A COMPARISON OF A QUATERNARY AMMONIUM COMPOUND AND A CALCIUM HYPOCHLORITE COMPOUND AS A GERMICIDAL AGENT.

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SUMMARY.

A calcium hypochlorite compound, used at a concentration of about 50 p.p.m. available chlorine, and a di-isobutyl cresoxy ethoxy ethyl di-methyl benzyl ammonium compound, used at 200 p.p.m., were tested by the Weber-Black method with a laboratory strain of Escherichia coli as the test organism.

The hypochlorite showed the greater efficiency, probably because of the lower resistance of the gram-negative test organism.

INTRODUCTION.

Methods suggested for the evaluation of the performance of germicides fall into two classes. The first includes all tests of the phenol-coefficient-type where the germicidal value of the test substance is compared with that of a standard disinfectant. The second includes those tests which determine the rate of death of the test organism under standard conditions.

Some investigators, dissatisfied with the abstract nature of the above tests, have attempted to create in the laboratory a microcosm of the macrocosm, thereby subjecting the germicidal agents under test to as many as possible of the variables liable to be encountered under practical conditions. Johns (1947) suggested the use of glass slides on which the test organism had been dried. Mallmann and Hanes (1945) used glass rods or rings similarly treated.

Weber and Black (1948), after a careful study of existing methods, devised a procedure which involves the performance of "straight" laboratory tests with the interpretation of the results in terms of an arbitrary standard based upon the time required to sanitise heavily contaminated food utensils under practical conditions. A 100 per cent. "kill" in less than 30 seconds in the laboratory is regarded as strong presumptive evidence that "in actual food utensil sanitising procedures an exposure of two minutes would be satisfactory for ordinary non-sporing pathogenic organisms." This method was used in the experiment reported here.

MATERIALS AND METHODS.

Germicides.

Two germicides were compared, viz., (1) a quaternary ammonium compound—di-isobutyl cresoxy ethoxy ethyl dimethyl benzyl ammonium chloride monohydrate; (2) calcium hypochlorite.

Weber-Black Method and Inhibitor.

The Weber-Black method employed is outlined hereunder:—

Five millilitres of a 2X concentration of the test germicide at 25 deg. C. are added to 5 ml. of a 2X suspension of the test organism at the same temperature as the sweep second hand of the laboratory timer reaches a pre-determined starting point. One millilitre of the test mixture is removed at exactly 15 seconds, 60 seconds, 120 seconds, and 300 seconds respectively and blown into separate 9 ml. inhibitor blanks. One millilitre and ·1 ml. of each inhibitor blank are then pipetted into petri dishes, resulting in 10–1 and 10–2 dilutions. Plates are poured with tryptone-glucose-extract medium (American Public Health Association, 1948). Initial and final plate counts of the bacterial suspension of the test organism are determined by appropriate dilutions in 99 ml. phosphate buffer dilution blanks. The pH of the test mixture is determined electrometrically.

After 300 seconds excess of the inhibitor is added to the test mixture and the walls of the tube swabbed to release any surviving bacteria. The swab is placed in a petri dish, cut with a sterile cutting instrument, and the plate poured with tryptone-glucose-extract medium. All plates are incubated for 24 hours at 35–37 deg. C.

Both lecithin and sodium oleate were used as inhibitors in separate trials with the quaternary ammonium compound in the ratio of inhibitor to quaternary of 100–1 in the 9 ml. dilution blanks. As far as could be ascertained, the inhibitors were equally effective. No information could be gained regarding the inhibitory effect, if any, of the resulting complex in either case. In the hypochlorite trials, sodium thiosulphate ($Na_2S_2O_3.5H_2O$) was used in the preparation of the 9 ml. blanks in the concentration recommended by the American Public Health Association (1948).

Recording of Results.

After incubation the plates were counted and the results recorded in accordance with the methods of the American Public Health Association (1948) Results of the individual trials were recorded as in Table 1.

Table 1.

SAMPLE DATA RECORDING SHEET.

Germicide : Q.A.C. 200 p.p.m. Date : 22–3–50.

Escherichia coli (test strain).

5 ml. Q.A.C. 400 p.p.m.

5 ml. *E. coli* 200 x 10

pH = 7·9 at 25°C.

Exposure in Seconds.	0.	15.	30.	60.	120.	300.	Tube.	Swab
10^{0}	100 x 10^{6}	..	..	..	..	..	..	..
10^{-1}	..	0	14	1	1	0	0	T.N.C.
10^{-2}	..	0	2	0	0	1	..	..

T.N.C. = Too numerous to count.

STERILITY CONTROLS.

Agar control—0.

Water control (distilled)—0.

Dilution blank control—0.

RESULTS.

Quaternary Ammonium Compound.

Table 2 summarizes the results of 20 trials conducted with the quaternary ammonium compound using the recommended concentration of 200 p.p.m.

Calcium Hypochlorite.

Table 3 summarizes the results of 20 trials conducted with the calcium hypochlorite compound using concentrations about 50 p.p.m. available chlorine. Concentrations of available chlorine were determined by titration with N/100 sodium thiosulphate standardized against potassium permanganate (Cumming and Kaye, 1934, p. 126).

DISCUSSION.

From Table 4 it will be seen that 15 per cent. of the trials in the case of the quaternary ammonium compound and 75 per cent. in the case of the calcium hypochlorite compound gave a 100 per cent. kill within the 30 second level, which is the criterion set down by Weber and Black. The concentrations of the germicides used are those generally recommended. Quaternary ammonium compounds are generally used at a concentration of 200 p.p.m., while the Ordinance and Code Regulating Eating and Drinking Establishments of the U.S. Public Health Service recommend a minimum concentration of 50 p.p.m. available chlorine for hypochlorite compounds. The test mixture in the case of the quaternary was slightly alkaline and decidedly alkaline in the case of the hypochlorite. The concentration of the test organism in the majority of trials in both cases was about 100 million per ml.

Table 2.

SUMMARY OF QUATERNARY AMMONIUM COMPOUND TRIALS.

Experiment No.	Conc. p.p.m.	pH.	Plate Count After—										Count of Sterility Control.				
			Initial Count.	15 sec.		30 sec.		60 sec.		120 sec.		300 sec.		Tube.	Swab.	Agar.	Water.	Blank.
				10–1.	10–2.	10–1.	10–2.	10–1.	10–2.	10–1.	10–2.	10–1.	10–2.					
1 ..	200	7·8	103×10^6	5	0	10	0	1	1	1	0	0	0	4	70	0	0	0
2 ..	200	7·9	100×10^6	0	0	14	2	1	0	0	1	1	0	0	T.N.C.	0	0	0
3 ..	200	8·4	132×10^6	1	0	20	0	0	1	6	0	0	0	0	0	0	0	0
4 ..	200	8·4	78×10^6	11	2	14	2	30	5	21	2	3	0	0	0	0	0	0
5 ..	200	7·8	88×10^6	74	7	48	1	4	0	20	3	3	1	0	1	0	0	0
6 ..	200	8·0	166×10^6	26	3	0	0	28	4	0	0	0	1	0	T.N.C.	0	0	0
7 ..	200	7·9	176×10^6	44	1	32	0	0	0	12	8	2	0	0	0	0	0	0
8 ..	200	8·0	59×10^6	8	5	6	1	0	1	0	1	0	0	1	0	0	0	0
9 ..	200	8·4	103×10^6	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0
10 ..	200	7·9	98×10^6	0	0	1	0	1	0	1	0	0	1	0	0	0	0	0
11 ..	200	8·2	130×10^6	0	0	1	0	1	0	1	0	0	1	0	0	0	0	0
12 ..	200	8·0	146×10^6	0	1	1	0	1	0	27	0	6	4	0	T.N.C.	0	0	0
13 ..	200	8·4	133×10^6	0	0	1	0	1	0	1	0	1	1	0	0	0	0	0
14 ..	200	7·6	94×10^6	72	9	1	0	2	0	0	0	0	0	0	0	0	0	0
15 ..	200	7·7	91×10^6	7	1	1	0	1	0	0	0	0	0	0	0	0	0	0
16 ..	200	8·3	115×10^6	13	1	1	0	0	0	0	0	0	0	0	0	0	0	0
17 ..	200	8·3	115×10^6	6	2	0	0	0	0	0	0	0	0	0	0	0	0	0
18 ..	200	7·6	102×10^6	15	1	0	0	0	0	0	0	0	0	0	0	0	0	0
19 ..	200	7·6	102×10^6	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0
20 ..	200	7·8	130×10^6	37	15	2	0	1	0	2	0	0	0	0	0	0	0	0

Table 3.

SUMMARY OF CALCIUM HYPOCHLORITE TRIALS.

Experiment No.	Conc. p.p.m.	pH.	Plate Count After—										Count of Sterility Control.				
			Initial Count.	15 sec.		30 sec.		60 sec.		120 sec.		300 sec.						
				10–1.	10–2.	10–1.	10–2.	10–1.	10–2.	10–1.	10–2.	10–1.	10–2.	Tube.	Swab.	Agar.	Water.	Blank.
	p.p.m.	pH																
1 ..	53	10·8	67×10^6	20	0	0	0	0	0	0	0	0	0	0	2	0	0	0
2 ..	53	10·9	75×10^6	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0
3 ..	62	11·0	123×10^6	13	1	3	0	0	0	0	0	0	0	T.N.C.	8	0	0	0
4 ..	62	11·0	98×10^6	20	2	2	0	0	0	0	0	0	0	8	3	0	0	0
5 ..	53	11·4	120×10^6	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6 ..	62	10·8	132×10^6	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0
7 ..	53	11·4	78×10^6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8 ..	53	10·9	88×10^6	0	1	6	2	0	1	0	0	0	0	0	1	0	0	0
9 ..	53	11·3	176×10^6	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
10 ..	49	12·2	98×10^6	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0
11 ..	50	10·8	134×10^6	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
12 ..	55	11·3	165×10^6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13 ..	53	10·9	120×10^6	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14 ..	48	11·2	130×10^6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15 ..	57	11·4	126×10^6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16 ..	57	11·4	126×10^6	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17 ..	57	11·2	126×10^6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18 ..	60	11·3	108×10^6	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
19 ..	60	11·3	108×10^6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20 ..	60	11·4	108×10^6	5	2	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4.

ANALYSIS OF RESULTS.

Germicide.	Conc. p.p.m.	pH Range. 25°C.	Range of Initial Concentration.		100% Kill Within.				
			Max.	Min.	15 sec.	30 sec.	60 sec.	120 sec.	300 sec.
Quaternary Ammonium Compound	200	7·6–8·4	130×10^6	59×10^6	10	15	30	50	60
Calcium Hypochlorite ..	48—62	10·8–11·4	176×10^6	67×10^6	55	75	80	100	..

The greater efficiency of the hypochlorite may in fact be due to the lower resistance of the gram-negative test organism to the hypochlorite than to the quaternary ammonium compound. Since the gram-negative organisms are the chief quality affecting types in the dairying industry, hypochlorites would thus appear to possess an intrinsic advantage over the quaternary ammonium compounds for dairy sanitation.

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