Microbiological Report

Antimicrobial Performance of the Medixair UVc Air Sterilisation Device

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1.0 Introduction

1.1 Biography

My name is R.D.O'Connor, I hold B.Sc. (Hons) degree in applied environmental microbiology and I am a Chartered Biologist (Ci.Biol). I currently work as a practising microbiologist and Managing Director of a UKAS accredited, DEFRA (formerly MAFF) authorised contract laboratory, capable of handling up to Class II pathogens.

This document presents the experimental design and data resulting from work commissioned by Brandenburg UK Ltd in respect of their invention, which is known as the Medixair UVc air sterilisation unit. This is a multi lamp UVc emitter which passes air through a decontamination chamber by means of a fan and which is intended for use in atmospheric control of biohazards.

1.2 Brief

My basic brief was to demonstrate the efficiency of this device in relation to the eradication of airborne pathogens. Specifically I was instructed to investigate such by employing Bacillus anthracis surrogates (B subtilis, B globigii B megaterium) and other class II pathogens of my choice. Bacillus anthracis in vegetative form is reported to require a lethal UVc dose of between 10,000 and 15,000 uW/seconds, while in the case of the spore phase, the energy requirements may be as much as three times higher .The surrogates employed (Appendix A) are known to exhibit similar UVc susceptibility to that of Bacillus anthracis.

1.3 Experiments

This study has focused on determining the microbiological performance of the Medixair device by investigation of the device in the three following areas;

a. *In vitro* verification of the antimicrobial potential of the energy generated by a single UVc lamp employing surrogate cultures for Bacillus anthracis (as spores) and other class II pathogens in vegetative form

b. *In vitro* volumetric antimicrobial performance in relation to the removal of surrogates for Bacillus anthracis (as spores).

c. Performance in an environmental situation-employing surrogate cultures for Bacillus anthracis (as spores) and other class II pathogens.

2.0 In vitro Performance Trials of a Medixair lamp

2.1 Conditions

In this section of work our goal was to establish that a single lamp, of the type employed in the construction of the Medixair unit, was capable of producing satisfactory levels of lethality both with the Bacillus anthracis surrogates (in spore form) and the other class II pathogens (in vegetative form) as listed in appendix A. This bench level work was considered a necessary prelude to larger scale investigations and was primarily intended to establish that the selected surrogates could be inactivated within reasonable time periods.

These trials were conducted by inoculating the surface of Tryptone Soya Agar plates with aliquots of either mid exponential cultures of vegetative cell cultures or spore suspensions (surrogates). In the latter case spore suspensions were generated by primary culture in Brain heart infusion containing 1- % starch. Prior to inoculation the surrogate cultures were heat treated at 63.5'c for 35 minutes to destroy the vegetative cells.

All inoculated plated were conditioned at 30'c for 2 hours prior to UVc treatment.

The lamp employed in the trial was mounted in a bespoke chamber, which facilitated the positioning of an exposed agar plate in a manner such that the surface of the plate was 50mm from the UVc source. Prior to initial use the lamp was conditioned for 4 days. Prior to plate exposure the lamp was stabilised for 30 minutes. Applicable safety procedures were in force during this and all other experiments.

Exposure of inoculated plates occurred over successive 15-second increments up to and including the 60-second mark. Cell or spore density was estimated by deployment of sterile bores capable of obtaining 5 cm^2 sections of agar to a depth of 5 mm. The resulting core was subject to serial dilution with subsequent recovery of isolates on appropriate agars. All analyses were conducted in duplicate. Each test organism was trialed with between 9 and 11 replicates at each interval. Media blanks were run and produced no isolates. In house Q.C. indicated that all media employed at this and at all subsequent stages gave an Ecometric recovery quotient of > 95 %.

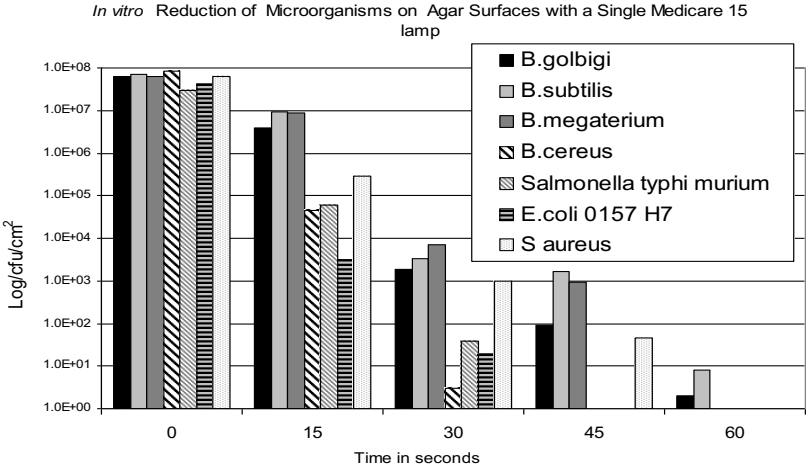
2.2 Experimental Results

The mean lethality data (survivors cfu/cm^2 v Exposure Time) obtained at each increment of exposure for all organisms are given below (Table 1 Graph 1). Additional the over all percentage kill is reported and the point where a kill of > 99.999 % was obtained has been highlighted.

Table 1 In vitro performance trials of a Medixair lamp

Exposure Time cfu/cm^2 agar/recovered				Time seconds					% Reduction
Organism	ID	Form of Organism	N	0	15	30	45	60	
B.golbigii	1	Spore	9	6.40E+07	3.80E+06	1900	90	2	>99.999
B.subtilis	2	Spore	11	7.10E+07	9.20E+06	3300	1700	8	>99.999
B.megaterium	3	Vegetative	11	6.20E+07	8.80E+06	7200	940	0	>99.999
B.cereus	4	Vegetative	11	8.30E+07	4.50E+04	3	0	0	>99.999
Salmonella typhimurium	5	Vegetative	11	2.90E+07	6.20E+04	38	0	0	>99.999
E.coli 0157 H7	6	Vegetative	11	4.30E+07	3.10E+03	19	0	0	>99.999
S aureus	7	Vegetative	11	6.30E+07	2.90E+05	960	46	0	>99.999

Table 1 In vitro performance trials of a Medixair lamp



Volumetric, Antimicrobial Performance of the Medixair Device.

3.1 Conditions

These trials were conducted in a microbiologically sealed PVA construction consisting of two chambers of identical dimension. These chambers were connected horizontally by the Medixair device. In this manner, during operation atmosphere was transferred from chamber A to B by means of the fan incorporated in Medixair device. An atmosphere return tube (150 ml diameter) connected chambers A and B, giving an over all operating volume of 54 $m^{3.}$ Each chamber contained four floor-mounted fans to assist with microbial dispersion and also a silica gel unit to prevent excessive humidity build up. All surfaces (excluding the internal surfaces of the Medixair unit) were sprayed with an anti static treatment. Pressure equalisation occurred via four apertures secured by 0.2-micron membrane filters.

This facility was equipped both for the introduction of microbial aerosols (Chamber A) and for volumetric recovery of atmosphere in volumes of an appropriate diluent medium (Chamber B). To permit uniform microbial dispersion and to facilitate study of microbial dynamics in the absence of UVc doses, the Medixair device was modified, at my request, so that the fan could be run with the lamp out of circuit. I was satisfied this modification would not effect the efficiency of normal intended usage.

All test organisms were obtained either as calibrated dry spore suspensions (powder). Spore suspensions were obtained by heat treatment (63.5 ‘c for 35 minutes) of mid exponential liquid cultures in Brain heart infusion containing 1 % starch. Heat-treated cultures were then lyophilised and assayed. Assays were conducted daily on spore stocks to assure viability (viable titre by enumeration on TSA with confirmation)) and vigour (impedance curve; onset of exponential growth and curve slope).

In the case of spores the environmental challenge was introduced by a positive air pressure jet while in the case of vegetative cultures dispersion was achieved by use of a fogging device delivering a particle size range of 5-15 micron. The target level of inoculation in all cases was in the 10e7 /cfum3 range.

Prior to introduction of the of any test organisms the lamp complex was run in circuit for 4 hours to eliminate airborne contamination resident within the system. Control plates showed that all occasions this conditioning sterilisation action did reduce internal contamination to <10 cfu/m^3.

All studies were conducted over 8 hours and each study was repeated nine times over consecutive days. All organisms were studied in monoculture. Additionally, as it was predicted that spore precipitation would occur due to gravity or electrostatic attraction, we conducted monitoring exercises with the UVc lamps out of circuit. Thus we obtained lethality figures due to system artefacts and this data was employed to correct the lethality data obtained when the UVc lamps were in operation.

Sampling was achieved by aspiration of a 1-m3 atmosphere volume through 100 ml of diluent (Peptone Saline recovery Broth) which formed the initial test dilution. Recovery of isolates was obtained by serial dilution and plating on appropriate agars. All analyses were conducted in duplicate with appropriate controls.

The organisms assessed in this trial were

- **Bacillus globigii**
- **Bacillus megaterium**
- **Bacillus subtilis** (See Appendix A)

All isolates obtained were confirmed by prescribed biochemical and morphological characteristics.

3.2 Experimental Results

The following tables (Tables 2-4) and their associated graphs summarise the
mean data obtained for the 8 hour trial series for each test organism. Each table returns the results for microbial reduction purely due to precipitation or other system artefacts and with UVc doses corrected for precipitation.

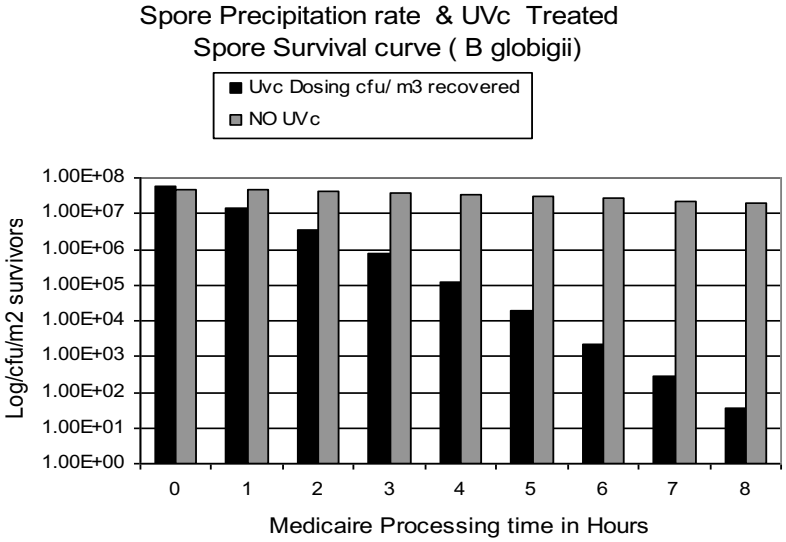
Table 2. Mean data for the recovery of Bacillus globigii in a 54 m^3 closed system over 8 hours with and without UVc treatment by the Medixair device.

	Control	UVc Dosing	Hourly Kill Rate
Sample point in hours	cfu/ m^3 recovered	cfu/ m^3 recovered	Log reductions per hour
0	4.90E+07	5.60E+07	0.00
1	4.50E+07	1.50E+07	0.57
2	4.10E+07	3.56E+06	1.20
3	3.80E+07	7.26E+05	1.89
4	3.50E+07	1.22E+05	2.66
5	3.00E+07	1.88E+04	3.47
6	2.60E+07	2.27E+03	4.39
7	2.20E+07	2.68E+02	5.32
8	1.90E+07	3.40E+01	6.22

Overall % kill in 8 hours = 99.9999 % Equivalent to 6.2 Log cycles

Mean Population precipitation rate = 10.8 % per hour

Graph 2. *Mean data for the recovery of Bacillus globigii in a 54 m^3 closed system over 8 hours with and without UVc treatment by the Medixair device.*



Mann-Whitney Test: Bacillus globigii

Do the median values for Bacillus globigii survival with no UVc doses and UVc doses differ significantly?

The two-tailed P value is 0.0400, considered significant.
The P value is exact.

Calculation details
Mann-Whitney U-statistic = 17.000
U' = 64.000
Sum of ranks in Column A = 109.00. Sum of ranks in Column B = 62.000.

Summary of Data

Parameter	Column A	Column B
Mean:	2.8830 E+07	8.3810E+06
Number of points:	9	9
Std deviation:	1.3950 E+07	1.8510E+07
Std error:	4.6531 E+06	6.1712E+06
Minimum:	4.900	34.000
Maximum:	4.5500E+07	5.6000E+07
Median:	3.0200E+07	1.2200E+05
Lower 95% CI:	1.8100E+07	-5.8498E+06
Upper 95% CI:	3.9560E+07	2.2610E+07

Spearman Rank Correlation Number of points = 18

Spearman r = -0.6470 (corrected for ties)
95% confidence interval: -0.8594 to -0.2439

Test: is r significantly different than zero?

The two-tailed P value is 0.0037, considered very significant.

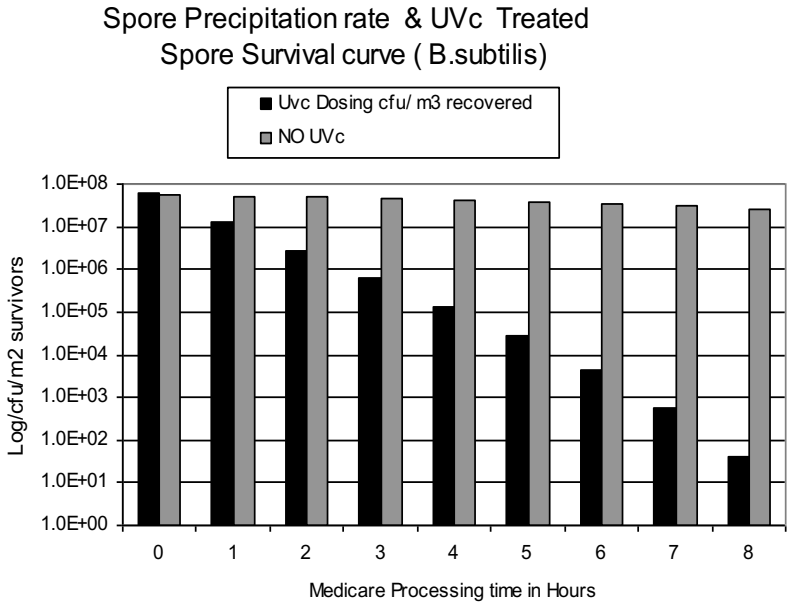
Table 3 Mean data for the recovery of Bacillus subtilis in a 54 m^3 closed system over 8 hours with and without UVc treatment by the Medixair device

Sample point in hours	Control	UVc Dosing	Hourly kill rate
	cfu / m^3 recovered	cfu / m^3 recovered	Log reductions per hour
0	5.6E+07	6.1E+07	0.00
1	5.3E+07	1.3E+07	0.67
2	4.9E+07	2.7E+06	1.35
3	4.7E+07	6.4E+05	1.98
4	4.3E+07	1.4E+05	2.64
5	4.0E+07	2.7E+04	3.35
6	3.5E+07	4.5E+03	4.13
7	3.0E+07	5.7E+02	5.03
8	2.6E+07	3.9E+01	6.19

Overall % kill in 8 hours = > 99.9999 % Equivalent to 6.19 Log cycles

Mean population precipitation rate = 9.2 % per hour

raph 3 *Mean data for the recovery of Bacillus subtilis in a 54 m^3 closed system over 8 hours with and without UVc treatment by the Medixair device.*



Mann-Whitney Test: Bacillus subtilis

Do the median values for Bacillus subtilis survival with no UVc doses and UVc doses differ significantly?

The two-tailed P value is 0.0400, considered significant.
The P value is exact.

Calculation details
Mann-Whitney U-statistic = 17.000 U' = 64.000
Sum of ranks in Column A = 109.00. Sum of ranks in Column B = 62.000.

Summary of Data

Parameter:	Column A	Column B
Mean:	3.589E+07	8.6125E+06
Number of points:	9	9
Std deviation:	1.6110E+07	2.0090E+07
Std error:	5.3708E+06	6.6980E+06
Minimum:	5.6000E+00	3.9000E+01
Maximum:	5.3000E+07	6.1000E+07
Median:	4.0000E+07	1.4000E+05
Lower 95% C	2.3500E+07	-6.8331E+06
Upper 95% CI:	4.8270E+07	2.4000E+00

Number of points = 18

Spearman r = -0.6470 (corrected for ties)
95% confidence interval: -0.8594 to -0.2439

Test: Is r significantly different than zero?

The two-tailed P value is 0.0037, considered very significant

Table 4 Mean data for the recovery of Bacillus megaterium in a 54 m^3 closed system over 8 hours with and without UVc treatment by the Medixair device.

	Control	UVc Dosing	Hourly Kill rate

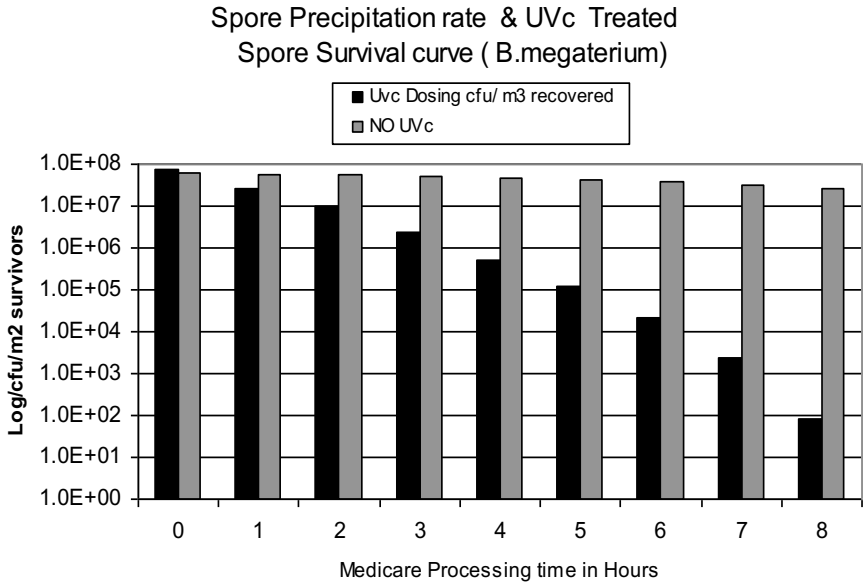
Sample point in hours	cfu/ m^3 recovered	cfu/ m^3 recovered	Log reductions per hour
0	6.2E+07	7.3E+07	0.00
1	5.8E+07	2.5E+07	0.47
2	5.4E+07	9.8E+06	0.87
3	5.0E+07	2.3E+06	1.50
4	4.6E+07	5.0E+05	2.16
5	4.2E+07	1.2E+05	2.78
6	3.7E+07	2.2E+04	3.52
7	3.3E+07	2.3E+03	4.50
8	2.6E+07	8.4E+01	5.94

Overall % kill in 8 hours = > 99.9999 % Equivalent to 5.94 Log cycles

Mean population precipitation rate = 10.2 % per hour

ɔh 4 Mean data for the recovery of Bacillus megaterium in a 54 m^3 closed system over 8 hours with and without UVc treatment by the Medixair device.

Mann-Whitney Test: Bacillus megaterium



Do the median values for Bacillus megaterium survival with no UVc doses and UVc doses differ significantly?

The two-tailed P value is 0.0400, considered significant.
The P value is exact.

Calculation details
Mann-Whitney U-statistic = 17.000
U' = 64.000
Sum of ranks in Column A = 109.00. Sum of ranks in Column B = 62.000.

Number of points = 18

Spearman r = -0.6470 (corrected for ties)
95% confidence interval: -0.8594 to -0.2439

Test: Is r significantly different than zero?

The two-tailed P value is 0.0037, considered very significant.

The P value is approximate because exact calculations would have been too exhaustive at this juncture

4.0 Performance of the Medixair Unit in the Reduction of Airborne Microbial Contaminants.

4.1 Conditions

This trial was conducted employing the space afforded by a laboratory waste handling facility with a working volume of 216 m3. The area is employed for the containment and thermal decontamination of Class II Biological waste and has the facility both for the introduction of aerosols and as detailed above for the volumetric recovery of atmosphere in sample diluent.

This space was selected for demonstration of the performance of the Medixair unit in the removal of airborne microbiological contaminants.

All test organisms were obtained either as calibrated dry spore suspensions (lyophilised powder) or as calibrated mid exponential cultures in brain heart infusion. In the case of spores, the environmental challenge was introduced by supplying a positive air pressure jet while in the case of vegetative cultures dispersion was achieved by use of a fogging device delivering a particle size range of 5-15 micron. As in the earlier trial test environment was conditioned for 4 hours prior to the introduction any test culture

Throughout the trial the dispersion and continued uniform atmospheric dispersion of the test organisms were maintained by a series of floor mounted industrial fans. The target level of inoculation in all cases was in the 10e6 /cfu/M^3. Dosing trials were conducted to establish loading volumes.

All trials were conducted employing monocultures and performance of the unit with each organism was assessed on three separate occasions. The mean data is presented. In this instance no attempt was made to establish loss of culture from the atmosphere due to precipitation. This was because we had already shown that the lethality of the device was significantly greater in comparison to when removal of air borne organisms was purely due to precipitation or adhesion effects in the environment.

After charging, the environment was sampled and thereafter four hourly basis over a 24 hour period.

The operating target was to obtain a reduction of 99.999 % with respect to each target organism.

4.2 Experimental Results

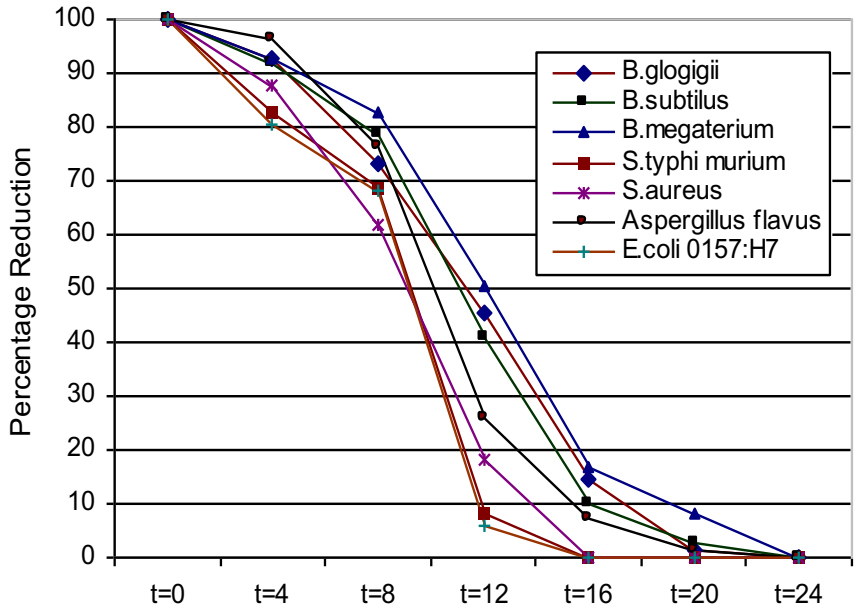
The table (5) below details the range of organisms and initial atmospheric loading for each, employed during the environmental assessment of the Medixair unit. Additionally the mean cumulative percentage reduction of atmospheric contamination incremented over the six hour period is given . The table also illustrates the sampling interval at which microbial lethality reached => 99.999 %.

Table 5 Performance of the Medixair in the removal of Airborne pathogens in a 216 m^3 room space.

Organism	Mean Organism load level cfu/m^3	Time in hours vs % reduction						
		t=0	t=4	t=8	t=12	t=16	t=20	t=24
B. globigii	4.00E+06	0	7.2	26.9	54.6	85.3	98.6	99.999
B. subtilus	3.60E+06	0	8.3	21.4	59.3	90.1	97.2	99.999
B. megaterium	7.10E+06	0	7.5	17.5	49.7	83.4	92	99.999
S. typhi murium	3.40E+06	0	17.4	31.2	91.6	99.999	99.999	99.999
S. aureus	2.90E+06	0	12.2	38.4	82	99.999	99.999	99.999
Aspergillus niger	3.10E+06	0	3.7	23.6	73.9	92.7	98.6	99.999
E.coli 0157:H7	5.20E+06	0	19.6	31.7	94.3	99.999	99.999	99.999

Graph 5. Performance of the Medixair in the removal of Airborne pathogens in a 216 m^3 contained room space.

Medixair device could in isolation bring about significant microbial



5.0 Data Discussion

Our early work in this trial hoped to confirm that a single lamp from the reduction. As such this was a Quality Assurance check on the lamps supplied by the manufacturer and an important milestone in knowing whether or not to proceed with more complex and demanding trials.
To this end all candidate surrogates and a range of class two pathogens were subject to doses of UVc irradiation whilst dispersed in high levels on the surface of agar plates. Our data has shown with good replication that all isolates so examined were reduced in numbers by => 99.999 % prior to or at the 60 second exposure marker. These results were considered favourable and the more extensive trials as detailed above were executed.

Our second trial more nearly mimicked a real 'in use' situation but at the same time facilitated examination of the Medixair device in the presence of high levels of surrogate organisms with a minimum of external variables. The test apparatus had a total internal volume of 54m^3. Over the 8-hour trial period of the test we calculated the air contained within the test chamber was passed through the device 3.56 times. Therefore based on a minimum of three passes the minimum cumulative dose would be 113,000uW.s/cm^2

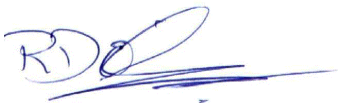
Evidently, according to the data obtained, and talking into account microbial depletion not due to UVc doses, we obtained greater than 99.999 % kill rates with UVc doses by the 8 hour mark for all organisms examined. As the initial spore challenge levels were in excess of 1.0 $x10^{7/}$cfu /m^3 this represents a 5 log reduction of contaminants over an 8 hour period. Furthermore in the case of each surrogate the microbial reduction obtained by UVc doses was statistically significantly different from the level of reduction obtained purely by other factors.

In a simulated real use situation, the Medixair device was operated over a 24 hour period in a environment with atmospheric contamination commencing at a level in excess of 1.0 $x10^6$ / cfu /$m^{3.}$ Given that the volume of the waste room was 216m^3 we anticipated 2.67 air changes during the 24hour test period

Evidently within this scope of operation the dosages of UVc were sufficient to bring about a 99.999 % reduction of all challenge organisms including Bacillus anthracis surrogates by the 24-hour marker.

6.0 Conclusion

I believe our data illustrates that the Medixair device represents a significant advance in atmospheric treatment. Our data suggests a sensible and effective combination of airflow rate to UVc dosage has been conceived in a system, which should integrate efficiently, and effectively into environmental biohazard protection systems.



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D.O'Connor B.Sc. Ci.Biol M.I.F.S.T.

APPENDIX A

1. 0 Organisms Employed ;

Bacillus megaterium	NCTC 10342
Bacillus globigii	ATCC 49822
Bacillus subtilis	ATCC 19659
Bacillus cereus	NCTC 2599
Salmonella typhi murium	NCTC 74
Ecoli 0157 H7	NCTC 12079 (ATTENUATED STRAIN: EX PUBLIC HEALTH SERVICE CULTURE)
Staphylococcus aureus	NCTC 8532
Aspergillus niger	NCPF 2275

APPENDIX B

Microsearch Quality System Extracts*

*N.B. Attached as a separate folder.

Notes: