µg/cm^2
Citrinin	< 0.0050	< 0.0050	µg/cm^2
Gliotoxin	< 0.025	< 0.025	µg/cm^2
Griseofulvin	< 0.00050	< 0.00050	µg/cm^2
iso-Satratoxin G	< 0.0010	< 0.0010	µg/cm^2
Ochratoxin A	< 0.0025	< 0.0025	µg/cm^2
Ochratoxin B	< 0.0025	< 0.0025	µg/cm^2
Patulin	< 0.0020	< 0.0020	µg/cm^2
Penitrem A	< 0.0050	< 0.0050	µg/cm^2
Roridin A	< 0.0020	< 0.0020	µg/cm^2
Roridin E	< 0.0020	< 0.0020	µg/cm^2
Roridin L-2	< 0.0010	< 0.0010	µg/cm^2
Satratoxin G	< 0.0020	< 0.0020	µg/cm^2
Satratoxin H	< 0.0020	< 0.0020	µg/cm^2
Sterigmatocystin	< 0.00050	< 0.00050	µg/cm^2
Verrucarin A	< 0.00050	< 0.00050	µg/cm^2
Verrucarin J	< 0.0010	< 0.0010	µg/cm^2
Zearalenone	< 0.0010	< 0.0010	µg/cm^2
Phenylspirodrimane Compounds	**0.015***	**<0.001***	µg/cm^2

*Estimated Detection Limits

Figures

Cultivation of Stock Organisms

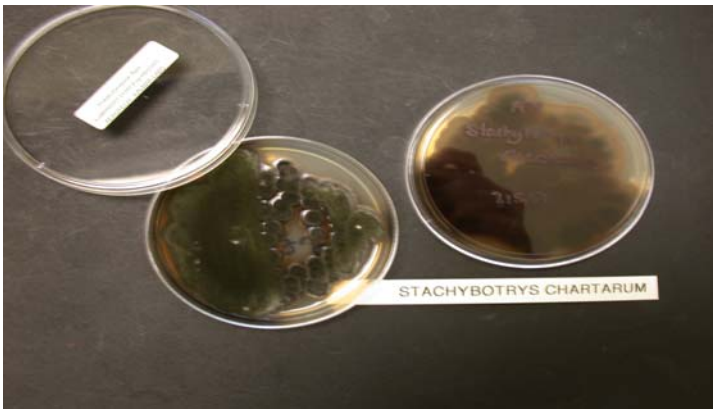


Figure 1. *Stachybotrys chartarum*

Drywall Preparation

Figure 2. Application of harvest cultures onto drywall

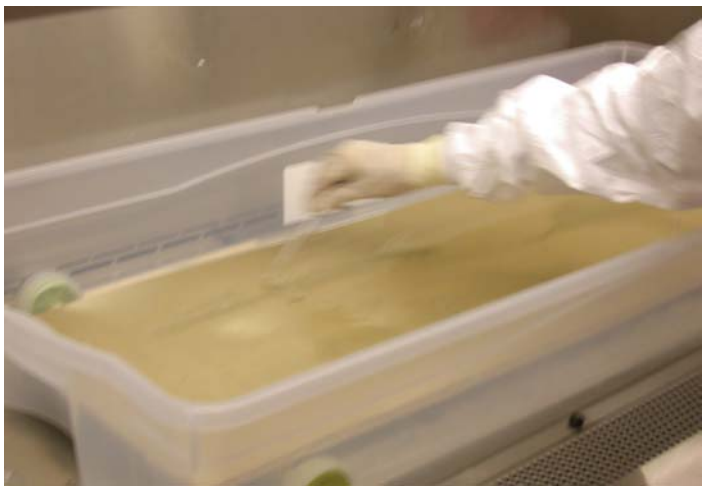


Figure 3. Spreading of cultures to ensure even distribution

VIII. Figures Continued

Sampling

Figure 4. Sampling of viable *Stachybotrys chartarum*

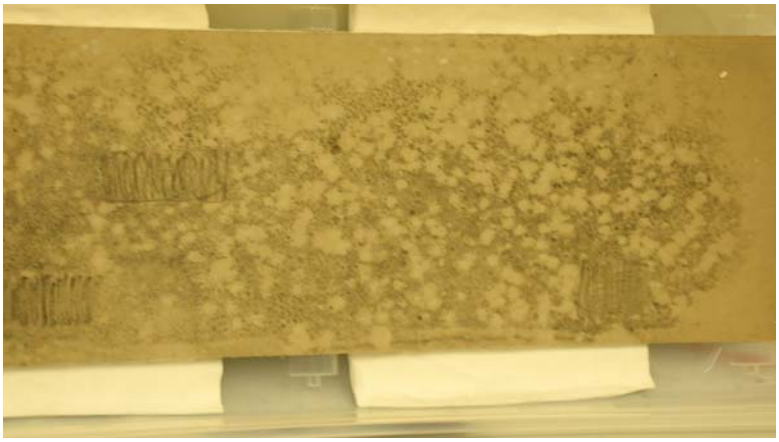


Figure 5. Drywall with *Stachybotrys chartarum* post ozone treatment

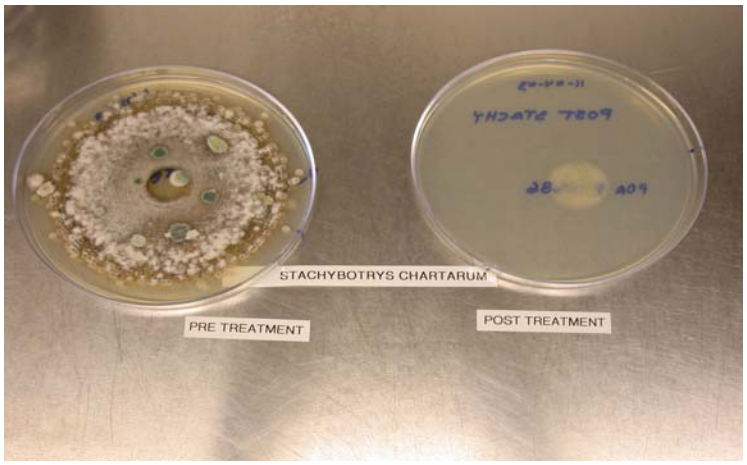
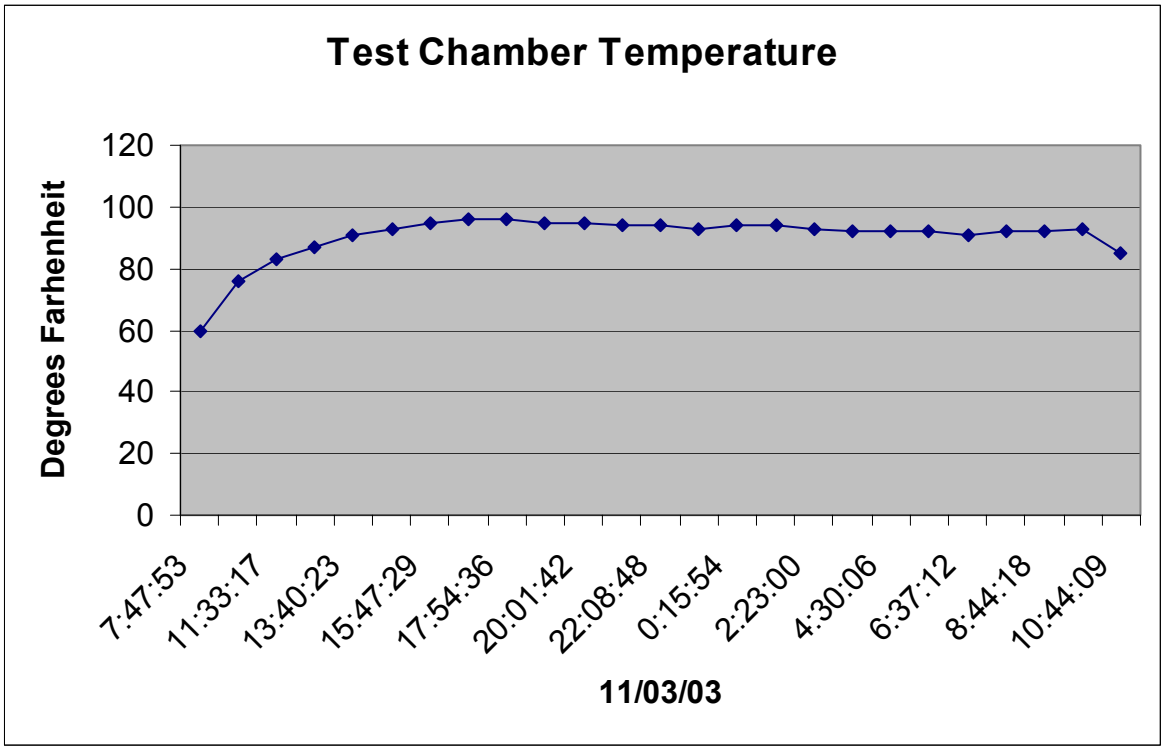
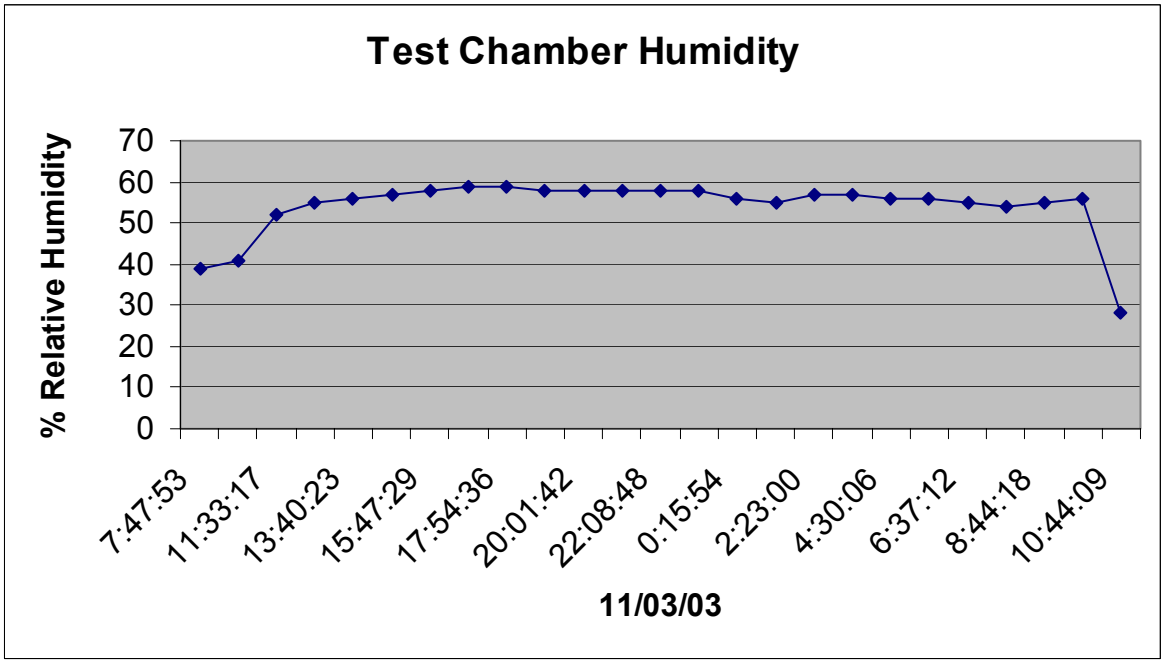


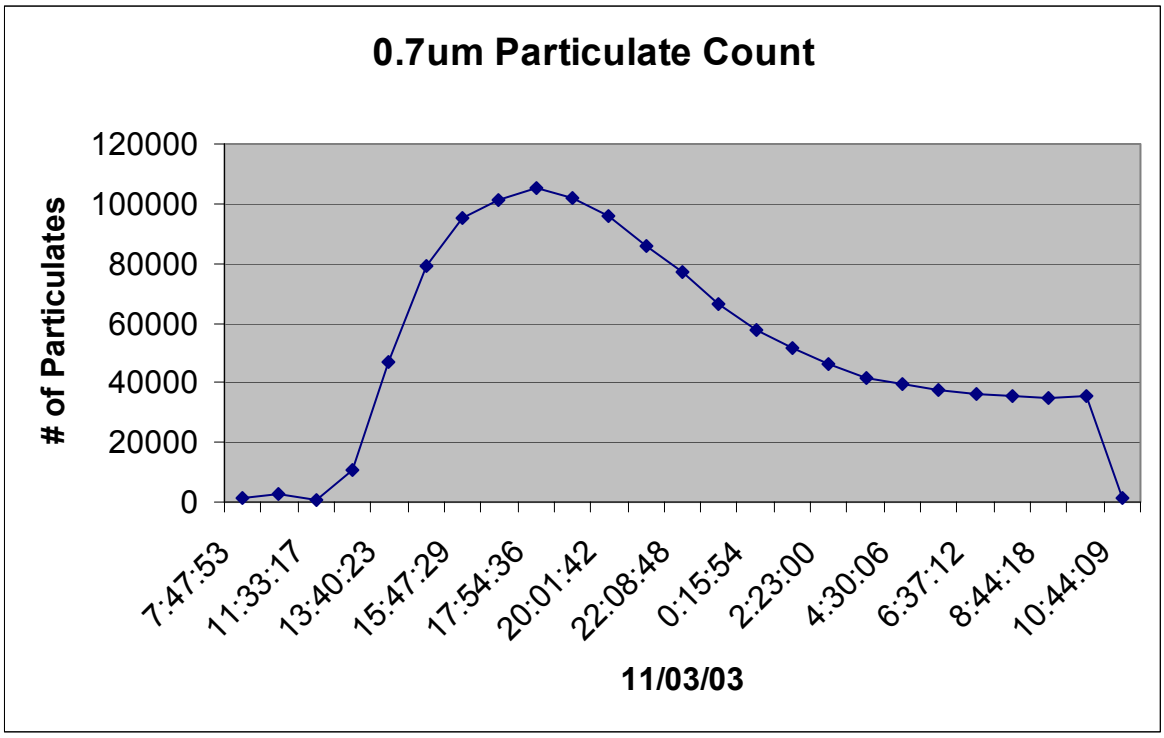
Figure 6. *Stachybotrys chartarum* culture plates, Pre and Post ozone

Appendices

Appendix A: Test Chamber Conditions



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60000
40000
20000
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15:47:29
17:54:36
20:01:42
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0:15:54
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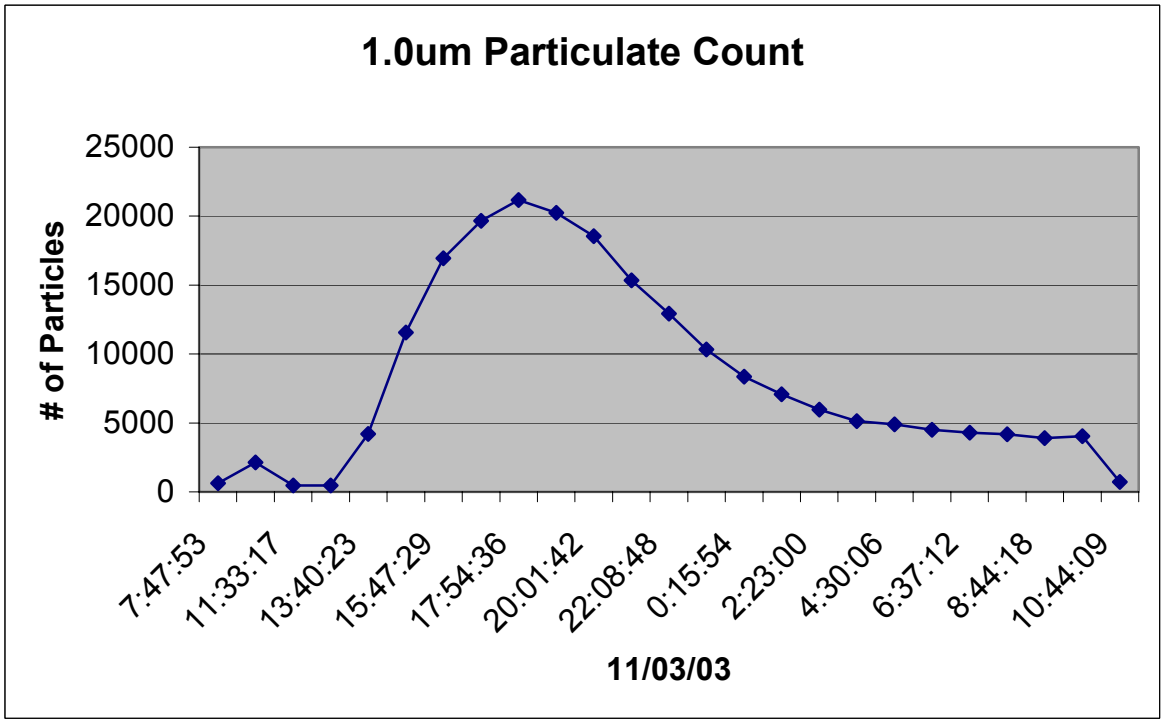
6:37:12
8:44:18
10:44:09
11/03/03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15000
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17:54:36
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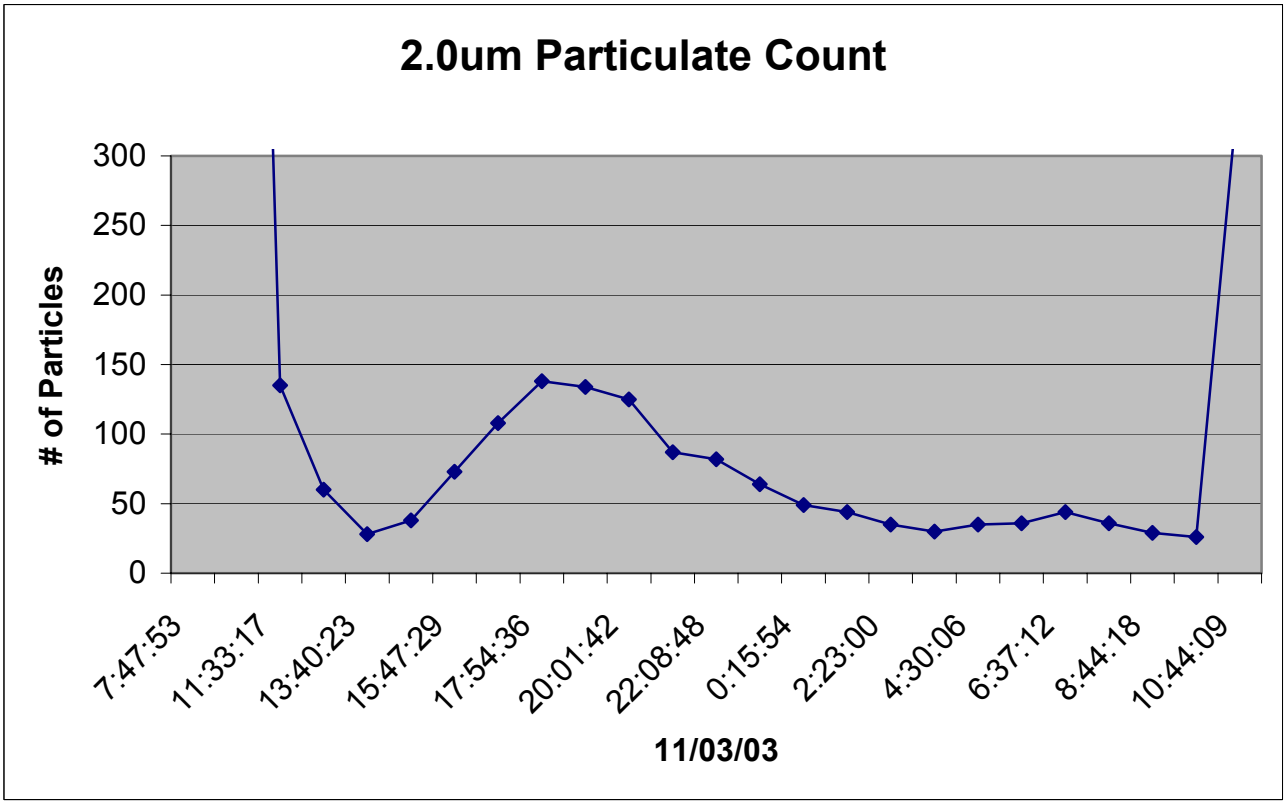
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100
50
0
7:47:53
11:33:17
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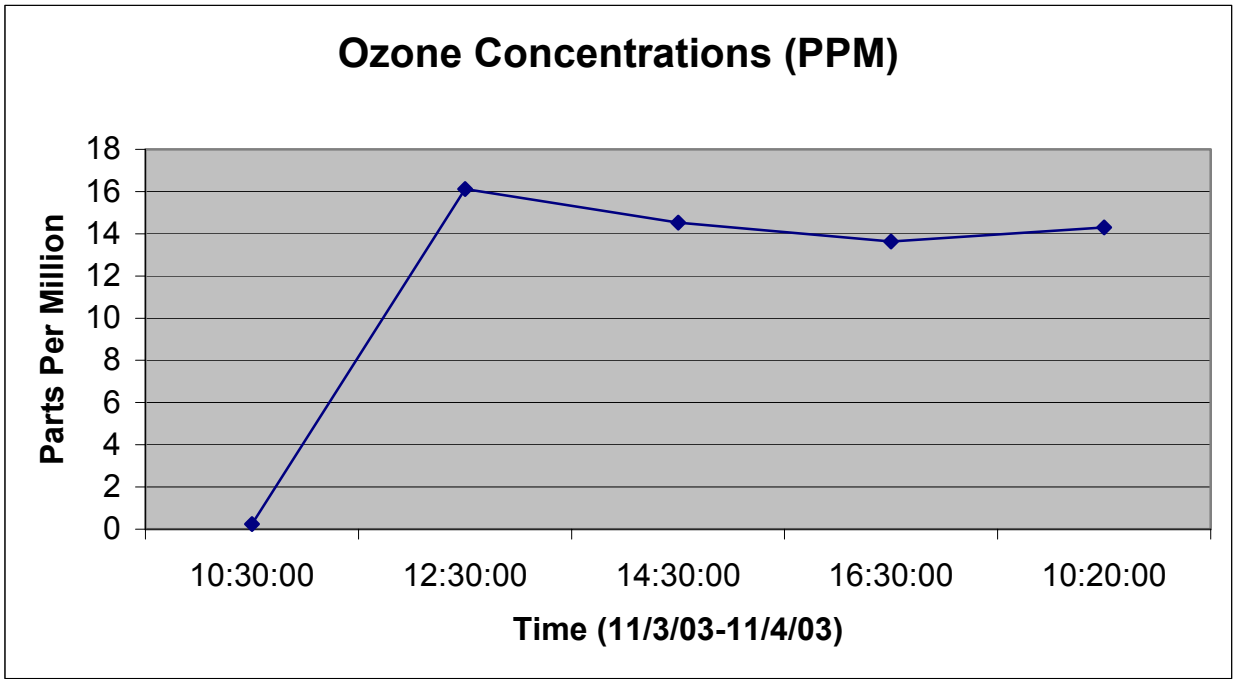
11/03/03


Ozone Concentrations (PPM)
Parts Per Million
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16
14
12
10
8
6
4
2
0
10:30:00
12:30:00
14:30:00
16:30:00
10:20:00
Time (11/3/03-11/4/03)