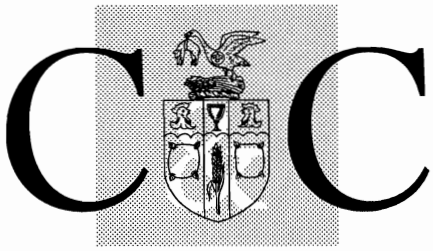
R&D REPORT NO. 13

The Evaluation of Air Disinfection Systems

June 1995



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The Evaluation of Air Disinfection Systems

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SUMMARY

The aim of this work was to evaluate some of the different methods for disinfection of air including chemical fogging, UV light and ozone. There are conflicting reports on the effectiveness of these techniques for the control of airborne microorganisms. A purpose built aerobiology cabinet was constructed in order to study the destruction of airborne microorganisms by disinfectant fogging, ozone and UV light. Survival was assessed using a variety of methods including settle plates, precipitation onto metal strips, impaction onto agar (SAS sampler) and impingement in a glass cyclone sampler.

From the results, ozone appeared to be both effective and reproducible in its effect on airborne microorganisms. UV was also effective, but in practice shadowing could pose problems in a factory environment. Disinfectant fogging was the least predictable, controllable and effective. In some trials 100% kill was achieved whereas in others no destruction was observed. The droplets generated at the fogging nozzle were photographed using a high speed camera and this highlighted a defect in the spray nozzle which was traced to a perforated disc inside the nozzle. Replacement of the component produced a finer aerosol which was more effective. The results indicate that further work is required on fogging and its effectiveness to ascertain the optimum conditions for fog generation and microbial destruction.

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INTRODUCTION

The use of high care or clean room technology to prevent recontamination of assembled foods is being increasingly applied in the food industry (Fitzpatrick, 1990) to control airborne pathogens and extend the shelf life of perishable products. In many instances clean air is provided by HEPA filters but other methods of reducing the numbers of viable microorganisms in the air include chemical fogging, UV light, heat, electrostatic precipitation and ozone (Hedrick, 1975). Chlorine fogs of 500 ppm are claimed to reduce the airborne count but levels over 10ppm cause human discomfort. Destruction of organisms by UV light is claimed to be 50-90% effective (Hedrick, 1975). Other workers (Edwards, 1978) point to the ineffectiveness of room fogging with phenols, quaternary ammonium compounds or iodophors for disinfection of air. Some disinfectants can support bacterial growth and themselves become infectants. Daschner *et al.* (1987) claim that disinfectant fogging has only ritualistic value and UV light is also ineffective. In view of the conflicting reports a purpose built aerobiology cabinet was constructed in which the effectiveness of disinfectant fogging, ozone and UV light could be studied.

In the development of the cabinet experiments, several methods were used to measure the number of viable microorganisms in the air. These included precipitation onto agar or metal strips and into diluent, impaction onto agar (SAS sampler) and impingement in a glass cyclone (Cox, 1987; Kang and Frank, 1989). Precipitation methods are easy and inexpensive to set up but do not measure airborne organisms quantitatively (numbers/m^3). However they may provide an estimate of deposition of viable microorganisms on surfaces as found in a food processing environment. While agar or diluent in Petri dishes can provide an estimate of the number of viable microorganisms deposited in a set time period, metal strips more closely simulate food machinery where organisms may dry out and die after deposition.

The SAS sampler and cyclone enabled the concentration of microorganisms in the cabinet air to be measured. Kang and Frank (1989) reviewed several of the commercially available aerosol samplers. Each sampler has limitations in assessing the numbers of airborne microorganisms. It is advisable in any experimental approach to utilise different methods but this can complicate the results and conclusions since each method will give different results.

Aerosolisation produces mechanical and physiological stresses on microorganisms which affect their recovery. Stersky and Hedrick (1972) demonstrated that recovery of *Escherichia coli* and *Salmonella newbrunswick* ranged from <1% to 122% in selective

media compared to standard plate count agar. Nutrient agar (NA) was used as the standard recovery medium for the test organism (*Pseudomonas aeruginosa*) used in the aerobiology chamber experiments. Two factory trials included the use of Malt Extract Agar (for yeasts and moulds), KG Streptococcus agar (for *Streptococcus* spp) and Listeria Oxford Agar (for *Listeria* spp) in the SAS sampler but there was insufficient time to assess in detail the use of selective media in this project.

Another factor which can affect survival is the diluent used for production of the aerosol and for liquid impingers such as the cyclone. Phosphate/peptone diluents are used most commonly (Kang and Frank, 1989) but it has been demonstrated (Stersky *et al.*, 1972) that *Salmonella newbrunswick* survives much longer when aerosolised from skim milk than in distilled water. There have been no studies on differing survival of microorganisms when aerolised from food products other than milk. It is feasible that some food products (eg those with fatty components) may protect organisms from desiccation in aerosols and extend their survival. Little is known of the survival of microorganisms aerosolised from food environments.

MATERIALS AND METHODS

1. Materials

Test Organism:	*Pseudomonas aeruginosa* (NCIB 10421, Campden RA No. 731)
Aerobiology Cabinet:	Bassaire Ltd., Duncan Road, Swanwick, Southampton, SO3 7ZS
Ozone Generator (Triox trucker):	Triox Ltd., Blagrove House, 2-3 Newport Street, Swindon, Wilts, SN1 3DX.
UV Equipment:	Hanovia Ltd., 145 Farnham Road, Slough, Berks, SL1 4XB.
Cyclone Sampler:	Hampshire (R&D) Glassware Ltd, 77-79 Dukes Road, Southampton, Hants, SO2 0ST.
SAS Air Sampler:	Cherwell Laboratories Ltd., 114 Churchill Road, Bicester, Oxon, OX6 7XB.

Collison Aerosol Nebuliser:	Biral, PO Box 2, Portishead, Bristol, BS20.
Cyclone Pump:	Casella London Ltd., Regent House, Britannia Walk, London, N1 7ND.
Peristaltic Pumps:	Smith and Nephew Watson-Marlow, Falmouth, Cornwall, TR11 4RU.
Disinfectant Spray:	H&M Disinfection Systems Ltd., 2 Dalby Court, Gadbrook Business Centre, Gadbrook Road, Northwich, Cheshire, CW9 7TN.
55mm Contact Plates:	Fisons Scientific Equipment, Bishop Meadow Road, Loughborough, Leics, LE11 0RG.
Air Circulation Fan:	80 x 80mm, 240v 12w, airflow 14 l/s RS509030 Radiospares, PO Box 99, Corby, Northants, NN17 9RS.
Laboratory Air Conditioning:	Supplied by Major Refrigeration and Air Conditioning Services, Evesham, Worcs, WR11 6BH.
Hygrometers:	Ebro RHT 200, Camlab Instruments, Cambridge, CB4 1TH. Fischer Hair Hygrometer, Fisons Scientific Equipment, Loughborough, Leics, LE11 0RG. Masons wet and dry bulb hygrometer, Fisons Scientific Equipment, Loughborough, Leics, LE11 0RG.
Temperature Measurement:	Electronic thermometer 2003, Jenway Instruments, Dunmow, Essex, CM6 3LB.

2. Construction of aerobiology chamber

The aerobiology chamber provided by Bassaire Ltd consisted of two HEPA filters on each end of an enclosure of 0.36m³ (60 cm x 60cm x 100cm; w x d x l). Air circulation inside the cabinet was achieved using a small fan fixed inside the roof of the chamber. The manufacturer's (Radiospares Ltd) literature stated an airflow of 14 l/s. This was calculated to be sufficient to mix the air in the cabinet once every 25s, and be sufficient to create a uniform aerosol. Uniformity of the aerosol was considered essential because the sampling positions were located in different positions in the chamber. Figure 1 shows the relative positions of the aerosol nebulisor, disinfectant spray, fan and various sampling points.

The Collison aerosol nebulisor and disinfectant nozzles were located in one top corner of the chamber. Both units were connected to a compressed air supply for operation.

Air samples could be withdrawn from both sides of the chamber using either a cyclone air sampler or SAS air sampler. Samples from the cyclone were collected using two peristaltic pumps and were then diluted and plated out using Nutrient Agar (Oxoid CM3) (NA). Results from the SAS sampler were counted after incubation of the 55mm Petri dishes used in the apparatus. No dilution was possible for the SAS plates.

Exposure of the aerosolised bacteria to UV light was achieved by mounting four high intensity UV lamps horizontally in the chamber. The distance of the lamps from the floor of the chamber could be adjusted using four threaded studs fixed to two mounting brackets. The whole chamber was shrouded for the UV experiments to protect the operators who also wore UV eye protection. The control systems for the UV lamps (Hanovia Ltd) were mounted on top of the cabinet.

Ozone was generated using a Triox 'Trucker' system which was connected to the cabinet by a plastic tube. Triox also supplied a meter to measure ozone concentration. The 'Trucker' unit is capable of generating 250mg/h ozone. The recommended exposure limit for ozone is 0.2mg/m³ (HSE, 1983) for 8 hour time-weighted average concentration and 0.6mg/m³ for short term (15 min) exposure. The 'Trucker' unit was capable of exceeding even the short term exposure limit for the volume of the room (29m³) containing the aerobiology chamber. For safety reasons the air outlet filter was ducted to atmosphere using 20cm diameter ducting for the ozone experiments and laboratory ozone levels were monitored using a meter supplied by Triox to ensure that maximum permitted levels were not exceeded.

The room in which the aerobiology cabinet was located was equipped with air conditioning to enable constant temperatures to be maintained during experiments.

LEGEND TO FIGURE 1

COORDINATES OF SAMPLING POINTS, COLLISON NEBULISER, DISINFECTANT SPRAY AND CIRCULATION FAN

Item		Co-ordinates		
		x	y	z
Collison nebuliser nozzle		2	2	1
Disinfectant spray nozzle		1	1	1
Cyclone air sampler port		6	3	1
SAS air sampling port		5	3	6
Wall mounted coupons	1	3	2	1
	2	8	2	1
	3	3	2	6
	4	8	2	6
Floor mounted coupons	1	4	6	3
	2	7	6	3
(control)	3	5	6	2
Floor mounted exposure plates	1	10	6	6
	2	10	6	1
	3	1	6	1
	4	1	6	6
(control)	5	5	6	5
Circulation fan		5/6	1	3/4
Glove ports	1	3	5	1
	2	8	5	1
	3	3	5	6
	4	8	5	6

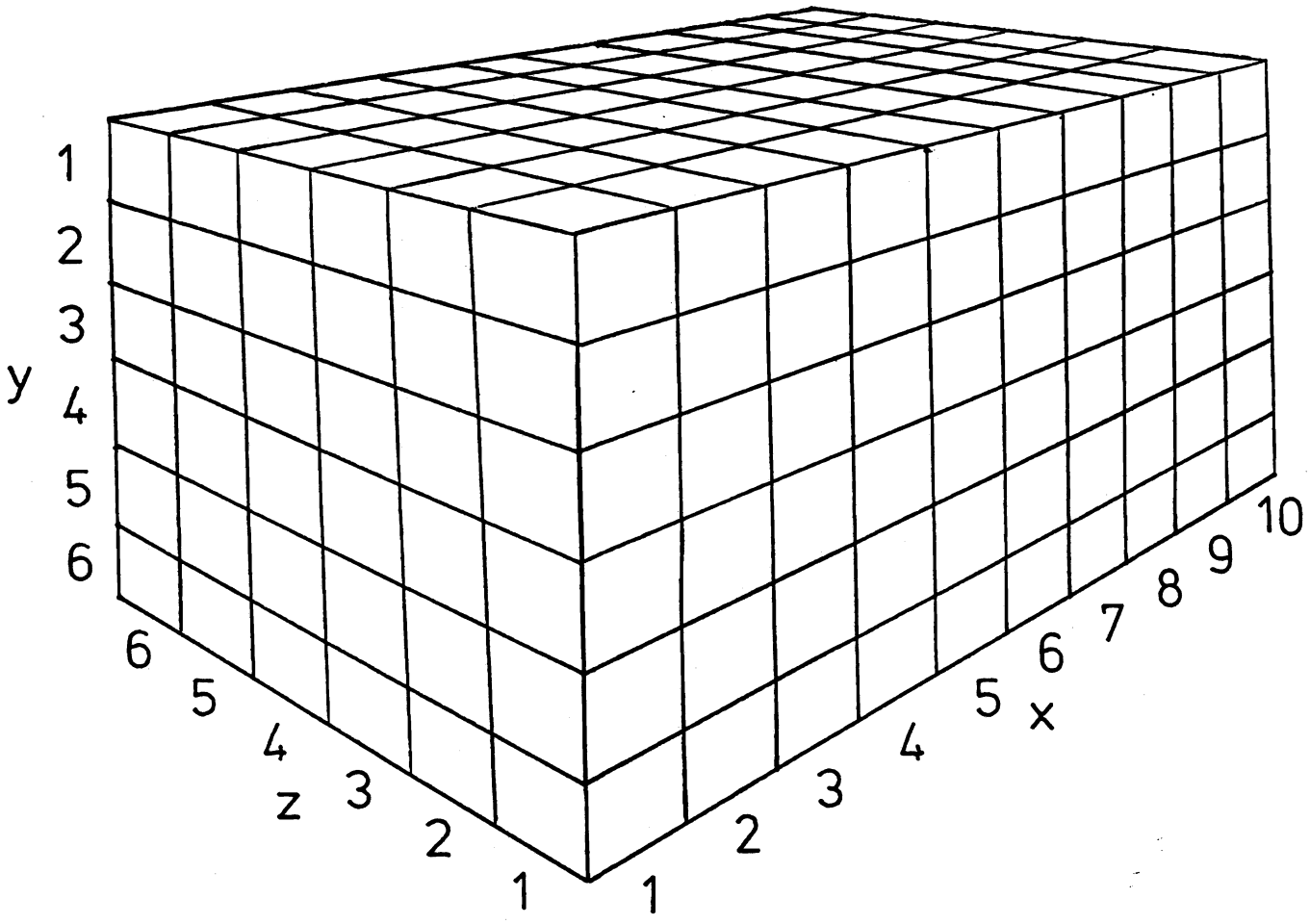


Figure 1. Location of Sampling Points, Collison Nebuliser, Disinfectant Spray and Circulation Fan

3. Disinfectant Fogging Procedure

a. Preparation of Culture

An overnight (30°C) culture of *P.aeruginosa* was centrifuged in a MSE Mistral 2000 centrifuge at 4500rpm for 11 min. The supernatant was discarded and the pellet of microorganisms resuspended in 100ml phosphate buffer (34g KH_2PO_4, 500ml distilled water, adjusted to pH 7.2 with NaOH and made up to 1000ml). The suspension was then placed in the Collison aerosol nebuliser which was attached to the aerobiology chamber. By experiment it was found that an average 1.518ml of suspension was sprayed into the chamber in 5 min. *P.aeruginosa* was chosen because it was often isolated in the factory surveys and is a recognised European disinfectant testing strain.

b. Stainless Steel Coupons

Stainless steel (316 with a 2B finish of 4 x 2cm) coupons were positioned on the floor and walls of the chamber to simulate machinery surfaces. The aim was to determine the total number of organisms deposited on the surfaces and the number of organisms remaining viable after deposition. The coupons were washed in detergent, rinsed and autoclaved prior to use. Two coupons were each stuck into Petri dishes using Blu-Tack and placed on each of the two sides of the aerobiology chamber and three were placed in Petri dishes on the floor of the chamber. After fogging treatment the coupons were removed from the Petri dishes using sterile forceps and placed in Universal bottles containing 18ml of diluent (Bacteriological peptone (Oxoid L37), 1.0g; NaCl, 8.5g; distilled water, 1000ml) and 2ml of inactivator (lecithin, 3.0g; polysorbate 80 (USP), 30ml; sodium thiosulphate, 5.0g; L-histidine, 1.0g; phosphate buffer (KH_2PO_4,$34gl^{-1}$) 0.25N, 10.0ml; distilled water, 1000ml). The bottles were placed in an ultrasonic bath for 1 min to remove the bacteria from the coupon and the resulting suspension was serially diluted and plated in Nutrient Agar (Oxoid CM3). After incubation at 30°C for 48h the viable count was determined. A sample of the suspension was also counted microscopically using a counting chamber to determine the total number of microorganisms.

c. Settle Plates

Settle plates containing Nutrient Agar (Oxoid CM3) were used on the floor and, in some experiments, on the walls of the aerobiology cabinet to assess the viable count of organisms deposited on the agar surface during the experiment In later experiments, diluent in Petri dishes was used to assess the viable count. It was found that the agar

settle plate count was often too high to count so the use of diluent which could be further diluted was evaluated. Incubation was again at 30°C for 48 hours.

d. Cyclone Air Sampler

Glass cyclones, tubing and collection vessel were washed with detergent, rinsed and autoclaved prior to connection to the aerobiology cabinet. During sampling, air was removed from the top of the cyclone as phosphate buffer plus inactivator (9:1 ratio) was pumped into the inlet of the cyclone through a hypodermic needle. The cyclone was operated for 2 min. The quantity of phosphate buffer plus inactivator pumped through the cyclone in 2 min was determined by weighing the final collection vessel. Samples (1ml) were plated out in Nutrient Agar (Oxoid CM3) and incubated at 30°C for 48 hours. The total count for the 2 min air sample was calculated by multiplying the plate count by the weight of buffer in the collection vessel.

e. SAS Air Sampler

An SAS air sampler (Cherwell Ltd) which was set to sample 60L of air was connected to the chamber at the opposite side to the cyclone. Contact plates containing Nutrient Agar (Oxoid CM3) were used for collection of microorganisms.

The SAS sampler was also used to take air samples from outside the chamber to ensure that the test organism was not escaping from the chamber.

f. Disinfectant Spray

Three disinfectants were tested: sodium hypochloritem a quaternary ammonium compound (QUAT) and an amphoteric disinfectant. Solutions containing 2% v/v of the disinfectants were sprayed into the chamber for 1 minute, while the mixing fan in the roof of the chamber was operated. During the first series of experiments the outlet HEPA filter was operated at its lowest setting to create a slight negative pressure within the chamber for operator safety. However, it was subsequently discovered that this adversely affected the distribution of aerosols within the chamber and later disinfectant experiments, and ozone and UV experiments were conducted with the HEPA filter off. Between experiments, the chamber was flushed out using the HEPA filters on maximum flow rate. The temperature and relative humidity were recorded but not controlled for the disinfectant fogging trials. The measuring equipment is listed under the materials section.

4. Ultraviolet Disinfection Procedure

Culture preparation was as described for disinfectant fogging. Aerosols were produced over a 5 min period of fogging. Four UV lamps were positioned centrally in the chamber. Settle plates containing either diluent or Nutrient Agar (Oxoid CM3) were positioned at the four corners on the floor of the chamber. To prevent UV light reaching the settle plates when not required, opaque plastic covers were placed over the Petri dishes. Diluent settle plates were used in order to obtain counts when high numbers of viable organisms were sampled. By experience it had been found that agar techniques (agar settle plates and SAS plates) were only useful for assessing low (<300) numbers of organisms. In many experiments the numbers of viable airborne organisms was much higher than could be counted on the agar plates. Diluent settle plates could be further diluted. At the end of each treatment with UV, settle plates were exposed for 5 min, followed by cyclone sampling for 2 min and SAS for 20 sec (60L) The electric circulation fan was used in all experiments to produce a uniform aerosol.

In addition to UV alone, one experiment using UV in combination with 2% hypochlorite disinfectant spray was done to assess any combined effect.

5. Ozone Disinfection Procedure

An evaluation of ozone disinfection was carried out using a Triox 'Trucker' unit which was connected to the aerobiology cabinet. Levels of ozone during the tests were monitored. Aerosols of *P.aeruginosa* were exposed to ozone for up to 10 min and survivors enumerated using agar settle plates. An aerosol was generated in the chamber for 5 min, the ozone generator was switched on for the appropriate exposure time (1-10 min), agar settle plates were then uncovered for 5 min, and the air was sampled using the cyclone air sampler for 2 min. Following recovery in Nutrient Agar (Oxoid CM3) survivors were enumerated after incubation at 30°C for 2 days. For safety reasons, the outlet filter was ducted to atmosphere and the cyclone pump was also ducted to atmosphere. The SAS sampler was not used because of potential exposure of the operator to levels of ozone above permitted safe levels.

Trials were also done to evaluate the combined use of UV and ozone for aerosol disinfection. In these experiments the UV lamps were switched on during the ozone treatment.

6. Use of Selective Media for Counting of Airborne Pathogens

In two factory trials it was necessary to use selective media in an attempt to determine whether *Enterococcus* species or *Listeria* species were present as airborne contaminants. In both trials the SAS sampler was used as the test equipment. The medium chosen for *Enterococcus* spp was KF Streptococcus Agar (Oxoid CM701) (KFSA) and for *Listeria* spp was Listeria Selective Agar - Oxford Formulation (Oxoid CM856) plus selective supplement (Oxoid SR140). Red or pink colonies on KFSA were recorded as presumptive faecal streptococci while black colonies on the Listeria Agar were recorded as presumptive *Listeria* species.

RESULTS AND DISCUSSION

Disinfectant Fogging Experiments

Results from the disinfection trials are presented in Tables 1 to 4. The viable counts, determined as described in the methods section, taken after disinfection (Table 2) were subtracted from the results before disinfection (Table 1) to enable percentage reduction in viable counts to be calculated (Table 3). Mean percentage reduction figures were then calculated for the different disinfectant fogging systems (Table 4).

One of the most difficult aspects of the disinfectant fogging experiments was ensuring a uniform aerosol which would remain airborne for the duration of the experiment. This applied to both the bacterial and disinfectant aerosols. During the trials using UV lamps, for example, over a 20 min period after fogging the chamber with bacterial aerosol, settle plates recorded drops in counts of 1 to 4 log reductions (Table 9). No explanation for these differences was found. Addition of wetting agent to the bacterial suspension with the aim of altering the surface tension and hence droplet size proved inconclusive.

High speed photography of both the Collison bacterial aerosol generator and disinfectant spray (Figures 2, 3) illustrates the difference in aerosol generated by the two devices. The photographs of the disinfectant fogging spray highlighted a problem with the spray nozzle which was traced to a damaged perforated disc inside the nozzle. Replacement of the component produced a finer aerosol. Droplet size distribution can be affected by spray pressure, nozzle size and spray head geometry (Chaya and Hills, 1991). Marthi *et al.* (1990) observed that survival of *Pseudomonas syringae* was reduced in small droplets. Aerosol droplets also settle after a period of time even in a fan circulated chamber. Spurlock and Zottola (1991) found that aerosols of *Listeria monocytogenes* settled out

within 100-200 min. In all experiments it could be expected that some fallout of airborne microorganisms was taking place. In order to monitor fallout, a series of light emitting diodes and detectors were arranged vertically in the cabinet and connected to an oscilloscope. The system was sufficiently sensitive to detect the steam from a beaker of hot water 10 metres away. Initial trials suggested that with further development it may be possible to quantify fallout in the aerobiology cabinet using this technique. At present the system only gives a visual display on an oscilloscope of changes in aerosol density.

In the disinfectant trials the mean percentage reduction in viable counts varied from 69-89% with the Quat to 72-98% with amphoteric and 89-93% with hypochlorite (Table 4). However, closer examination of the data (Tables 1-3) demonstrated the difficulty in reproducing results from experiment to experiment and from sample to sample. This was ascribed to the fact that up until experiment 31, the outlet HEPA filter was switched on its lowest setting to create a negative pressure on the chamber. This was to ensure operator protection. However after experiment 31 the cabinet seals were improved and the HEPA filter was switched off. The result was a rise in the settle plate counts to uncountable levels. Results from later experiments (Table 10) appeared to give more consistent results.

In general, recovery of organisms on stainless steel coupons, settle plates, and in the cyclone and SAS samplers were not comparable. Organisms appeared not to be recovered consistently on the stainless steel. Drying out of the organisms on the stainless steel probably accounted for some of the low recoveries using this method. The settle plate technique suffered from the drawbacks of being only semi-quantitative and from uncountable numbers at high aerosol concentration. The use of diluent (which could be further diluted) in the settle plates (Tables 5-7) appeared to overcome the problem of high numbers, but the reproducibility still relied on the manual dexterity of the operator in covering the plates at the end of the exposure period. Counts would also be affected by deposition or fallout rate of organisms from the aerosol. The cyclone method was calculated to withdraw all the air from the chamber in the 2 min sampling period. Counts were generally, but not always, higher than with the settle plates. A high settle plate result would suggest that the aerosol had deposited quickly on the floor of the chamber leaving fewer organisms in the air for the cyclone to recover. The SAS method was not very useful for the aerobiology cabinet work. Numbers were much too high in the chamber even when the sampler was set to its lowest level (60 L air). In conclusion it appeared that comparisons of results from one method to another would not be meaningful. Hypochlorite and the amphoteric appeared to be better at killing airborne microorganisms than QUAT but as the mean reductions were of the same log order the

differences between chemicals is insignificant. However, the variability of the disinfection results with different recovery methods indicates that measurement of fogging effectiveness in factory situations requires careful evaluation and several replicates.

With the variabilities associated with the stainless steel coupons and settle plates it was difficult to measure the efficacy of the disinfectant fogging via a strict mass flow approach. The results from the stainless steel and settle plates do, however, give confidence in the reduction of airborne microorganisms by disinfectant fogging as no evidence was found to indicate a build up of microorganisms on the chamber floor or walls, ie loss of viability was apparent rather than settlement. This observation also applies to the UV and ozone data.

A survey of airborne microorganisms (Holah, 1995) indicated that mean numbers could range from less than 10/60L to over 500/60L. High risk area counts averaged 51/60L. To completely eliminate airborne bacteria in these situations would require at least 2-3 log reductions (99-99.9% reduction). While disinfectant fogging achieved this on occasions, the mean percentage reduction figures were all lower than 99%. It would appear therefore that disinfectant fogging alone may be insufficient to completely eliminate airborne microorganisms even in a high risk area where low counts would be expected prior to disinfection.

TABLE 1

RESULTS OF VIABLE COUNTS TAKEN BEFORE DISINFECTION USING DIFFERENT METHODS OF ASSESSING NUMBERS OF AIRBORNE BACTERIA

		QUAT											Amphoteric					
Expt. No		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Stainless steel coupons	W	40	220	30	-	-	0	80	0	240	20	10	1900	0	80	180	0	20
	W	1300	190	0	0	0	0	50	10	330	30	0	310	0	30	11	10	0
	W	0	0	0	0	0	0	130	0	0	20	20	30	10	0	90	0	980
	W	10	10	0	0	330	0	0	20	50	270	10	1000	0	20	20	2200	70
	F	*	10	520	0	0	0	690	90	40	0	20	1800	0	2.3E6	11	0	0
	F	10	260	0	710	0	0	280	0	50	0	30	890	0	0	40	10	10
	Control	0	0	0	0	20	0	10	10	0	0	30	2300	0	0	0	0	0
Settle plates	Control	269	704	68	24	18	9	0	1	*	178	0	33	6	2	31	73	42
	1	460	*	*	1000	2500	2200	*	620	406	1	1400	2400	308	1340	*	*	*
	2	320	*	*	1100	2600	*	*	1140	200	1	2320	2460	132	1930	*	*	*
	3	888	*	*	1E4	5550	1670	*	952	72	144	528	928	76	243	*	*	*
	4	1220	*	*	2600	2380	1710	*	704	60	580	74	1000	170	596	*	*	*
Cyclone	-	0	0	5500	1.5E4	240	5.6E6	1.1E8	1.2E8	3.1E8	2.8E9	1.4E5	7.4E8	2.1E8	9.9E8	0	1000	2600
SAS	-	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Suspension count		5.1E9	7.7E9	2.7E9	9.3E8	1.2E9	2.3E9	1.2E10	3.6E8	9.7E8	4.4E9	3.5E9	2.7E9	5.2E9	1.7E9	4.6E9	7.7E9	6.3E9

* Too many to count
- = No result

W = wall sample
F = floor sample

TABLE 1 (CONT ...)

RESULTS OF VIABLE COUNTS TAKEN BEFORE DISINFECTION USING DIFFERENT METHODS OF ASSESSING NUMBERS OF AIRBORNE BACTERIA

		Hypochlorite															Amphoteric	QUAT
Expt. No		18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
Stainless steel coupons	W	300	270	560	0	0	130	20	0	0	0	0	20	20	0	10	40	20
	W	1600	110	30	0	20	20	40	0	0	0	20	10	20	0	0	120	60
	W	200	130	10	60	0	10	0	0	0	0	40	10	30	0	30	60	40
	W	400	20	20	30	0	10	140	0	0	0	0	180	0	0	0	160	10
	F	1000	30	0	0	0	20	10	0	0	0	20	20	20	0	10	140	50
	F	920	20	20	0	0	0	10	0	0	0	0	40	10	40	120	200	60
	Control	10	30	10	0	10	10	200	0	20	0	0	10	80	10	30	10	38
Settle plates	Control	2320	123	73	71	0	18	0	0	0	61	8	5	1	0	7	68	83
	1	*	1760	*	*	336	*	462	135	2	224	8	2	5	17	*	*	*
	2	688	1310	*	*	420	*	704	222	0	288	164	6	8	36	*	*	*
	3	*	5920	*	*	98	484	42	23	0	25	37	0	0	0	*	*	*
	4		1970	*	*	126	692	226	39	0	44	45	1	0	0	*	*	*
Cyclone	-	0	110	1.7E7	0	1.2E9	4.6E7	420	360	97	31	0	2.7E4	1.4E6	1.6E6	1.2E5	3.3E5	9.1E5
SAS	-	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Suspension count		5.1E9	4.2E9	2.3E9	1.9E9	2.7E9	2.1E9	5.0E9	2.9E9	1.8E7	1.1E9	2.6E9	2.5E9	1.6E9	6.5E9	3.5E8	1.3E9	8.3E7

TABLE 2

VIABLE COUNTS BEFORE DISINFECTION MINUS VIABLE COUNTS AFTER DISINFECTION FROM DIFFERENT METHODS OF ASSESSING NUMBERS OF AIRBORNE BACTERIA

(Numbers in brackets indicate count higher after disinfection)

		QUAT											Amphoteric						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
Stainless steel coupons	W	30	220	30	-	-	0	60	(130)	240	(2.2E5)	(30)	1840	(10)	70	180	(20)	20	
	W	(1700)	190	0	0	0	0	(440)	(6890)	330	(130)	(30)	290	(280)	30	11	10	0	
	W	(20)	0	0	0	-	0	90	(60)	(10)	20	(70)	(40)	(10)	0	90	0	980	
	W	0	(160)	0	0	-	0	(620)	10	40	250	(310)	(500)	(10)	(80)	20	2200		
	F	*	0	520	0	0	0	620	90	40	(10)	0	1770	(90)	2.3E6	11	0	0	
	F	10	260	0	710	-	0	250	0	50	(20)	10	830	(10)	0	40	10	10	
	Control	-	-	-	-	-	(1400)	10	(20)	0	0	0	2290	0	0	0	0	(160)	
Settle plates	Control	-	-	-	-	-	(65)	(215)	(152)	-	(195)	(2)	(147)	(58)	(151)	13	23	(*)	
	1	122	*	-	1000	-	2200	*	620	406	1	1400	2400	308	1340	*	*	*	
	2	(352)	*	*	1100	2600	*	*	1139	199	1	2302	2453	130	1930	*	*	*	
	3	544	*	*	1E4	3550	1422	-	935	64	(612)	417	839	62	243	*	*	*	
	4	1140	*	*	2600	2300	1615	-	704	60	(244)	48	944	166	596	*	*	*	*
Cyclone	-	0	0	*	1.2E4	110	2.6E6	(2.0E7)	(5E7)	2.9E8	1.8E9	(6.1E5)	(2.5E8)	1.95E8	3.6E8	0	(400)	2290	
SAS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* Too many to count
- = No result

W = wall sample
F = floor sample

TABLE 2 (CONT...)

VIABLE COUNTS BEFORE DISINFECTION MINUS VIABLE COUNTS AFTER DISINFECTION FROM DIFFERENT METHODS OF ASSESSING NUMBERS OF AIRBORNE BACTERIA
(Numbers in brackets indicate count higher after disinfection)

Expt. No		Hypochlorite															Amphoteric	QUAT
		18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
Stainless steel coupons	W	300	270	560	0	(10)	130	20	0	0	0	0	10	20	0	10	20	-
	W	1600	110	30	0	20	10	20	0	0	0	20	10	10	0	0	(180)	60
	W	200	90	10	60	0	0	0	0	0	0	40	180	30	0	30	(6.3E8)	40
	W		20	20	30	(10)	10	140	0	0	0	0	40	(60)	(10)	(460)	160	10
	F	1000	30	0	0	0	20	(90)	0	0	0	20	20	20	0	10	130	50
	F	92010	20	0	0	0	10	0	0	0	0	10	10	40	120		190	40
	Control	0	20	10	0	10	10	150	(10)	20	0	0	0	70	0	30	(50)	30
Settle plates	Control	2241	1	34	32	0	13	(8)	(4)	0	52	(9)	3	(3)	(17)	7	(128)	83
	1	*	1760	*	*	336	*	462	135	2	224	8	2	3	16	*	*	*
	2	*	1310	*	*	420	*	704	222	0	288	164	1	8	9	*	*	*
	3	198	5920	*	*	98	460	16	23	0	25	37	0	(2)	(4)	*	*	*
	4	*	1970	*	*	126	482	215	37	0	44	15	1	0	(1)	*	*	*
Cyclone	-	2010	110	1.6E7	0	(7E8)	4.3E7	420	334	70	31	(150)	2.7E4	1.4E6	1.3E6	5.1E4	2.3E5	8.4E5
SAS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

TABLE 3

PERCENTAGE REDUCTION IN VIABLE COUNTS AFTER DISINFECTION FROM DIFFERENT METHODS OF ASSESSING NUMBERS OF AIRBORNE BACTERIA

		QUAT											Amphoteric					
Expt. No		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Temp °C		-	-	16.5	-	-	-	15.8	19.1	18.6	19.2	17.8	15.8	18.5	-	17.1	16.0	18.1
R.H %		-	-	75.9	-	-	-	84.8	74.6	83.4	75.4	76.1	80.5	46.4	-	79.3	70.8	73.2
Stainless steel coupons	W	75	100	100	-	-	-	75	-	100	-	-	97	-	88	100	-	100
	W	-	100	-	-	-	-	-	-	100	-	-	94	-	100	100	100	-
	W	-	-	-	-	-	-	69	-	-	100	-	-	-	-	100	-	100
	W	0	-	-	-	-	-	-	50	80	93	-	-	-	-	100	100	100
	F	100	0	100	-	-	-	90	100	100	-	0	98	-	100	100	-	-
	F	100	100	-	100	-	-	89	-	100	-	33	93	-	-	100	100	100
Settle plates	1	27	100	0	100	98	100	97	100	100	100	100	100	100	100	100	100	100
	2	-	100	100	100	100	98	88	100	100	100	99	100	98	100	100	100	100
	3	61	100	100	100	64	85	0	98	89	-	79	90	82	100	100	100	100
	4	93	100	100	100	97	94	0	100	100	-	65	94	98	100	99	99	99
Cyclone	-	-	-	65	77	46	46	-	-	95	64	-	-	93	36	-	-	88

- = No result

W = wall sample
F = floor sample

TABLE 3 (CONT ...)

PERCENTAGE REDUCTION IN VIABLE COUNTS AFTER DISINFECTION FROM DIFFERENT METHODS OF ASSESSING NUMBERS OF AIRBORNE BACTERIA

		Hypochlorite															Amphoteric	QUAT
Expt. No		18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
Temp °C		16.6	17.1	19.1	16.3	16.9	16.6	17.3	15.3	17.8	14.1	15.6	-	18.3	17.9	17.3	18.0	17.0
R.H %		82.3	81.5	73.9	79.4	61.0	62.2	36.1	37.5	40.0	91.0	55.4	-	-	-	77.0	61.3	77.5
Stainless steel coupons	W	100	100	100	-	-	100	100	-	-	-	-	50	100	-	100	50	-
	W	100	100	100	-	100	50	50	-	-	-	100	100	50	-	-	-	100
	W	100	69	100	100	-	0	-	-	-	-	100	100	100	-	100	-	100
	W	100	100	100	100	-	100	100	-	-	-	-	100	-	-	-	100	100
	F	100	100	-	-	-	100	-	-	-	-	100	100	100	-	100	93	100
	F	100	50	100	-	-	-	100	-	-	-	-	100	100	100	100	95	67
Settle plates	1	100	100	100	100	100	100	100	100	100	100	100	100	60	94	100	89	-
	2	100	100	100	100	100	100	100	100	-	100	100	17	100	25	100	93	-
	3	29	100	100	100	100	95	38	100	-	100	100	-	-	-	98	99	-
	4	66	100	100	100	100	70	95	95	-	100	33	100	-	-	98	100	-
Cyclone	-	91	100	97	-	-	93	100	93	72	100	-	100	100	83	43	70	92

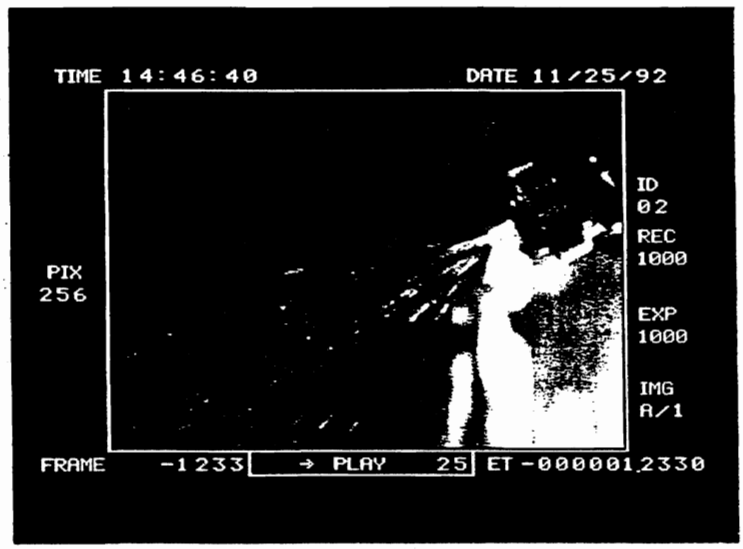
TABLE 4

MEAN PERCENTAGE REDUCTION IN VIABLE COUNTS AFTER DISINFECTION FROM DIFFERENT METHODS OF ASSESSING NUMBERS OF AIRBORNE BACTERIA

	QUAT		Amphoteric		Hypochlorite	
	Mean	Range	Mean	Range	Mean	Range
Coupons	89	0-100	96	50-100	92	0-100
Settle Plates	84	0-100	98	82-100	93	17-100
Cyclone	69	46-95	72	36-93	89	43-100

FIGURE 2

HIGH SPEED (1/1000 SEC) PHOTOGRAPHS OF AEROSOL PRODUCED BY COLLISON AEROSOL NEBULISER



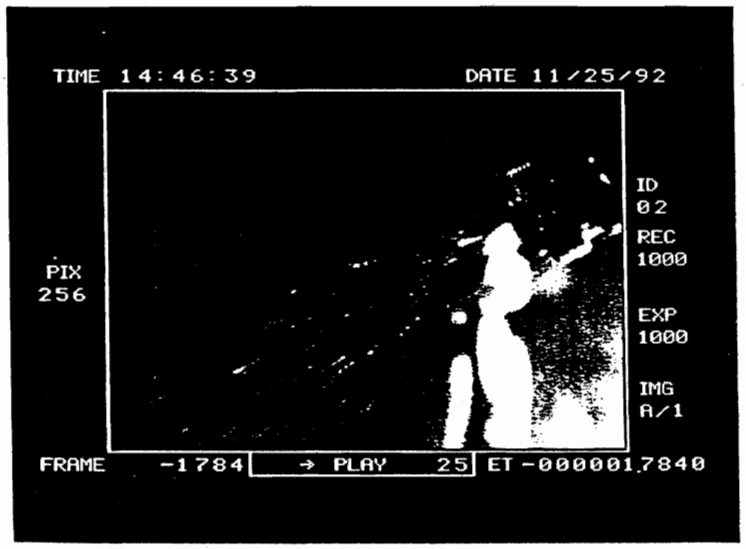


FIGURE 3

HIGH SPEED (1/1000 SEC) PHOTOGRAPHS OF DISINFECTANT FOGGING SPRAY

UV Experiments

Results for UV experiments (Tables 5 - 11) again illustrate the differences in results from different sampling methods (diluent plate count, agar plate count, SAS and cyclone). It is therefore necessary to consider results from each sampling method individually to estimate numbers of log reductions achieved, and then compare trends from the different methods. It is not valid to compare directly results from one method with another since each method uses different principles of sampling (eg settling or impaction). It is possible, for example, for settle plate results to be high while SAS or cyclone counts are low because organisms have settled out quickly. Conversely settle plate results may be low compared to SAS or cyclone counts because organisms have remained airborne. Table 10 shows the numbers of organisms recovered using settle plate techniques at 5 min intervals for up to 20 min. These trials were done immediately after the experiments using 1, 2 and 3 UV lamps using the same suspension. Zero counts were those obtained from the UV experiments. In two of the three trials, results remained consistent, but in the third, organisms were not recovered to the same extent. Because of the experimental procedure it was not possible to deduce precisely why organisms were apparently 'lost' from the chamber. Cyclone results for 3 lamps (Table 7) remained relatively high for up to 20 min exposure suggesting that perhaps the aerosol was not settling out on the agar plates.

UV exposure was easier to control than the disinfectant fogging. The important factors appeared to relate to fallout rate of the bacterial aerosol (Tables 8 - 10). UV proved effective in reducing the airborne microorganism count by up to 4 log reductions over a 20 min period. Increasing the number of lamps illuminated from 1 to 4 progressively increased the rate of kill. There was also some evidence (Table 11) that UV and hypochlorite fogging had a synergistic effect since the counts with the combined treatment were lower than each treatment separately. The other noticeable factor was that the results were more consistent than in the disinfectant fogging trials discussed earlier.

TABLE 5

RESULTS OF EXPOSURE OF AEROSOL TO ONE UV LAMP FOR DIFFERENT TIME PERIODS

Exposure Time (min)	Diluent Plate Count (5 min)	Agar Plate Count (5 min)	SAS # Count (per 60L)	Cyclone # Count (2 min)
0	9.45×10^2 2.27×10^4 2.43×10^4 3.59×10^4	* * * *	*	4.68×10^5
5	2 10 1 5	62 83 58 62	500	9.40×10^4
10	2 5 26 2	24 22 34 19	375	4.50×10^4
15	1 1 2 1	22 9 13 14	132	1.26×10^4
20	1 0 0 0	1 0 0 0	3	5.23×10^2

* Too many to count

\# Taken at end of exposure period

TABLE 6

RESULTS OF EXPOSURE OF AEROSOL TO TWO UV LAMPS
FOR DIFFERENT TIME PERIODS

Exposure Time (min)	Diluent Plate Count (5 min)	Agar Plate Count (5 min)	SAS # Count (per 60L)	Cyclone # Count (2 min)
0	4.40×10^4 4.21×10^4 3.71×10^4 3.76×10^4	* * * *	*	8.65×10^4
5	21 9 11 5	21 20 17 10	250	6.93×10^3
10	2 3 7 9	6 3 10 9	103	3.62×10^3
15	3 0 9 0	3 1 2 2	43	2.71×10^3
20	1 6 0 0	2 0 0 1	21	2.53×10^3

* Too many to count

\# Taken at end of exposure period

TABLE 7

RESULTS OF EXPOSURE OF AEROSOL TO THREE UV LAMPS
FOR DIFFERENT TIME PERIODS

Exposure Time (min)	Diluent Plate Count (5 min)	Agar Plate Count (5 min)	SAS # Count (per 60L)	Cyclone # Count (2 min)
0	8.01×10^3 1.69×10^4 1.16×10^4 8.73×10^4	- - - -	75	2.43×10^4
5	<9 <9 900 <9	0 0 0 0	2	2.96×10^2
10	<9 <9 <9 <9	2 0 0 1	3	8.19×10^2
15	<9 <9 <9 <9	0 0 0 0	0	5.40×10^2
20	<9 <9 <9 <9	0 0 0 0	0	2.21×10^3

Taken at end of exposure period

TABLE 8

SETTLE PLATE COUNTS AFTER DIFFERENT UV (4 LAMP) TREATMENTS

UV Exposure Time (Min)	Ranging Experiment Settle Plate Counts (4 min exposure)			
0	*	*	*	*
1	*	*	*	*
10	1.6×10^3	149	135	124

* Too many colonies to count.

TABLE 9

RESULTS OF EXPOSURE OF AEROSOL TO FOUR UV LAMPS
FOR DIFFERENT TIME PERIODS

UV Exposure Time (Min)	Agar Settle Plate Counts (5 min exposure)				SAS Counts per 60L	Cyclone Counts (2 min)
0	4000	4000	4000	4000	*	3.4×10^6
5	25	23	3	18	96	-
10	24	33	99	53	224	-
15	7	31	7	9	85	-
20	156	1	1	1	17	7.5×10^3

* Too many to count

Initial suspension sprayed into chamber = 1.4×10^9 organisms/5 min aerosol

TABLE 10

RESULTS FROM DILUENT SETTLE PLATES EXPOSED FOR 5 MIN INTERVALS AT DIFFERENT TIME INTERVALS AFTER FOGGING THE CHAMBER WITH MICROBIAL AEROSOL

	Time elapsed after initial 5 min fogging period				
	0	5	10	15	20
Trial 1 (one UV lamp)	9.45×10^2 2.27×10^4 2.43×10^4 3.59×10^4	2.21×10^3 2.98×10^3 5.90×10^3 6.03×10^3	5.00×10^2 2.16×10^3 1.22×10^3 1.49×10^3	9.90×10^2 7.20×10^2 5.40×10^2 9.45×10^2	8.10×10^2 1.04×10^3 3.15×10^2 1.03×10^3
Trial 2 (two UV lamps)	4.40×10^4 4.21×10^4 3.71×10^4 3.76×10^4	1.76×10^4 1.84×10^4 1.40×10^3 1.69×10^3	3.56×10^4 7.01×10^3 3.92×10^4 9.68×10^3	7.02×10^3 1.04×10^3 4.86×10^3 7.43×10^3	2.75×10^3 1.89×10^3 2.12×10^3 2.03×10^3
Trial 3 (three UV lamps)	8.01×10^3 1.69×10^4 1.16×10^4 8.73×10^3	405 540 405 315	135 315 90 405	0 45 0 90	135 0 0 45

TABLE 11

COMPARISON OF RESULTS OF AEROSOLS TREATED WITH EITHER UV OR 2% HYPOCHLORITE AND IN COMBINATION

a. Exposure Plates Containing Diluent

No Treatment (5 min aerosol) (5 min exposure)	UV (4 lamps) 5 min aerosol 1.5 min UV	2% hypochlorite 5 min aerosol 30 sec disinfectant	UV + hypochlorite 5 min aerosol 30 sec disinfectant + 1.5 min UV
7.0×10^4	44	58	0
6.8×10^4	53	0	0
6.6×10^4	80	0	0
9.8×10^4	35	1	0

b. Exposure Plates Containing Agar

No Treatment (5 min aerosol) (5 min exposure)	UV (4 lamps) 5 min aerosol 1.5 min UV	2% hypochlorite 5 min aerosol 30 sec disinfectant	UV + hypochlorite 5 min aerosol 30 sec disinfectant + 1.5 min UV
*	*	5	1
*	*	0	2
*	*	0	4
*	*	70	119

* Too many to count

c. SAS results

No Treatment (5 min aerosol) (5 min exposure)	UV (4 lamps) 5 min aerosol 1.5 min UV	2% hypochlorite 5 min aerosol 30 sec disinfectant	UV + hypochlorite 5 min aerosol 30 sec disinfectant + 1.5 min UV
*	*	*	*

* Too many to count

d. Cyclone results

No Treatment (5 min aerosol) (5 min exposure)	UV (4 lamps) 5 min aerosol 1.5 min UV	2% hypochlorite 5 min aerosol 30 sec disinfectant	UV + hypochlorite 5 min aerosol 30 sec disinfectant + 1.5 min UV
1.56×10^6	8.22×10^3	448	53

Ozone Experiments

Exposure to ozone above 4ppm (5 min, 10 min exposure) generated by the Triox 'Trucker' was effective in significantly reducing the numbers of airborne *P.aeruginosa.* Cyclone results indicated that 2-4 log reductions were possible in 5-10 mins operation of the unit in combination with the volume of the aerobiology chamber. There appeared, however, to be some variation from one experiment to another in the levels of ozone generated in the cabinet after comparative time intervals (Table 12). This may explain some of the differences between replicate trials (Table 13).

There may be some synergism between ozone and UV (Table 14) but insufficient data has been obtained to draw any firm conclusions. This aspect requires further investigation.

On the basis of the limited trials which were done with ozone, it was effective in reducing airborne contamination with *P.aeruginosa* and also had an advantage over disinfectant fogging in that levels of ozone could be readily monitored and more even dispersion of ozone was possible. The effectiveness of disinfectant fogging relies very much on the dispersion of the droplets of disinfectant. Ozone is a dry disinfectant procedure which has particular advantages in factory situations where electrical equipment is involved. It also has an advantage over UV since it is unaffected by shielding. UV light is only effective in the areas where the light reaches.

Comparisons

Of the various methods of disinfection examined, ozone appeared to be both effective and reproducible in its effect. In the conditions of the trials, UV was also effective but shadowing of organisms could be a problem in a factory environment. The least predictable method of disinfection was the fogging technique. Sometimes the fogging was effective and produced 100% kill and sometimes no destruction was observed. On the basis that 2-3 log reductions (99 - 99.9% reduction) appears to be the minimum required to eliminate airborne contamination as shown by the figures in section 1, both UV and ozone were the most predictable and controllable and could potentially achieve the desired reduction in count. Disinfectant fogging was the least predictable, controllable and effective. Further work on methods of air sterilisation appears to be necessary in order to improve both the methods of disinfection themselves and the methods of monitoring the effectiveness, particularly in factory situations.

Selective Media Experiments

The use of selective media in sampling for airborne factory contaminants has attracted little research. However there are occasions when it is necessary to determine whether airborne pathogens are present, particularly in high care factory production areas. Using KF Streptococcus Agar and Listeria Agar (Table 15) for both air sampling and contact plates indicated that selective media could be used for detection of airborne pathogens. The recovery rate could not be determined in these experiments. Further work is necessary to quantify the recovery rate of airborne organisms using selective media. In a factory situation it is very important that the recovery potential of the method is known in order to avoid the situation where false negative results or low recovery gives a false sense of security.

TABLE 12

OZONE LEVELS (PPM) IN AEROBIOLOGY CHAMBER AFTER OPERATING TRIOX 'TRUCKER' OZONE GENERATOR FOR DIFFERENT TIME INTERVALS

Time (sec)	Experiment Number						
	1	2	3	4	5	6	7
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	-	0.2	0.4	0.1	-	0.2	0.2
48	-	0.4	0.8	0.9	-	0.5	0.6
72	-	0.9	1.1	1.4	2.8	0.6	1.4
96	-	1.5	1.5	1.8	3.9		1.8
120	2.6	2.1	2.0	2.3	5.0		2.5
144	3.3	2.9	2.3	2.9	6.2		3.0
168	3.9	3.5	2.8	3.4	7.3		3.4
192	4.1	4.2	2.9	3.8	8.2		4.0
216		4.7		4.3	9.2		4.5
240		5.2		4.4	10.1		4.6
264		5.6		4.5	10.9		4.6
288		6.1		4.2	11.8		4.9
312		6.4		4.0	12.0*		5.2
336					11.7		5.2
360					11.3		5.6
384					10.9		5.9
408					10.5		6.1
432					10.2		6.2
456					9.9		6.4
480					9.5		6.7
504					9.1		7.1
528					8.8		7.4
552					8.4		7.2
576					8.0		7.1
600					7.7		7.0

* Ozone generator switched off at this point.

TABLE 13

RESULTS FROM OZONE DISINFECTION TREATMENTS

Variable	After 5 min aerosol	After treatment time (no ozone)	After treatment time (with ozone)	Ozone level at end of treatment time (ppm)
1. 1 min exposure				
Settle plate results	480 456 572 300	75 79 69 68	51 39 45 46	0.6
Cyclone results		1.56×10^5	4.34×10^3	
2. 3 min exposure				
	Run 1 Run 2	Run 1 Run 2	Run 1 Run 2	Run 1 Run 2
Settle plate results	111 * 118 * 133 * 139 *	53 * 47 * 47 * 51 *	14 224 9 280 7 328 12 316	4.1 2.9
Cyclone results		8.34×10^3 1.20×10^6	2.91×10^3 2.05×10^3	
3. 5 min exposure				
Settle plate results	1160 * 1008 * 968 * 864 *	66 * 54 * 81 * 384 *	0 11 0 27 0 20 0 4	6.4 4.0
Cyclone results		1.09×10^4 1.88×10^6	5.08×10^2 3.90×10^2	
4. 10 min exposure				
Settle plates	272 264 288 348	2 0 2 4	0 0 0 0	7.0
Cyclone results		4.38×10^3	2.42×10^3	

* = Too many too count

TABLE 14

RESULTS FROM COMBINED OZONE/UV EXPERIMENTS

Variable	After 5 min aerosol	After treatment time (no ozone or UV)	After treatment time (ozone + UV)	Ozone level at end of treatment time (ppm)
1 min exposure				
a Experiment 1 (one UV lamp)				
Settle plate results	38	1	0	1.6
	56	8	0	
	94	10	0	
	48	5	0	
Cyclone results		1.16×10^3	1.47×10^3	
b Experiment 2 (3 UV lamps)				
Settle plate results				
	280	0	121	0.9
	556	1	0	
	122	0	0	
	91	1	0	
3 min exposure				
a Experiment 3 (one UV lamp)				
Settle plate results	45	2	0	4.7
	41	3	0	
	94	1	0	
	55	4	0	
Cyclone results		5.54×10^2	2.42×10^1	
b Experiment 4 (3 UV lamps)				
Settle plate results				
	*	712	0	1.6
	*	768	0	
	*	904	0	
	*	576	0	

* = Too many too count

TABLE 15

RESULTS FROM USE OF SELECTIVE MEDIA IN FACTORY TRIALS

No. of Samples	KF Streptococcus Agar		Listeria Agar		
	No. of Positive samples	Colony Counts from Positive plates	No. of samples	No. of Positive Samples	Colony Counts* from Positive Plates
1. Air Samples (per 60L) 10	3	6, 6 (2) 33	24	3	1 6 8
2. Contact plates 14	2	1 14	23	10	1 3, 3 22 38 72 100, 100 120 270

* *L. monocytogenes* was only confirmed in 2 of the contact plate samples.

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Biotest (UK) Ltd., Solihull for supply of RCS air sampler.
Triox Ltd, Swindon for loan of Triox trucker ozone apparatus.
Hanovia Ltd, Slough for loan of UV equipment.
Cherwell Laboratories Ltd, Bicester, for supply of SAS air sampler.
H&M Disinfection Systems Ltd, Northwich for supply of disinfectant spray.