Vet.Med.J.,Giza. Vol.47, No.3. (1999) : 405 - 413 .

HYGIENIC EVALUATION OF SOME WATER SANITIZERS USING STANDARD EVALUATION TESTS

M. M. ALI

Dept. of Hygiene, Management and Zoonoses
Fac. Vet. Med., Cairo University.

Received: 20. 4. 1999
Accepted: 25. 5. 1999

SUMMARY

This experiment was done to evaluate four different water sanitizers (sodium hypochlorite, calcium hypochlorite, iodophore and Biocid 30) using standard evaluation tests. The tested sanitizers were used in three different dilutions 2, 3 & 5%, and evaluated under conditions simulating those present in the environment. The obtained results revealed that although the phenol coefficient of the all tested sanitizers was the same in their germicidal power (5), yet the use dilution and capacity tests succeeded in evaluation between them, as sodium hypochlorite and iodophore were capable of destroying the tested microorganisms within 7 minutes in 3 % concentration.

Meanwhile there was no growth at all on using Biocid 30 (3%), while calcium hypochlorite failed to realize this result on the basis of use dilution test. The capacity of both sodium hypochlorite and iodophore was the same for 8 minutes only at 3% conc. but Biocid 30 had a capacity for 8, 18 and even 28 minutes at that concentration (3%). Calcium hypochlorite had a capacity for 8 minutes only at 5% conc. on using capacity test. Although Biocid 30 realized this result, it failed to give any capacity on using at dilutions (1:1500, 1: 2500 and 1: 4000). The recommended dilution of Biocid 30 (1:2500) realized 70% reduction in the total viable colony count of drinking water after 30 minutes exposure, meanwhile, this percentage reached 87.64% on using a dilution of 1: 1500 and its efficiency reduced (31.91%) reduction on using higher dilution (1: 4000).

INTRODUCTION

The general health condition together with efficient production among animal species depends on many factors including combating of diseases and elimination of disease producing agents, available healthy food and clean sufficient water.

Water to be safely used should be clean. Cleanliness of water depends on its periodical sanitization using available sanitizing agents to break the cycle of infectious diseases and elimination of infective agent which are disseminating through it (Linton et al., 1987).

Recently pharmaceutical companies produced many sanitizers, which have a characteristic range of antimicrobial activity under optimal conditions. The efficacy of these compounds may be altered by many factors relating to the numbers and state of microorganisms as well as the interfering compounds in the environment as presence of organic matter, and the type of water in which these compounds are diluted before their application as well as the nature of microorganisms disseminating in the water source or supply (Block, 1983; A.P.H.A., 1989; El-Agrab, 1991; Sahoo et al., 1995; Bergh & Jelmert, 1996 and Willinghan et al., 1996).

Hypochlorites and iodine compounds are among the sanitizers in common use. The evaluation of these preparations should be resolved with three major considerations, firstly, the selection of method which will provide magniful results, secondary the precis application of the method selected and lastly, the accurate interpretation of the obtained results (Vimla et al., 1997 and Rajkowski, 1998).

This trial was done to evaluate the potency of four available sanitizers used for sanitization of water intended for animal use under conditions simulating those present in the environment (normal environmental conditions).

MATERIAL and METHODS

The following standard evaluation tests were used to evaluate the four different preparations used in this study.

I. Phenol Coefficient test.
II. Use dilution test.
III.Capacity test.

The laboratory work depended on:

I. Preparation of culture:

The following prepared culture were used:

a. Salmonella typhi
b. Mixed culture (Escherichia coli, Proteus vulgaris "Gram negative"), Staphylococcus aureus "Gram positive" and yeast.

The used cultures were isolated at Animal Health Research Institute, Dokki, Giza, Egypt, and were used to prepare a broth of cell density $1.7x10^8$/ml at 37°C for each culture (A.O.A.C., 1984).

2. Preparation of Sanitizers:

The following water sanitizers were used:

a. Sodium hypochlorite (10% active chlorine), El- Nasr Co.

b. Calcium hypochlorite (Bleaching powder, 35% active chlorine) El- Nasr Co.

c. Iodine antigerm (Iodophore 12%), a product of Orion Cl. LTD., UK, m.p.h., Egypt.

d. Biocid 30 (2.75% available iodine), a product of Pfizer Pharmaceutical Co.

Each sanitizer was used in three different concentrations (2, 3 and 5%) and was diluted in distilled water (Phenol Coefficient test) and hard water (total hardness = 650 mg/L as $CaCO_3$) obtained from driven wells located at El-Ayatt locality- Giza- Egypt. (Use dilution and capacity tests).

Biocid 30 was subjected to further evaluation using three dilutions (1:1500, 1: 2500 and 1: 4000) prepared in available drinking water.

The procedure adopted was that described by Linton et al., 1987.

Statistical analysis was done according to Snedecor and Cochran (1989).

The obtained results are shown in tables (1-5).

RESULTS

Table (1): Phenol Coefficient:

(A)- Phenol.

Contact time (Min.)	Disinfectant dil			
	1/90	1/95	1/100	1/105
2.5	-	+	+	+
5	-	-	+	+
7.5	-	-	-	+
10	-	-	-	+
12.5	-	-	-	-
15	-	-	-	-

(+) Growth (-) No growth Phenol Coefficient (P.C.)= 5

(B): Sodium hpochlorite

Contact time (Min.)	Sanitizer dil		
	2%	3%	5%
2.5	+	+	+
5	+	+	+
7.5	+	+	-
10	+	-	-
12.5	+	-	-
15	-	-	-

(+) Growth (-) No growth Phenol Coefficient (P.C.)= 5

(C) Calcium hypochlorite:

Contact time (Min.)	Sanitizer dil		
	2%	3%	5%
2.5	+	+	+
5	+	+	+
7.5	+	+	-
10	+	-	-
12.5	+	-	-
15	+	-	-

(+) Growth (-) No growth Phenol Coefficient (P.C.)= 5

(D)- Iodophore:

Contact time (Min.)	Sanitizer dil		
	2%	3%	5%
2.5	+	+	+
5	+	+	+
7.5	+	+	-
10	+	-	-
12.5	+	-	-
15	+	-	-

(+) Growth (-) No growth Phenol Coefficient (P.C.)= 5

(E)- Biocid 30:

Contact time (Min.)	Sanitizer dil		
	2%	3%	5%
2.5	+	+	+
5	+	+	+
7.5	+	+	-
10	+	-	-
12.5	-	-	-
15	-	-	-

(+) Growth (-) No growth Phenol Coefficient (P.C.)= 5

Table (2): Use dilution test:

(A)- Sodium hypochlorite:

Contact time (Min.)	Sanitizer dil		
	2%	3%	5%
1	+	+	-
2	+	+	-
3	+	+	-
4	+	+	-
5	+	+	-
6	+	+	-
7	+	+	-
8	+	-	-
9	+	-	-
10	+	-	-

(+) Growth (-) No growth

(B)- Calcium hypochlorite:

Contact time (Min.)	Sanitizer dil		
	2%	3%	5%
1	+	+	+
2	+	+	+
3	+	+	+
4	+	+	+
5	+	+	+
6	+	+	+
7	+	+	+
8	+	+	+
9	+	+	-
10	+	+	-

(+) Growth (-) No growth

(C)- Iodophore:

Contact time (Min.)	Sanitizer dil		
	2%	3%	5%
1	+	+	-
2	+	+	-
3	+	+	-
4	+	+	-
5	+	+	-
6	+	+	-
7	+	+	-
8	+	-	-
9	+	-	-
10	+	-	-

(+) Growth (-) No growth

(D)- Biocid 30:

Contact time (Min.)	Sanitizer dil		
	2%	3%	5%
1	+	-	-
2	+	-	-
3	+	-	-
4	+	-	-
5	+	-	-
6	+	-	-
7	+	-	-
8	+	-	-
9	+	-	-
10	-	-	-

(+) Growth (-) No growth

Table (3) Capacity test:

(A)- Sodium hypochlorite:

Dil / Time(Min.)	Sanitizer dil		
	2%	3%	5%
8	+	-	-
	+	-	-
	+	-	-
	+	-	-
	+	-	-
18	+	+	-
	+	+	-
	+	+	-
	+	+	-
	+	+	-
28	+	+	-
	+	+	-
	+	+	-
	+	+	-
	+	+	-

(+) Growth (-) No growth

(B)-Calcium hypochlorite:

Time(Min.) \ Dil	Sanitizer dil 2%	3%	5%
8	+ + + + +	+ + + + +	- - - - -
18	+ + + + +	+ + + + +	+ + + + +
28	+ + + + +	+ + + + +	+ + + + +

(+) Growth (-) No growth

(C)- Iodophore:

Time(Min.) \ Dil	2%	3%	5%
8	+ + + + +	- - - - -	- - - - -
18	+ + + + +	+ + + + +	- - - - -
28	+ + + + +	+ + + + +	- - - - -

(+) Growth (-) No growth

(D)- Biocid 30:

Time(Min.) \ Dil	2%	3%	5%
8	+ + + + +	- - - - -	- - - - -
18	+ + + + +	- - - - -	- - - - -
28	+ + + + +	- - - - -	- - - - -

(+) Growth (-) No growth

Table (4): Capacity test on Biocid 30 tested dilutions (1:1500, 1: 2500 & 1: 4000).

Time(Min.) \ Dil	1:1500	1:250	1:4000
8	+ + + + +	+ + + + +	+ + + + +
18	+ + + + +	+ + + + +	+ + + + +
28	+ + + + +	+ + + + +	+ + + + +

(+) Growth (-) No growth

Table (5): Effect of different dilutions of Biocid 30 on total viable colony count of drinking water.

Dilution	Befor Treatment		Total Viable Colony Count/ml After Treatmnet in Minutes								
			5			15			30		
	A	B	A	B	R%	A	B	R%	A	B	R%
1: 1500	$9.2x10^3$	8.9	243.3	5.57	37.41	43.6	2.10	76.40	23	1.1	87.64
1: 2500	$9.2x10^3$	8.9	1185	6.44	28.0	126.7	3.42	61.57	76.70	2.60	70.0
1: 4000	$9.2x10^3$	8.9	$8.6x10^3$	8.00	10.11	$2x10^3$	6.96	21.79	430	6.06	31.91

A= Raw data B= Statistically transformed data R%= Reduction%.

DISCUSSION

Four different water sanitizers were laboratory tested to detect their efficacy against microorganisms, the evaluating standard tests used were, phenol coefficient, use dilution and capacity tests.

Table 1 (A-E) recorded the time at which Salmonella typhi was destroyed by using the four previously mentioned preparations. Accordingly phenol coefficient of cach tested sanitizer was calculated.

From the obtained data it can be demonstrated that the phenol coefficient for all tested sanitizers was the same number (5).

These results are in agreement with those reported by Linton et al. (1987) who indicated that the efficiency of the disinfectant depends on the concentration of the compound and the exposure time, Moreover, all disinfectants needed a minimum time of 5 - 10 minutes to destroy various types of microbes in absence of organic matter.

The results obtained in table 2 (A-D) cleared that both sodium hypochlorite and iodophore succeeded in destroying the microorganisms under test within 7 minutes in 3% concentrations, while calcium hypochlorite failed to destroy the microorganisms in 3% concentration and only killed them after 8 minutes by using 5% concentration. Biocid 30 produced no growth of the tested microorganisms in 2% concentration after 9 minutes exposure and no growth at all could be seen on using 3 & 5% concentration of the compound.

The results for use dilution test were better to a great extent than those obtained on using phenol

coefficient method as the efficiency of each sanitizer begins to be clear, these coincide with the reports of Brown (1973) and Block (1983) who indicated that phenol coefficient method must be replaced by A.O.A.C. use dilution method.

Finally in the capacity test, the sanitizer is challenged by addition of bacterial suspension until it is capable to kill organisms. The ability of sanitizer to retain its antibacterial activity in the presence of an increasing bacterial challenge is the capacity of the sanitizer. Berrang et al., 1997; Furuta et al. 1997 and Valerio et al. (1997).

Table 3 (A-D) showed that both sodium hypochlorite and iodophore had capacity for 8 minutes at 3% concentration and have a capacity for 8, 18 and 28 minutes at 5% concentration, Biocid 30 had a capacity for 8, 18 and 28 minutes at both 3 and 5% concentrations, while calcium hypochlorite has capacity for 8 minutes only at 5% concentration.

The pronounced weak effect of calcium hypochlorite may be due to its weak solubility in water (as it is sold in powder form) and consequently weak releasing of active chlorine in comparison with the other tested sanitizers.

Although Biocid 30 was considered to be the most efficient tested sanitizer (table 4) yet this preparation gave no capacity at all on using at a dilutions 1:1500, 1: 2500 & 1:4000 and only produce a reduction percentage of 87.64%, 70% and 31.91% respectively for total viable colony count of drinking water after 30 minutes exposure (table 5).

Finally it can be concluded that:

Use dilution test as well as capacity test gave reliable results in evaluation of the tested sanitizers, on contrary to phenol coefficient test which failed to evaluate between them.

Biocid 30 showed high efficiency in comparison with other tested sanitizers, followed by both sodium hypochlorite and iodophore which gave the same efficacy and finally calcium hypochlorite.

Although Biocid 30 has a capacity even for 28 minutes in 3% concentration, it failed to realize that in other tested dilutions (1:1500, 1: 2500 & 1: 4000) and realized only reduction percentage of 87.64%, 70.0% and 31.91% respectively for total viable colony count of drinking water after 30 minutes exposure.

REFERENCES

American Public Health Association (1989): Standard Methods for Examination of Water and Waste Water. 17th. Ed., A.P.H.A., W.P.C.F.; Inc. Washington D. C., USA.

Bergh, Q. and Jelmert, A. (1996): Iodophore disinfection of eggs of Atlantic halibut. Journal of Aquatic Animal Health, 8 (2) 135.

Berrang, M. E.; Frank, J. F.; Duhr, R. J.; Baily, J. S.; Cox, N. A. and Mauldin, J. M. (1997): Microbiology of sanitized broiler hatching eggs through the egg production period. Journal of Applied Poultry Research. 6 (3), 298.

Block, S. S. (1983): Disinfection, sterilization and preservation. 3rd Ed. LEA and Febiger, Philadelphia, USA.

Brown, E. (1973): Disinfectant regislation soap, Chem. SPA (49).

El-Agrab, H. M. (1991): Studies on the disinfecting and growth promoting effect on chloride and iodine compounds in broiler farm. Benha Vet. Med. J. (2), 1, 60.

Furuta, K. and Pshizawa, M. (1997): Effect of high concentration of disinfectant solutions on reduction of viable bacterial count. Japanese Poultry Science 34 (2), 132; 1977. Cited in Vet. Bulletin, 68 (7), 1998.

Linton, A. H.; Hugo, W. B. and Russell, A. D. (1987): "Disinfection in Veterinary and Farm Animal Practice." pp. 76 .

Official Methods of Analysis of the Association of Official Analytic Chemists. AOAC (1984): 14th Ed. Arlington, Virginia, USA.

Rajkowski, K. T.; Eblen, E. and Laubauch, C. (1998): Efficacy of washing and sanitizing trailers and for swine transport in reduction of salmonella and E. coli. Journal of Food Protection. 61 (1) 31.

Sahoo, P. K.; Chawak, M. M.; Reddy, M. R.; Mohapatra, S. C.; Mishra, S. K.; and Bhanja, S. K. (1995): Effect of common sanitizers and antibiotics treatment of eggs on surface contaminants and hatchability. Indian Journal of Poultry Science. 30 (3) 261.

Snedecor, G. W. and Cochran, W. G. (1989): Statistical Methods. 8th Ed., Iowa State University Press, Ames, Iowa, USA.

Valerio, J. L.; Larios, J. L.; Vidal, J.; Gutierrez, C. B. and Radriguez-Ferri, E. F. (1997): Study of the purification of water supplies for fowls using N-duopropenide. Medicina Veterinaria. 14 (1), 23. Cited in Vet. Bull. Vol. 67 (12).

Vimla, M. P.; Ranadas, B. and Balaji, S. (1997): Bacteriological efficiency of stabilized chlorine dioxide against common pathogens. Indian Veterinary Journal. 74 (2), 123.

Willinghan, E. M.; Sander, J. E.; Thayer, S. G.; and Wilson, J. L. (1996): Investigation of bacterial resistance to hatchery disinfectants. Avian Diseases. 40 (3), 510.