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Ortho-phthalaldehyde: a possible alternative to glutaraldehyde for high level disinfection

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S.E. WALSH, J.-Y. MAILLARD AND A.D. RUSSELL. 1999. *Ortho*-phthalaldehyde (OPA) was tested against a range of organisms including glutaraldehyde-resistant mycobacteria, *Bacillus subtilis* spores and coat-defective spores. Glutaraldehyde (GTA) and peracetic acid (PAA) were tested for comparative purposes. Both suspension and carrier tests were performed using a range of concentrations and exposure times. All three biocides were very effective ($\geqslant$ 5 log reduction) against *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* in suspension tests. OPA and GTA (PAA was not tested) were also very effective against *Staph. aureus* and *Ps. aeruginosa* in carrier tests. OPA showed good activity against the mycobacteria tested including the two GTA-resistant strains, but 0·5% w/v OPA was found not to be sporicidal. However, limited activity was found with higher concentrations and pH values. Coat-defective spores were more susceptible to OPA, suggesting that the coat may be responsible for this resistance. The findings of this study suggest that OPA is effective against GTA-resistant mycobacteria and that it is a viable alternative to GTA for high level disinfection.

INTRODUCTION

Glutaraldehyde (GTA) has been used as a disinfecting/sterilizing agent for over 30 years (Russell 1994). Alkaline GTA (2% v/v) has a broad range of activity and rapid antimicrobial action as well being non-corrosive to metals, rubber and lenses. However, potential mutagenic and carcinogenic effects have been reported (Hugo and Russell 1999) as well as skin and eye irritation and respiratory disorders (Russell 1994). The risk to personnel and the increasing frequency of GTA-resistant *Mycobacterium chelonae* (Griffiths *et al*. 1998a) has highlighted the need for a replacement.

One possible alternative to GTA is *ortho*-phthalaldehyde (OPA). OPA is already used in amino acid analysis (Carlomagno *et al*. 1985; Blundell and Brydon 1987) but 0·5% (w/v) OPA has also been demonstrated to be bactericidal. When assessed in the disinfection of 100 endoscopes, OPA was found to be effective without activation and was stable over a 14-day usage cycle (Alfa and Sitter 1994). Mycobactericidal activity has also been demonstrated against *M. bovis* with a 5 log reduction after 5 min exposure to 0·2% OPA (Roberts and Chan-Myers 1998).

Another possible GTA alternative is peracetic acid (PAA). PAA was introduced as an antibacterial agent in 1955; it has a broad spectrum of activity including bacteria, spores, moulds, yeasts, algae and viruses (Hugo and Russell 1998). PAA is a powerful oxidizing agent and can be corrosive to some metals. However, corrosive problems can be reduced by using commercial formulations and 13 months usage of PAA gave no overtly visible signs of corrosion of flexible endoscopes using the Steris system (DiagMed Ltd, Norby, UK) (Mannion 1995). 'Nu-Cidex', an equilibrium mixture of acetic acid, PAA, and hydrogen peroxide has been shown to be rapidly mycobactericidal, including against drug-resistant isolates of *Mycobacterium tuberculosis* and *M. avium-intracellulare,* after only 5 min (Holton *et al*. 1995).

This study was initiated to investigate the biocidal properties of OPA against a range of non-acid-fast non-sporulating organisms, GTA-sensitive and resistant mycobacteria and *B. subtilis* spores. A suspension test and a carrier test were used to evaluate the activity of the biocides; this was considered necessary as it has been shown that disinfectants with high activity in suspension tests are not necessarily as active on contaminated surfaces (Best *et al*. 1988). Spores treated with

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urea/dithiothreitol/sodium lauryl sulphate (UDS), pH 10·3, were also used. The removal of protein from the spore coat has been shown to dramatically increase the sensitivity of *B. subtilis* to alkaline GTA (Gorman *et al.* 1984) and the same effect was thought likely to occur with OPA. Comparisons with GTA and PAA were carried out to assess OPA activity.

MATERIALS AND METHODS

Test organisms

Three organisms were used in the suspension tests, *Escherichia coli* ATCC 9481, *Staphylococcus aureus* NCTC 6571 and *Pseudomonas aeruginosa* PA01, a laboratory strain.

These bacteria were inoculated into 10 ml Nutrient broth (Oxoid) and grown overnight for 18 h at 37 °C in a Gallenkamp water bath (Crawley, UK) shaking at 100 rev min^{-1}.

The carrier tests also used five mycobacterial strains and spores of *B. subtilis* 168. The mycobacteria used were *M. terrae* NCTC 10856, *M. chelonae* var. *abcessus* NCTC 10882, *M. chelonae* NCTC 946 and two glutaraldehyde-resistant washer isolates, *M. chelonae* Harefield and Epping (obtained from Dr P.A. Griffiths, Hospital Infection Research Laboratory, City Hospital NHS Trust, Dudley Road, Birmingham). *Escherichia coli* was omitted from the carrier tests as preliminary experiments showed low survival when dried on to the carriers.

The mycobacteria were inoculated into 10 ml 7H9 broth in vented cell culture flasks (Costar) and incubated at 30 °C for 14 d, except for *M. terrae* and *M. chelonae* var. *abcessus* which were incubated at 37 °C for 21 and 14 d, respectively. Cultures of *B. subtilis* 168 were grown for 18 h at 37 °C in Nutrient broth (Knott 1993). Aliquots (5 ml) of these suspensions were used to inoculate Roux flasks containing Nutrient agar (Oxoid) and 0·0001% w/v manganese sulphate (BDH). The flasks were incubated for 7 d at 37 °C before harvesting using 28 ml sterile deionized water. The resulting suspensions were centrifuged three times at 500 *g* for 10 min and then once for 25 min, re-suspending in 10 ml sterile deionized water. The spore suspensions were stored at 4 °C in 1 ml aliquots.

Disinfectants

Three high level disinfectants were tested, *ortho*-phthalaldehyde (Johnson & Johnson), glutaraldehyde (Sigma) and peracetic acid (Aldrich). However, only OPA and GTA were used in the carrier tests. Biocide solutions were diluted to the required concentration with sterile deionized water, and GTA was activated by increasing the pH of the solution to 8 using sodium hydrogen carbonate (Fisher). The same procedure was used to increase the pH of OPA for some of the carrier tests.

Preparation of test suspensions

Suspension tests. Aliquots (1 ml) of the bacterial suspensions were taken from the overnight cultures and used as the test inocula. The final concentration of bacteria in the biocide at zero time was approximately 7–8 $\log_{10}$ cfu ml^{-1}.

Carrier tests. For *E. coli*, *Staph. aureus* and *Ps. aeruginosa*, 8 ml of each culture were centrifuged twice at 2000 *g* to remove the growth medium. The first resuspension was carried out in 8 ml and the second, in 0·5 ml sterile deionized water to increase the number of cfu ml^{-1}.

With the mycobacterial suspensions, 5 ml of each culture was centrifuged twice at 2000 *g* to remove the growth medium. The first resuspension was carried out in 5 ml and the second, in 0·5 ml sterile deionized water. For spores, the 1 ml aliquots were centrifuged to produce a pellet which was resuspended in 0·5 ml sterile deionized water. The final number of bacteria in the biocide at zero time was approximately 8–9 $\log_{10}$ cfu ml^{-1}.

Preparation of coat-defective spores (Gorman *et al.* 1984)

A UDS solution was prepared containing urea 8 mol l^{-1} (BDH), dithiothreitol 50 mmol l^{-1} (Sigma) and sodium lauryl sulphate (SLS) 1% w/v (Sigma) in 0·1 mol l^{-1} sodium chloride (Fisher) at pH 10·3 and equilibrated at 37 °C; 4·5 ml UDS were added to 0·5 ml of spore test suspension and shaken at 37 °C for 180 min. The suspension was spun three times for 15 min at 2000 *g* to remove the UDS solution, and the final resuspension was carried out using 0·5 ml sterile deionized water. The UDS-treated spores were stored overnight at 4 °C before use.

The final number of bacteria in the biocide at zero time was approximately 7–8 $\log_{10}$ cfu ml^{-1}.

Preparation of carriers (based on a method developed by S. A. Sattar and V. Susan Springthorpe, University of Ottawa)

The suspensions were vortexed to mix them and transported to a laminar air flow safety cabinet; 10 μl volumes were pipetted into each sterile carrier (flat-bottomed glass bottles (Fisher, Loughborough, UK)), taking care not to touch the edges, and left to dry for 2–3 h. When the suspension was dry, sterile caps were screwed onto the bottles which were then removed from the cabinet.

Neutralization and recovery

Before testing the disinfectants, neutralization tests were carried out.

Suspension tests. For the suspension tests, 1 ml bacterial culture was added to 8 ml neutralizer, then 1 ml of the appropriate biocide was added and left for 10 min. Titre was verified by viable count. The log reduction of the culture was calculated by subtracting the final number of colony-forming units from the original number in the 18 h culture.

The appropriate concentrations (w/v) of neutralizers were determined to be: 0·5% sodium bisulphite (Aldrich) for OPA, 3% sodium thiosulphate (BDH) for PAA, 5% glycine for 0·1 ml GTA ($>$ 0·2% v/v) and 5% glycine for 1 ml GTA ($<$ 0·2% v/v).

The use of 0·1 ml GTA in the neutralization process with concentrations above 0·2% (v/v) meant that the sensitivity of the method was decreased by 1 log.

Carrier tests. For the carrier tests, 0·9 ml neutralizer and 0·1 ml biocide were added to the dried cultures and left for at least 10 min. Sterile glass-coated magnets (Fisher) were added to the bottles aseptically and a magnetic stirrer used to resuspend the cells from the base of the carrier; the resulting suspension was then vortexed. The number of cfu remaining was determined using the drop counting method on nutrient agar and subtracted from the number of cfu ml^{-1} in the centrifuged culture to calculate log reduction.

The use of 0·9 ml 0·5% (w/v) sodium bisulphite was found to be an effective neutralizer of OPA without inhibiting the growth of the test organisms, and 0·9 ml 5% (w/v) glycine was found to be an effective neutralizer of GTA, except for 2% (v/v) GTA with *Staph. aureus* and *Ps. aeruginosa*.

The use of a larger volume of glycine (9·9 ml) with *Staph. aureus* and *Ps. aeruginosa* decreased log reduction to a more acceptable level. However, using 10 times the volume of neutralizer decreased the sensitivity of the experiment by 1 log. Therefore, a lower concentration of GTA (0·2% v/v) was tested with 0·9 ml glycine to determine whether this decrease could be avoided in some experiments; this procedure was found to be satisfactory (less than 1 log reduction).

Suspension test

A 1 ml sample of culture was added to 9 ml biocide (prepared fresh for each experiment) and left for a specific contact time, after which 1 ml (for OPA, PAA and GTA 0·2% (v/v) or less) or 0·1 ml (for GTA $>$ 0·2% (v/v)) was removed and added to 9 ml or 9·9 ml of the appropriate concentration of neutralizer. Neutralization was allowed to occur for 10 min, and residual viability was determined by using the drop counting method on nutrient agar.

Log reduction was then calculated from $\log N_c - \log N_b$ where N_c and N_b represent the numbers of cfu ml^{-1} in the control and biocide solutions, respectively.

Carrier test

Test carriers were exposed to 0·1 ml biocide for the appropriate time before neutralization. Control carriers were exposed to 1 ml neutralizer for at least 10 min

Sterile glass-coated magnets were added aseptically to the bottles and a magnetic stirrer was used to resuspend the cells from the base of the carriers; the resulting suspensions were then vortexed. The number of cfu remaining was determined using the drop counting method on nutrient agar (or 7H11 agar for the mycobacteria). Log reduction was calculated using the equation above.

Spore suspension test

This was carried out identically to the carrier test except that the spore suspension was not dried onto the carrier. Instead, 10 μl volumes of suspension were pipetted onto the bottom of the carrier bottles just prior to testing.

RESULTS

Suspension tests

All three biocides were effective against the test organisms (Tables 1, 2 and 3) with concentrations at or below the recommended in-use concentrations resulting in a $\geqslant 5$ log reduction in viability within 1 min of exposure. Very low concentrations remained biocidal; 0·045% v/v GTA caused a $\geqslant 5$ log reduction in 5 min with all three organisms (Table 1), as did 0·0045% v/v PAA (Table 2) and 0·018% w/v OPA (Table 3).

Carrier tests

OPA 0·5% w/v was very effective against dried *Ps. aeruginosa* (Table 4) and *Staph. aureus* (data not shown), with a 5 log reduction in cfu ml^{-1} within 1 min exposure time and a $\geqslant 6$ log reduction within 2–5 min. This agreed with the results from the suspension tests ($\geqslant 5$ log reduction in 1 min), so drying the cells onto carriers appeared to have little effect on the efficacy of full strength OPA. However, the effectiveness of the lower concentration of OPA used (0·018% w/v) did seem to be reduced, although this could be attributed partly to the larger inoculum size. The time for a $\geqslant 5$ log reduction of *Staph. aureus* doubled from 5 to 10 min and for *Ps. aeruginosa*, increased from 1 min to 10 min.

The results for 2% (v/v) GTA showed a high level of activity against dried samples of these organisms with a $\geqslant 5$ log reduction within 1 min (*Staph. aureus*) or 2 min (*Ps. aeruginosa*) (Table 4). Again, with the lower concentration of biocide (0·09% v/v GTA), the time for a $\geqslant 5$ log reduction of *Staph. aureus* doubled from 5 to 10 min but with *Ps.*

Table 1 Effect of glutaraldehyde on *Pseudomonas aeruginosa* at 25 °C using the suspension test

Contact time (min)	Mean log reduction at GTA concentration (% v/v)							
	1·8	0·45	0·18	0·09	0·045	0·018	0·009	0·0045
1	⩾4	⩾4	4·08	2·72	0·99	0·074	0·01	0·01
2	⩾4	⩾4	4·22	4·51	2·36	0·18	0·06	0·02
5	⩾4	4·07	4·56	3·92	⩾5	0·76	0·14	0·05
10	⩾4	⩾4	5·41	5·27	—*	2·53	0·40	0·08
20	⩾4	—	—	—	—	3·33	0·44	0
30	⩾4	—	—	—	—	5·34	0·91	0·09

*—, Not done.

Table 2 Effect of peracetic acid on *Pseudomonas aeruginosa* at 25 °C using the suspension test

Contact time (min)	Mean log reduction at PAA concentration (% v/v)									
	0·9	0·09	0·045	0·018	0·009	0·0045	0·0018	0·0009	0·00045	0·000·18
1	⩾5	⩾5	⩾5	⩾5	4·47	4·83	2·94	1·26	−0·12	0·01
2	⩾5	⩾5	⩾5	⩾5	⩾5	5·44	2·90	1·99	0·27	−0·02
5	⩾5	⩾5	—*	—	⩾5	⩾5	4·77	3·09	1·46	−0·05
10	⩾5	⩾5	—	—	—	⩾5	4·50	3·75	1·87	0·02
20	⩾5	⩾5	—	—	—	—	4·33	4·26	2·12	0·08
30	⩾5	⩾5	—	—	—	—	5·09	4·52	2·11	0·19

*—, Not done.

Table 3 Effect of *ortho*-phthalaldehyde on *Pseudomonas aeruginosa* at 25 °C using the suspension test

Contact time (min)	Mean log reduction at OPA concentration (% v/v)							
	0·45	0·09	0·045	0·018	0·009	0·0045	0·0018	0·0009
1	⩾5	⩾5	4·30	⩾5	1·14	0·97	1·90	1·48
2	⩾5	⩾5	⩾5	⩾5	1·25	0·74	1·28	1·47
5	⩾5	—*	—	—	3·46	0·73	1·78	1·36
10	⩾5	—	—	—	⩾5	0·55	1·62	2·68
20	⩾5	—	—	—	⩾5	⩾5	⩾5	2·07
30	⩾5	—	—	—	⩾5	⩾5	⩾5	1·10

*—, Not done.

aeruginosa, activity was, if anything, enhanced (⩾ 6 log reduction within 5 min).

The results demonstrated the high activity of these biocides against the test organisms. Both OPA and GTA were still effective when the organisms were dried onto carriers in higher numbers than those used for the suspension tests.

OPA (0·5% w/v) was very effective against all of the mycobacteria tested (⩾ 5 log reduction within 10 min) including, importantly, the two GTA-resistant washer disinfector isolates *M. chelonae* Epping and Harefield (Table 5). The only organism against which 2% (v/v) GTA performed slightly better was *M. chelonae* var. *abcessus*. As expected, 2% (v/v) GTA was not effective against the Harefield and Epping strains (within the longest contact time of 30 min), but was

Table 4 Effect of OPA and GTA on viability of *Pseudomonas aeruginosa* at 25 °C by means of the carrier test

Contact time (min)	Mean log reduction (± S.D.)			
	0·5% (w/v) OPA	0·018% (w/v) OPA	2% (v/v) GTA	0·09% (v/v) GTA
1	⩾6 (0)	1·67 (0·37)	4·49 (0·75)	0·29 (0·03)
2	5·40 (1·03)	1·09 (0·41)	⩾5 (0)	1·40 (1·37)
5	⩾6 (0)	2·69 (3·27)	⩾5 (0)	⩾6 (0)
10	—*	⩾6 (0)	—	⩾6 (0)
20	—	⩾6 (0)	—	⩾6 (0)
30	—	⩾6 (0)	—	⩾6 (0)

*—, Not done.

Table 5 Effect of 0·5% (w/v) OPA on the viability of mycobacterial strains at 25 °C using the carrier test

Contact time (min)	*Mycobacterium chelonae* NCTC 946	*Mycobacterium chelonae* var. *abscessus* NCTC 10882	*Mycobacterium chelonae* Epping	*Mycobacterium chelonae* Harefield	*Mycobacterium terrae* NCTC 10856
1	⩾6 (0)	3·67 (0·62)	2·28 (1·19)	1·88 (1·77)	⩾6 (0)
2	⩾6 (0)	4·11 (0·74)	2·65 (0·97)	1·84 (1·53)	5·63 (0·64)
5	⩾6 (0)	3·62 (1·01)	5·32 (1·18)	3·70 (0·52)	⩾6 (0)
10	⩾6 (0)	5·56 (0·76)	⩾6 (0)	5·51 (0·85)	⩾6 (0)
20	⩾6 (0)	5·69 (0·53)	⩾6 (0)	⩾6 (0)	⩾6 (0)
30	⩾6 (0)	⩾6 (0)	⩾6 (0)	⩾6 (0)	⩾6 (0)

very effective against *M. terrae* and the other *M. chelonae* strains tested (Table 6).

Most importantly, there was a lack of cross-resistance to OPA of the GTA-resistant strains.

Spore carrier and suspension tests

OPA (0·5% w/v) was not sporicidal against *B. subtilis* within 270 min of exposure (Table 7), and longer exposure times of

Table 6 Effect of 2% (v/v) GTA on the viability of mycobacterial strains at 25 °C using the carrier test

Contact time (min)	*Mycobacterium chelonae* NCTC 946	*Mycobacterium chelonae* var. *abscessus* NCTC 10882	*Mycobacterium chelonae* Epping	*Mycobacterium chelonae* Harefield	*Mycobacterium terrae* NCTC 10856
1	⩾6 (0)	2·16 (1·68)	0·19 (0·17)	0·24 (0·31)	4·05 (0·20)
2	⩾6 (0)	3·78 (1·23)	0·22 (0·43)	0·38 (0·27)	5·11 (0·35)
5	⩾6 (0)	⩾6 (0)	0·23 (0·38)	0·22 (0·62)	⩾6 (0)
10	⩾6 (0)	—*	0·06 (0·25)	0·25 (0·38)	⩾6 (0)
20	⩾6 (0)	—	0·16 (0·25)	0·38 (0·25)	⩾6 (0)
30	⩾6 (0)	—	0·29 (0·14)	0·45 (0·31)	⩾6 (0)

*—, Not done.

Table 7 Effect of GTA and OPA on *Bacillus subtilis* spores at 25 °C using the carrier test

Contact time (min)	Mean log reduction (± S.D.)	
	2% (v/v) GTA	0·5% (w/v) OPA
120	3·56 (0·43)	−0·10 (0·22)
150	4·52 (0·59)	0·00 (0·23)
180	5·04 (0·69)	−0·01 (0·39)
210	5·25 (0·24)	−0·08 (0·24)
240	5·72 (0·37)	0·14 (0·38)
270	5·93 (0·32)	0·09 (0·08)

− Shows that there was an increase in mean log.

up to 12 h caused less than a 0·5 log reduction (data not shown). In contrast, 2% (v/v) alkaline GTA caused a 5 log reduction of *B. subtilis* within 180 min.

Experiments investigating the effect of raising OPA concentration and/or pH, as well as the effect of not drying the spores, are summarized in Table 8. Increasing the concentration of OPA increased its sporicidal activity from 0 to a 3 log reduction with 2% (w/v) OPA. Increasing the pH from its unadjusted level (about 6·5) to 8 further increased the activity of OPA. OPA 1% (w/v) pH 8 gave a 3 log reduction in 270 min, while 2% (w/v) OPA pH 8 gave $\geqslant 6$ log reduction (although 2% (w/v) OPA had to be heated to aid the dissolving process). Using spores that had not been dried onto the carriers did not increase the efficacy of OPA.

It was suspected that the spore coat might be the source of resistance to OPA. To test this hypothesis, coat-defective (UDS-treated) spores were used in the carrier tests, the results of which are presented in Table 9. Results for 2% (v/v) GTA are included for comparison.

As expected (Knott 1993), UDS-treated spores were far more sensitive to GTA, with a 5 log reduction in just 30 min. The activity of OPA also increased; 0·5% (w/v) became sporicidal (about 4 log reduction in 270 min) and again, increasing the concentration and/or pH increased activity.

DISCUSSION

Despite the high number of bacteria needed to measure large log reductions in these experiments, all three biocides tested were shown to be very effective against non-acid-fast non-sporulating organisms. OPA, like the other two biocides, was still very effective at concentrations far lower than its recommended in-use concentration of 0·5% (w/v) and was equally effective against both the Gram-negative and Gram-positive test bacteria.

Due to the increase in the number of organisms tested, a direct comparison between the results of the suspension tests and carrier tests could not be drawn. However, both concentrations of OPA and GTA were still effective ($\geqslant 5$ log reduction) with only 10 min of exposure time against both of the test organisms, and the recommended in-use concentrations of 0·5% (w/v) and 2% (v/v) for OPA and GTA, respectively, were effective within 2 min. Therefore, it can be concluded that drying of these test organisms did not significantly impair the action of either OPA or GTA despite the increased cell numbers.

Due to the worrying increase in isolation of GTA-resistant *M. chelonae* from washer disinfectors and processed endoscopes (Griffiths *et al.* 1998a), it was encouraging to note a lack of cross-resistance to OPA in the two GTA-resistant washer isolates tested. OPA (0·5 w/v) and 2% (v/v) alkaline GTA were also very effective against *M. terrae* NCTC 10856, which has been suggested as a possible surrogate testing organism for *M. tuberculosis*, although *M. avium-intracellulare* was found to be more resistant (Griffiths *et al.* 1998b). The other two *M. chelonae* strains tested were sensitive to both biocides, although GTA was slightly more effective against *M. chelonae* var. *abscessus*. The rapid action of GTA against *M. chelonae* NCTC 946 (Jetté *et al.* 1995; Lynam *et al.* 1995) was confirmed with a $\geqslant 6$ log reduction within 1 min of exposure.

Table 8 Effect of OPA concentration (% w/v) at different pH values on *Bacillus subtilis* spores at 25 °C

pH	Mean log reduction (± S.D.) after exposure to OPA					
	Suspension test			Carrier test		
	0·5%	1%	2%	0·5%	1%	2%
Unadjusted, 6·5	0·09 (0·08)	0·74 (0·18)	3·82 (0·63)	0·09 (0·08)	0·70 (0·02)	3·48 (0·16)
8	1·09 (0·49)	2·41 (0·72)	5·19 (0·62)	1·34 (0·79)	3·26 (1·43)	$\geqslant 6$ (0)

Contact time, 270 min.

Table 9 Effects of GTA and OPA on coat-defective spores of *Bacillus subtilis*

Contact time (min)	Mean log reduction (± S.D.)				
	2% (v/v) GTA	0·5% (w/v) OPA	0·5% (w/v) OPA pH8	1% (w/v) OPA	1% (w/v) OPA pH8
30	5·06 (0·85)	0·14 (0·21)	1·58 (1·04)	0·23 (0·14)	3·03 (1·27)
90	5·49 (0·88)	0·58 (0·17)	4·67 (0·77)	1·48 (0·14)	5·43 (0·99)
150	⩾6 (0)	1·22 (0·41)	5·62 (0·66)	3·54 (0·17)	⩾6 (0)
210	—*	1·98 (0·11)	5·62 (0·66)	5·14 (0·66)	—
270	—	3·99 (0·34)	⩾6 (0)	⩾6 (0)	—

*—, Not done.

The sporicidal activity of GTA was confirmed with the same 3 h exposure time found in other studies (Russell 1990). The lack of any sporicidal effect with 0·5% (w/v) OPA seemed to be connected with the spore coat as there was a large increase in activity when coat-defective (UDS-treated) spores were tested. Raising the pH of OPA to 8 increased its efficacy but not to the levels seen with 2% (v/v) alkaline GTA. Increasing the concentration of the OPA as well as the pH did produce sporicidal activity, although OPA took longer (preparation of 2% w/v OPA was aided by warming) to dissolve at these higher concentrations.

In conclusion, 0·5% (w/v) OPA is considered to be a viable alternative to GTA for high level disinfection where, by definition, the compound need not have a lethal action against high levels of bacterial spores (Russell 1994; Rutala and Weber 1995). OPA should not be used in situations where sterilization is required, but it might be particularly useful in washer systems where GTA-resistant organisms have developed.

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