

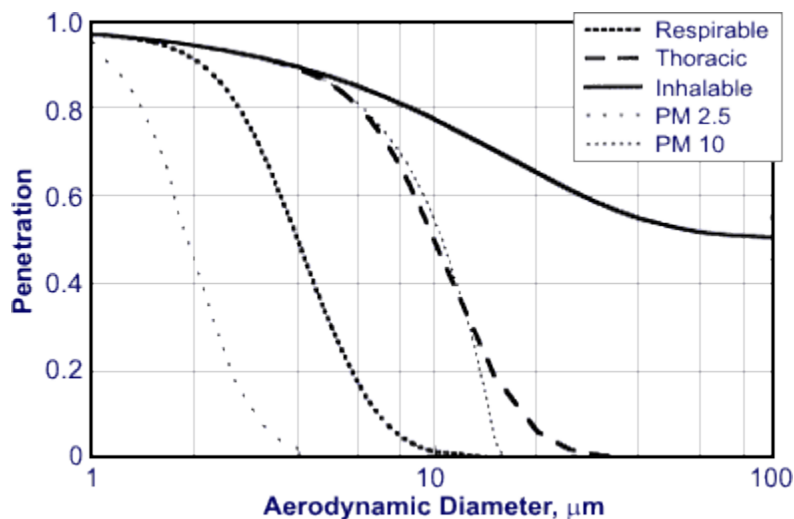
I. Particle Size

Acute Inhalation Injury and Particle Size of Inhaled Substances

The airways and lungs receive continuous first-pass exposure to non-toxic and irritant or toxic gases via inhalation. Smoke, chlorine, phosgene, sulfur dioxide, hydrogen chloride, hydrogen sulfide, nitrogen dioxide, ozone, and ammonia are common irritants. Damage can be widespread due to the gaseous nature of these elements. Acute inhalation injury may result from frequent and widespread inhalation of these elements, which are commonly caused by the use of household cleaning agents and industrial gases including chlorine and ammonia ([Wikipedia](#)).

Inhaled substances may affect the respiratory system at various levels according to various factors, an important factor of which is the particle size of that substance. Bigger particles have enough mass and inertia to be trapped in the airway between the nostril and bronchi when inhaled, while smaller particles are drawn deeper into the lungs. Particularly large particles tend to become trapped in the nose, and are expelled by sneezing or blowing the nose. Therefore, the bigger the particle of the inhaled substance, the less likely they are to cause any damage to the respiratory system ([Airborne dangers, EHS Today, May 1, 1999](#)).

Particle size in inhalation toxicity is usually defined by their mass median aerodynamic diameter (MMAD) and aerodynamic equivalent diameter (AED) in micrometers (μm). According to [Human Health Risk Assessment of Inhaled Materials](#), the term inhalable fraction refers to the mass fraction of particles capable of entering into the respiratory system. Among the inhalable fraction there are three categories, extrathoracic fraction, the thoracic fraction, and the respirable fraction. Particles of $>25\mu\text{m}$ AED generally fall into the extrathoracic fraction, the fraction of the inhalable particles that can deposit in the area of the respiratory tract lying between the nostrils/ mouth and the distal end of the larynx. Particles of $\leq 25\mu\text{m}$ AED fall into the thoracic fraction, fraction of inhalable particles that can penetrate the head airways and enter the airways of the lung. Particles of $\leq 10\mu\text{m}$ AED fall into the respirable fraction, fraction of particles capable of penetrating the respiratory tract to the level of the on-ciliated airways and gas-exchange regions of the lungs. Figure 1 below shows the relationship between the % penetration into the respiratory system vs the particle size of the inhaled particles. Tree pollens have a particle size of 10 – 100 μm and atmospheric dust has a particle size of 0.001 - 40 μm whereas viruses have a particle size of 0.002 – 0.03 μm . The US EPA generally controls substances with a particle size of less than 10 μm . The [SEHSC](#) recommends using 30 μm MMAD (Mass Median Aerodynamic Diameter) with no more than 1% of particles having an AED of $\leq 10\mu\text{m}$ as the cutoff when considering a consumer aerosol application to ensure all aerosol particles to be trapped in the nasopharyngeal region ([Guidance for Aerosol Applications of Silicone-Based Materials](#)). In general, inhaled particles with larger particle size of $>25\mu\text{m}$ are of a less concern because they tend to be trapped in the nasopharyngeal region and be expelled through sneezing and talking, thus less likely to harm the respiratory system.



Background of Aquaox ESS Sprayer and Dispensed Droplet Size

The Aquaox Electrostatic Sprayer features the ES 3001-5 Model. The device is a portable electrostatic aerosol applicator that utilizes a 3-nozzle air-assist design. The device is intended for applications of water-based formulations and is useful for dispensing most chemicals which are labeled aerosol or mist applications.

The device consists mainly of a motor/blower assembly, a high voltage power supply, a charging ring and electrode, a blower housing, a nozzle, a formulation tank and a metering valve. The flow rate of the liquid to be dispensed is regulated by a one-turn precision metering valve and determines the output particle sizes. The particle sizes of the dispensed particles typically range from 10 to 50 μm VMD (Volume Mean Diameter). There are three positions on the metering valve, positions 1, 2, and 3. Each position designates a different flow rate, which results in a different particle size range. Table 1 below shows the approximate flow rate and resulting droplet size of each position setting. Generally, the output droplet size increases with increasing flow rate.

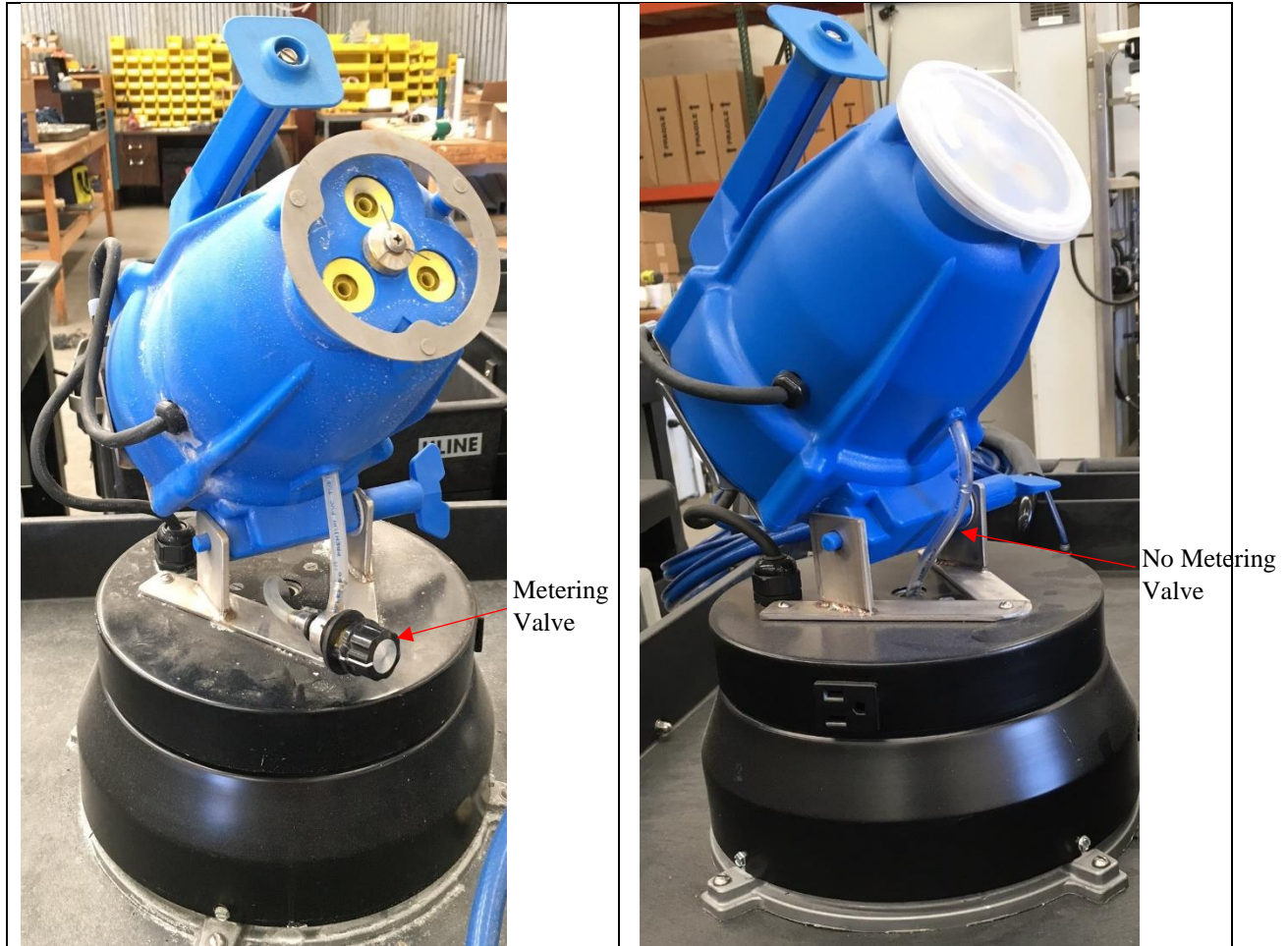
Table 1: Valve Position Settings, Approximate Flow Rates and Droplet Size

Position	Flow Rate	Droplet Size (μm VMD)
1	6 oz/min (177 mL/min)	10 – 20
2	7.5 oz/ min (222 mL/min)	20 – 30
3	9.5 oz/min (281 mL/min)	30 – 50

Aquaox's Modification and Droplet Size of the Aquaox ESS

The metering valve mentioned above functions as an obstruction to the liquid being delivered to the device, and thus regulates the liquid flow rate. Aquaox has removed this metering valve and the formulation tank as part of the customization of this device. The removal of the metering valve results in no obstruction of the liquid flow into the device, thus the liquid is delivered to the device with a flow rate of above 9.5 oz/min. According to Table 1 above, the flow rate of 9.5 oz/min correlates to a droplet size range of 30 – 50 μm VMD. Since increasing flow rate associates with increasing particle size, the particle size will definitely be larger than 30 μm VMD as a result of the removal of the metering valve.

Original Spray Head with Metering Valve	Modified Spray Head with No Metering Valve



Aquaox ESS and Effect of Sprayed Droplets on Respiratory System

The dispensed particles of the Aquaox Electrostatic Sprayer should not cause any harm to the respiratory system due to the following reasons, 1) the output particle size, 2) falling time of particles, and 3) electrostatic ion field. These rationales are further explained below.

The output droplet size of the Aquaox ESS should always be larger than 30 μm VMD due to the modification of the device. As explained in the previous page, inhaled substances with a particle size of larger than 30 μm tend to be trapped in the nasopharyngeal region and thus expelled through sneezing, thus less likely to harm the respiratory system, according to previous research literatures. Therefore, the droplet size of the Aquaox ESS should be large enough not to cause any respiratory issues.

Secondly, according to the WHO Pesticide Evaluation Scheme (Table 2 below), the time it takes for a droplet size ranging from 20 – 50 μm VMD to fall 10 meters ranges from 14 minutes to 135 seconds. Aquaox implements a 10-minute dwelling time before reentry into the sprayed area as part of Aquaox instructions in the operation of the sprayer. Therefore, by the time one reenters the sprayed area, most, if not all, particles should have fallen and deposited on the floor, and thus not likely to be inhaled.

Table 2: Time required for a droplet to fall 10 meters (WHO Pesticide Evaluation Scheme)

Droplet Size (μm VMD)	Time to fall 10 meters	Droplet Density (no/cm^3)
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1	93.7 hours	19120.0
5	3.7 hours	152.0
10	56 minutes	19.2
20	14 minutes	2.38
50	135 seconds	0.150
100	36 seconds	0.0192

Last but not least, the device features “electrostatic ion field,” which the dispensed droplets pass through as they exit the nozzles. This results in electrostatically charged droplets which gravitate to neutral objects to form a uniform coverage. Particles of the smaller particle size range will be attracted to the nearby surfaces as a result of the electrostatic charge. Thus, upon completion of the dwelling time, larger particles will have deposited onto the floor due to gravity while smaller particles will be attracted to nearby surfaces due to electrostatic charge. This results in very few, if not none, air particles floating in the air and to be inhaled when someone reenters the room after the dwelling time.

In conclusion, the output particle size of the Aquaox ESS should be above 30 μm VMD, which should be big enough to be trapped in the nasopharyngeal region and not likely to harm the respiratory system. Furthermore, large particles should have deposited onto the floor due to gravity and small particles should have attached onto nearby surfaces due to electrostatic charge upon collapse of the dwell time, which results in theoretically no particles floating in the air and being inhaled by someone reentering the sprayed area. All the above rationales support that the Aquaox ESS should not cause any harm to the respiratory system if used following our protocol.

Not vaporizer/ mister / fogger

II. Chlorine Exposure Limits

OSHA Standards on Chlorine Exposure Limits

The solutions that the Aquaox ESS dispenses include Aquaox Disinfectant 275 (AX275) and Aquaox Disinfectant 525 (AX525). The former solution contains 275 ppm Hypochlorous Acid (HOCl) while the latter contains 525 ppm HOCl as active ingredient. OSHA has not yet implemented a standard regulating HOCl exposure limits nor a method for determining HOCl concentration. Therefore, the standards for Chlorine have been adopted when concerning the safety of sprayed particles of the Aquaox ESS. Current OSHA permissible exposure limits (PEL) for Chlorine include a short-term exposure limit for up to a 15-minute exposure not to exceed 1 ppm (2.9 mg/m^3 where mg/m^3 is defined as mg Chlorine per m^3 of air), and a time-weighted average for up to 8 hours not to exceed 0.5 ppm (1.5 mg/m^3). Two experiments have been conducted internally to verify that the Aquaox ESS complies with the above required limits.

Experiment 1: Assessment of HOCl Concentration in Air Samples following NIOSH 7607 Method

The HOCl concentration in air samples in a sprayed area is assessed via the NIOSH 7607 Method. The Aquaox ESS is operated following the Aquaox ICS protocol in a 12ft x 18ft experimental room that has been constructed to mimic an average patient room in a hospital. Air samples are collected from the experimental room after a 5-minute spraying time and a 10-minute dwelling time. Upon completion of the dwelling time, air samples are collected for a period of 15-minutes (for determining the short term exposure level) and a period of 6 hours (for determining the time-weighted average level). Air sample is pumped into a pre-coated sample collection tube via a calibrated AirChek sample pump at a rate of 1 L/minute. Samples are collected internally at Aquaox and sent out to ALS Environmental at Salt Lake City for analysis.

The sample collection tubes are prepared and the analysis is done following the NIOSH 7607 method. The sample collection tube is a tube of silica gel coated with sulfamic acid and potassium iodide. The collection tubes do not contain the filter cassettes because we do not intend to analyze trichloramines in our samples. The treated silica gel is nonspecific and traps soluble chlorine compounds including mono- and dichloramines, hypochlorous acid, hypochlorites, and chlorine. The reaction of these chlorine compounds with potassium iodide in an acid medium yields chloride ion, which are then analyzed. Mobile Phase Ion Chromatography with suppressed conductivity detection is the technique used in the analysis. The analysis results should only capture hypochlorous acid, hypochlorites, and chlorine because there are no nitrogenous compounds in our samples to form any chloramines.

Test Result:

Specifications of Test Solutions:

1st Trial:

Air Sample Collection Date: October 5, 2015 (AX275)
 October 6, 2015 (AX525)

Specifications of Test Solutions:

	Aquaox Disinfectant 275	Aquaox Disinfectant 525
FAC (ppm)	300	546
pH	6.79	6.58
ORP	832	873
Conductivity	2323	3250

2nd Trial:

Air Sample Collection Date: October 8, 2015 (AX275)
 October 9, 2015 (AX525)

	Aquaox Disinfectant 275	Aquaox Disinfectant 525
FAC (ppm)	281	556
pH	6.78	6.52
ORP	842	877
Conductivity	2405	4832

Interpretation:

Experiment 2: Assessment of HOCl Concentration in Air Samples following the OSHA ID-101 Method

The above experiment is repeated internally following the OSHA ID-101 method and only short-term exposure is determined. The Aquaox ESS is operated following the Aquaox ICS protocol in a 12in x 18in experimental room as described above. Air samples are analyzed from the experimental room after a 5-minute spraying time and a 10-minute dwelling time. Upon completion of the dwelling time, air samples are collected for a period of 15-minutes for determining the short term exposure level. Air sample is pumped into a midjet fritted glass bubbler containing 0.1% sulfamic acid solution using a calibrated AirChek sample pump at a rate of 1 L/minute. The collected sample then reacts with DPD (N,N-diethyl-p-phenylenediamine) in the presence of potassium iodide to yield a red-colored product that absorbs at a wavelength of 540nm. The concentration of HOCl in the original air sample can be determined by determining the absorbance at 540nm.

The experiment is done as described below. A standard solution with a known HOCl concentration is first generated. The absorbances of different volumes of this standard solution will then be determined and that

will correspond to the different HOCl concentrations at different volumes of standard solutions (**Graphs 1 and 2**). The absorbances of different weights of the standard solutions will then be plotted against the corresponding HOCl concentrations (**Graph 3**) to generate a standard curve. The HOCl concentration of the test sample can be determined by comparing the absorbance of the test sample to the standard curve.

Test Result:

Air Sample Collection Date: October 7, 2015 (AX275)
 October 7, 2015 (AX525)

Specifications of Test Spray Solutions:

	Aquaox Disinfectant 275	Aquaox Disinfectant 525
FAC (ppm)	287	525
pH	6.82 / 25.7C	6.57 / 25.7C
ORP	852	878
Conductivity	2420	3162

Specifications of Standard Solution:

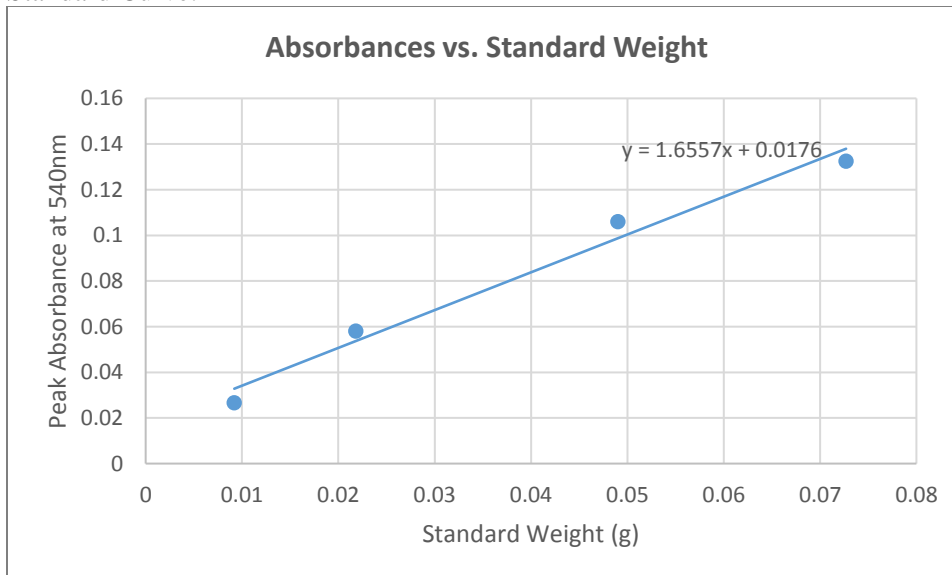
FAC (ppm)	295
pH	6.90 / 25.7C

Aquaox Disinfectant 275:

Absorbances of Standard and Collected Sample:

		Peak Absorbance at 540nm
295ppm Standard	0.0092g (10uL)	0.0266
	0.0218g (25uL)	0.0580
	0.0490g (50uL)	0.1060
	0.0727g (75uL)	0.1325
Collected Sample		0.0550

Standard Curve:



Calculations:

Absorbance of the Collected Sample:	0.0550
Corresponding Standard Weight: (determined using the linear equation $y = 1.6557x + 0.0176$)	0.0226g
HOCl Concentration of Standard:	295ppm
Corresponding Weight of HOCl in Air Sample:	$0.0226g \times 295ppm = 6.667ug$
Volume of Air Sample Collected:	15L
Molecular Weight of HOCl:	52.46 g/mol
* HOCl Concentration of Air Sample, mg/m ³ :	0.444mg/m³
* HOCl Concentration of Air Sample, ppm:	0.207ppm

* The formulae for direct comparison with OSHA PEL for gas and aerosol is used according to NIOSH Manual of Analytical Methods as explained below.

<u>Physical Form of Substance Sampled</u>	<u>Unit of Air Concentration</u>	<u>Formula for Direct Comparison With OSHA PEL Table</u>
Gas	ppm	$C_v = \frac{m \cdot 10^3}{V} \cdot \frac{24.46}{MW}$
Gas	mg/m ³	$C = \frac{m \cdot 10^3}{V}$
Aerosol	mg/m ³	$C = \frac{m \cdot 10^3}{V}$

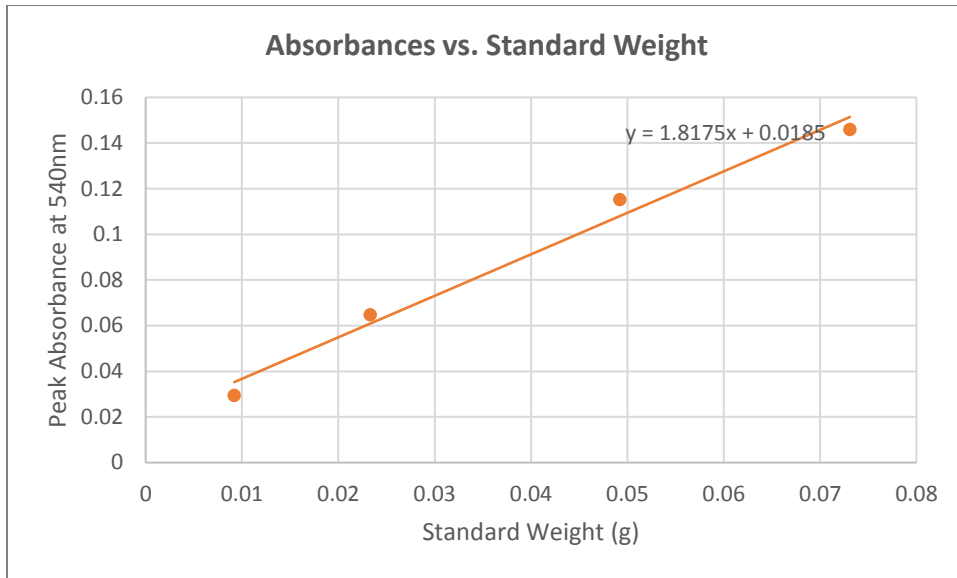
where: m = actual mass of substance, in mg, found on the sampling device
V = air volume, L, taken at the sampling site, ambient temperature and pressure
24.46 = the volume (L) of 1 mole of gas at 25 °C and 760 mm Hg
C_v = air concentration, ppm by volume, at 25 °C and 760 mm Hg
C = air concentration, mg/m³
MW = molecular weight, grams/mole

Aquaox Disinfectant 525

Absorbances of Standard and Collected Sample:

		Peak Absorbance at 540nm
295ppm Standard	0.0092g (10uL)	0.0295
	0.0233g (25uL)	0.0648
	0.0492g (50uL)	0.1152
	0.0731g (75uL)	0.1460
Collected Sample		0.0704

Standard Curve:



Calculations:

Absorbance of the Collected Sample:	0.0704
Corresponding Standard Weight: (determined using the linear equation $y = 1.8175x + 0.0185$)	0.0286g
HOCl Concentration of Standard:	295ppm
Corresponding Weight of HOCl in Air Sample:	$0.0286g \times 295ppm = 8.424ug$
Volume of Air Sample Collected:	15L
Molecular Weight of HOCl:	52.46 g/mol
* HOCl Concentration of Air Sample, mg/m^3 :	0.562mg/m^3
* HOCl Concentration of Air Sample, ppm:	0.262ppm

Interpretation:

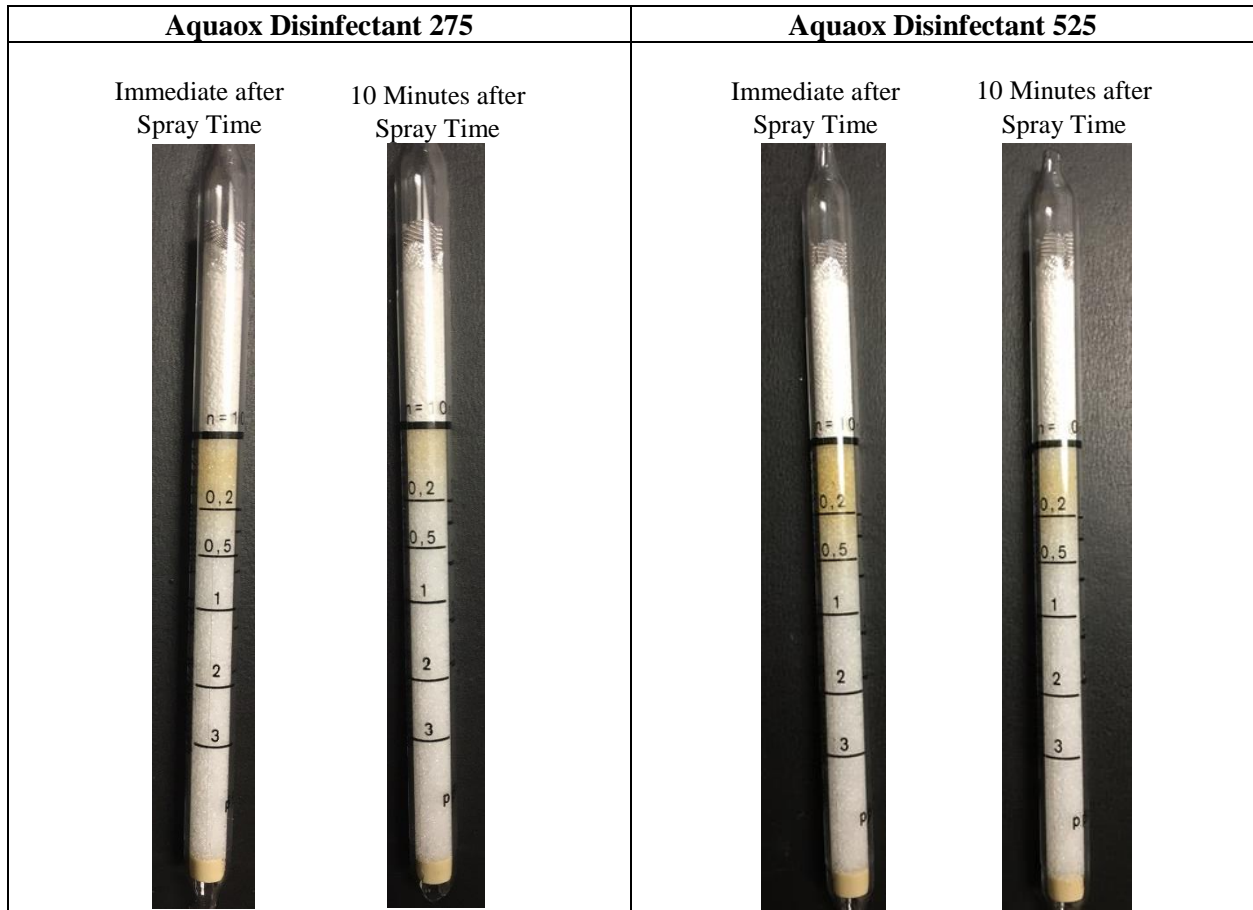
After a 5-minute spray time and a 10-minute dwell time using the AX275 solution, the HOCl concentration in the air sample collected in a 15-minute collection time is $0.444mg/m^3$ ($0.444mg$ HOCl per m^3 of air volume or $0.207ppm$). For the AX525 solution, the HOCl concentration is $0.562mg/m^3$ ($0.562mg$ HOCl per m^3 of air volume or $0.262ppm$). Since there is not a standard level established for Hypochlorous Acid, the current OSHA PEL for Chlorine, $1 ppm$ (or $2.9 mg/m^3$), is used. The HOCl levels in the collected air samples when using both test solutions are well below the OSHA PEL for Chlorine.

Experiment 3: Immediate Assessment of Chlorine Gas Concentration in Air Samples via the Draeger System

Chlorine gas concentration in immediate air samples is assessed via the Draeger Chlorine 0.2/a System. This system has a measurement range of $0.2 - 3 ppm$ for Chlorine gas and is widely used for detecting gases and vapors in industrial workspaces. The system contains the Draeger accuro pump and the Draeger tubes. The Aquaox ESS is operated following the Aquaox ICS protocol in the $12in \times 18in$ experimental room as discussed above. Air samples are collected from the experimental room at two different time frames, 1) immediately after the 5-minute spray time and 2) upon completion of the 10-minute dwelling time after the spray time. Air samples are collected into the Draeger tube and test results are interpreted by the length of color change in the tube. Samples are collected and analyzed internally at Aquaox.

The chemistry behind this method is further explained below. The Draeger tubes are glass vials filled with a chemical reagent that reacts to the target chemical (or family of chemicals) to be measured. In this case, the tubes contain the chemical o-tolidine, which when reacts with Chlorine will yield a yellow orange reaction product. The pump draws a calibrated 100 mL of air sample into the tube with each stroke, and 10 strokes are performed for each experiment. Any Chlorine in the air sample will react with the reagent and yield a yellow orange reaction product. The length of the color change in the tube indicates the amount of reaction product, and thus the Chlorine gas concentration in the original air sample.

Test Result:



	Aquaox Disinfectant 275	Aquaox Disinfectant 525
Test Date	October 8, 2015	October 7, 2015
Test Time	10:05am / 10:15am	9:30am / 9:40am
Immediately after Spray Time	0.2ppm	0.5ppm
10-Minute after Spray Time	<0.2ppm	0.2ppm

Specifications of Test Solutions:

	Aquaox Disinfectant 275	Aquaox Disinfectant 525
FAC (ppm)	281	525

pH	6.78	6.57
ORP	842	878
Conductivity	2405	3162

Interpretation:

After spraying with the AX275 solution for 5 minutes, a residual Chlorine of 0.2ppm immediately after the spray time and less than 0.2ppm was detected 10 minutes after the spray time. For the AX525 solution, a residual Chlorine of 0.5ppm immediately after the spray time and 0.2ppm was detected 10 minutes after the spray time. As a result, the residual Chlorine level in the air is always below the regulated concentration of 1 ppm under both scenarios (immediately or 10 minutes after),