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TECHNICAL REPORT
MICROBIOLOGICAL DETERIORATION SERIES NO. 8

LONG-TERM STORAGE STUDY OF DISINFECTANT, GERMICIDAL AND FUNGICIDAL

by

Elizabeth Pillion Morris R. Rogers

Arthur M. Kaplan

PIONEERING RESEARCH DIVISION

December 1965

U. S. Army Materiel Command
U. S. ARMY NATICK LABORATORIES
Natick, Massachusetts

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ABSTRACT

A five-year storage test was conducted on Disinfectant, Germicidal and Fungicidal, Phenolic, Dry-Type, Specification MIL-D-51061 in order to obtain data on storage stability and performance under adverse climatic conditions. The storage sites were located at Fort Churchill, Canada (arctic, cold-dry), Maynard, Massachusetts (temperate, cold-wet), Yuma, Arizona (desert, hot-dry) and Panama Canal Zone (tropic, hot-wet).

After five years of storage in arctic and temperate climates the disinfectant showed no significant change in appearance or composition, and no decrease in bactericidal activity.

The samples exposed in the hot environments showed varying degrees of alteration evidenced by darkening and liquefaction of the dry powder. Bactericidal activity was lower than control values but sufficient to meet the performance requirements.

LONG TERM STORAGE STUDY OF DISINFECTANT, GERMICIDAL AND FUNGICIDAL

INTRODUCTION

In June, 1958, under Test Plan FEA-58014, a five year storage test was initiated on Disinfectant, Germicidal and Fungicidal, Phenolic, Dry-Type, Specification MIL-D-51061. This material was developed for the disinfection of latrine buckets and as a general purpose housekeeping disinfectant. It was selected from a wide variety of candidate compounds as best fulfilling the requirement for a stable, water soluble concentrate, non-corrosive to metal, non-toxic in normal handling, and with high biological effectiveness in the presence of large amounts of organic material. It has a phenol coefficient of 71, and the composition specified in the following table from the specification:

Ingredient	% By Weight	
	Minimum	Maximum
Sodium orthophenylphenolate	20.0	---
Sodium 4-chloro-2-phenylphenolate	40.0	---
Sodium 6-chloro-2-phenylphenolate	13.0	17.5
Moisture	14.0	18.0

The disinfectant was produced under Contract No. DA-19-129-AM-1027 with Scientific Oil Compounding Company, Inc., Chicago, Ill. An experimental or semi-commercial production run of 10,000 pouches was packaged in a laminate of polyethylene/aluminum foil/polyethylene/kraft paper with lacquer coating. The pouches were 3 1/2 inches in width and 5 inches in length with 3/8 inch seams. Twelve pouches of one ounce capacity were packaged in an intermediate cardboard carton, and eight cartons were crated in a wooden box for a total of 96 pouches per box. The disinfectant was placed in test under FEA-58014, Test of Long Term Storage of Disinfectants to obtain information on its storage stability and performance under adverse climatic conditions.

PLAN OF TEST

Storage of Disinfectant

The U. S. Army Test and Evaluation Command (formerly QM Field Evaluation Agency), Fort Lee, Virginia, was authorized to arrange for the storage and withdrawal of the test items, over a five year period, at each of four storage sites representing the major climatic conditions to which military supplies are normally exposed. The storage sites were at the following locations:

1. Fort Churchill, Manitoba, Canada (arctic, cold-dry conditions). This facility was closed during the summer of 1962, and the storage items were transferred to Fort Wainwright, Alaska.

2. Maynard QM Test Activity, Maynard, Massachusetts (temperate, cold-wet conditions). This designation was changed after 1962 to U. S. Army Natick Laboratories Sudbury Annex.

3. Yuma, Arizona (desert, hot-dry conditions).

4. Corozal, Panama Canal Zone (tropic, hot-wet conditions).

Ten boxes of disinfectant were placed in storage at each of the above sites in June, 1958. At six month intervals one box was withdrawn from each of the sites and shipped to the U. S. Army Natick Laboratories for evaluation.

Analysis of Stored Samples

The exposed boxes received in the laboratory were opened, one carton was removed, and the appearance of pouches and contents was recorded. The sampling procedure in the early part of the test was to remove two pouches, one from the center and one from the end of the carton, and analyze each pouch for phenol components and moisture. When changes in the appearance of the powder were observed a pooled sample from five pouches was used for the analyses. On the final five year samples the contents of an entire carton (twelve pouches) were combined.

Moisture was determined according to MIL-D-51061, para. 4.4.2. A 10 gram sample was suspended in toluene and distilled until all the water was collected in a graduated receiving flask.

Phenol components were analyzed according to MIL-D-51061, para. 4.4.1.3.2. A 5 gram sample was dissolved in water, acidified and extracted with carbon disulfide. The extract was analyzed as a three component system by infrared spectrophotometry. A Beckman IR Spectrophotometer

was used to examine samples which had been in storage through a 2 1/2 year period. The remaining storage samples were analyzed with Perkin Elmer Models 137 and 237 Recording Spectrophotometers. The 3, 3 1/2 and 4 year samples were analyzed at the laboratories of DeBell and Richardson, Inc., Hazardville, Connecticut, under Contract No. DA-19-129-QM-1869, Task No. 3. All other samples were analyzed in the Germicides Laboratory, Pioneering Research Division.

The carbon disulfide extracts from the 4 1/2 and 5 year samples were also analyzed by gas chromatography using a Model 720 F & M Gas Chromatograph with thermal conductivity detector and a silicone nitrile column.

Bacteriological performance of the 5 year samples was determined by a modified phenol coefficient method using E. coli ATCC #26 as the test organism. Appropriate dilutions of the disinfectant with or without inactivators were inoculated, transferred after 5 minutes to a subculture of nutrient agar, and incubated 48 hours at 37°C.

RESULTS

Appearance

Throughout the storage period the pouch material showed no change except for discoloration of the white kraft paper on some of the samples stored at Panama. There was a change in the color and consistency of the disinfectant powder in all samples except those subjected to arctic exposure. The normal pale tan color darkened progressively to a deep brown. The powder gradually became liquified, changing into a dark viscous fluid. Discoloration was observed initially at points along the seams of the pouches, indicating that imperfections in the seal, providing access to air, were responsible to some extent for the changes.

The deterioration was related to temperature and humidity; the Churchill samples showing no change and the Maynard samples only moderate change at the end of five years. However, the samples stored at Panama were completely dark and viscous after two years. The samples from Yuma were intermediate, and the contents of individual pouches varied from partial discoloration to complete liquefaction.

Chemical Analysis

Results of analyses for phenol components and water are presented in Tables I and II. Table I lists the control values prior to storage and

Table II lists the results of analyses made at six-month intervals after storage began. The samples stored at Fort Churchill and Maynard showed little or no change in phenol composition. The variability in phenol measurement is due to error in the spectrophotometric analysis rather than to actual fluctuations in the concentrations.

TABLE I

COMPOSITION OF DISINFECTANT BEFORE STORAGE

COMPONENT	WEIGHT PERCENT*
Sodium orthophenylphenolate	28.1
Sodium 4-chloro-2-phenylphenolate	40.1
Sodium 6-chloro-2-phenylphenolate	14.4
Moisture	16.5

*Each value is an average of 8 determinations

The results of gas chromatographic analysis of the 4 1/2 and 5 year storage samples are listed in Table III. In the case of the Churchill and Maynard samples the results were in good agreement with the infrared analysis. In the Yuma and Panama samples, however, there was a large discrepancy between the two methods which is the reason for inclusion of the gas chromatographic data in this report. In these deteriorated samples the phenol concentrations dropped far below specification levels when measured by gas chromatography. But the loss was only partly indicated by the infrared procedure which showed some decrease in orthophenylphenol and very little change in the chlorophenylphenols. The infrared spectrograms were similar to those of the undeteriorated Churchill and Maynard samples except for the appearance of an absorption band at 1690 cm^{-1} (5.92 microns) indicating carbonyl formation. The color of the carbon disulfide solutions was deep red-brown in contrast to the normal amber, and the color intensity was suggestive of quinone formation. Whatever the oxidation product(s), it is probable that its presence caused spectrophotometric interference at the analytical wavelengths leading to high results in the infrared analyses. Notwithstanding the visual and spectral evidence, however, this product was not detected in the gas chromatograph at the sensitivity settings used for the analysis, and the amount present may have been small.

TABLE II

WEIGHT PERCENT OF PHENOL COMPONENTS AND WATER IN STORED SAMPLES OF DISINFECTANT

Storage Time (Yrs.)		1/2	1	1 1/2	2	2 1/2	3	3 1/2	4	4 1/2	5
Storage Site	Component	Weight Percent[a]									
Churchill	OPP[b]	28.3	28.9	31.2	27.6	27.1	23.0	29.6	26.8	28.3	26.9
	4-Cl[c]	39.2	38.8	38.3	40.1	39.7	34.1	42.5	39.8	40.8	39.1
	6-Cl[d]	13.8	13.7	13.3	14.1	13.8	11.7	14.3	11.1	13.6	13.6
	H_2O	17.6	--	17.4	18.6	18.7	16.2	17.3	18.8	16.8	19.0
Maynard	OPP	26.1	27.6	28.3	28.4	28.7	29.9	30.8	26.8	27.2	26.2
	4-Cl	41.5	41.0	41.0	41.5	39.3	41.2	43.2	38.9	39.9	37.8
	6-Cl	14.2	13.0	14.0	14.9	14.0	16.5	14.2	11.1	13.0	13.6
	H_2O	--	--	17.1	17.9	17.6	16.1	14.5	19.2	18.7	18.5
Yuma	OPP	26.7	27.4	27.1	27.7	27.9	29.4	29.2	22.4	24.6	20.9
	4-Cl	41.3	39.6	41.8	41.1	41.1	35.5	42.8	40.3	43.8	40.8
	6-Cl	14.3	14.8	15.6	15.4	15.1	7.5	16.5	11.7	16.3	16.2
	H_2O	--	--	16.4	15.9	15.7	13.7	15.0	12.6	12.7	12.5
Panama	OPP	25.4	26.8	30.2	20.4	21.4	24.3	29.3	20.1	22.2	19.1
	4-Cl	39.3	40.2	37.9	38.9	39.7	36.0	45.4	33.9	37.9	32.0
	6-Cl	13.3	12.5	13.4	15.4	14.6	12.4	15.1	10.7	15.3	16.4
	H_2O	--	--	19.0	25.5	22.0	21.6	18.0	19.8	18.7	18.3

a. Average of 2 Determinations
b. Sodium orthophenylphenolate
c. Sodium 4-chloro-phenylphenolate
d. Sodium 6-chloro-phenylphenolate

Specification Requirements:

	Min.	Max.
OPP	20.0	--
4-Cl	40.0	--
6-Cl	13.0	17.5
H_2O	14.0	18.0

TABLE III

PHENOL CONTENT OF STORED SAMPLES
BY GAS CHROMATOGRAPHY

Storage Site	Component	Weight Percent[a] 4 1/2 Yrs.	5 Yrs.
Churchill	OPP[b]	26.4	26.0
	4-Cl[c]	39.9	37.1
	6-Cl[d]	13.3	13.3
Maynard	OPP	25.4	25.8
	4-Cl	37.2	37.3
	6-Cl	12.5	12.6
Yuma	OPP	15.2	11.4
	4-Cl	32.5	28.3
	6-Cl	11.3	10.6
Panama	OPP	13.1	10.4
	4-Cl	27.5	22.8
	6-Cl	9.7	8.0

a. Average of 2 Determinations
b. Sodium orthohenylphenolate
c. Sodium 4-chloro-phenylphenolate
d. Sodium 6-chloro-phenylphenolate

It was further demonstrated by infrared spectrophotometry that substantial conversion from sodium salts to free phenols had taken place in the deteriorated samples. A large amount of the material was extractable with carbon disulfide before the acidification step which converts the salts to phenols. The liquefaction of the samples is accounted for by this phenol formation, since it results when 4-Cl-2-phenylphenol is in contact with either orthophenylphenol or 6-Cl-2-phenylphenol.

After analysis, the water layers from the carbon disulfide extractions of the five year Churchill and Panama samples were retained and reextracted with ether. Gas chromatography of the ether extract of the Churchill sample produced only a solvent peak, but the Panama extract contained several other peaks which have not been identified. Thus, the large drop in phenol concentrations indicated by the gas chromatograph may be attributed to the formation of water soluble products which are not extractable with carbon disulfide and remain in the aqueous layer.

In the samples from all the storage sites except Yuma there was an increase in water content of approximately 2%. The Yuma samples showed a water loss which appeared to level off in the fifth year at about 4%.

Bacteriological Analysis

Results of bactericidal testing of the five year storage samples are listed in Table IV together with control values. The test requirements are listed at the bottom of the table, the critical concentrations being 500 ppm without inactivator, 1000 ppm + 5.0% peptone, and 2000 ppm + 0.5% linseed oil soap. The difference in culture counts between controls and storage tests is not sufficient to affect the killing time.

The activity of the Churchill and Maynard samples was not altered during the storage period. In the Yuma and Panama samples some reduction in activity over the control values was observed, but in each case the performance was satisfactory. A slightly elevated count of 22 colonies was obtained with the Panama sample at 1000 ppm + 5% peptone. This count is within the variability of the method and corresponds to a kill of 99.99%. It was therefore considered acceptable since the tests with linseed oil soap and without inactivator were within the performance requirements.

It should be noted that the solubility of the Yuma and Panama samples was reduced because of the free phenol present. The material did not form a stable emulsion, and although the suspensions were stirred vigorously when aliquots were taken for the test the entire amount sampled may not have been available for biocidal action.

TABLE IV

PLATE COUNTS OF E. COLI AFTER 5 MINUTE CONTACT IN SOLUTIONS OF DISINFECTANT BEFORE AND AFTER 5 YEARS STORAGE

	CONTROL SAMPLES				5 YEAR STORAGE SAMPLES			
	#1	#2	#3	#4	Churchill	Maynard	Yuma	Panama
Culture Count (Organisms/ml)	70×10^7	128×10^7	55×10^7	120×10^7	15×10^7	22×10^7	28×10^7	15×10^7
100 ppm	TNC	TNC	TNC	TNC	TNC	TNC	TNC	TNC
250 ppm	TNC	TNC	73	220	0	12	TNC	TNC
500 ppm	0	0	0	0	0	0	0	1
500 ppm + 5.0% Peptone	0	0	1	17	0	0	TNC	TNC
1000 ppm + 5.0% Peptone	0	0	0	0	0	0	0	22*
1500 ppm + 5.0% Peptone	0	0	0	0	0	0	0	1
1000 ppm + 0.5% Soap	11	72	TNC	TNC	0	0	156	TNC
2000 ppm + 0.5% Soap	0	0	0	0	0	0	0	0
3000 ppm + 0.5% Soap	0	0	0	0	0	0	0	0

TNC = Too numerous to count (over 300)

Test Requirement: Not over 5 colonies per plate in three out of three tests at concentrations of: 500 ppm without inactivator, 1000 ppm with 5.0% Peptone, 2000 ppm with 0.5% Linseed Oil Soap.

* Average of 5 Plate Counts

SUMMARY AND DISCUSSION

After five years of storage in arctic and temperate climates the disinfectant showed no appreciable change in appearance or composition, except a 2% increase in water content. There was also no change in bactericidal activity.

In the disinfectant exposed to hot-dry conditions at Yuma there was a 4% decrease in water content, and considerable alteration in appearance and composition. The light tan powder became darkened and liquified from oxidation and conversion of the sodium salts to free phenol. The degree of alteration was observed to vary from pouch to pouch. Bactericidal activity was slightly reduced but the tests requirements were met in all cases.

Under tropical conditions at Panama the water content of the disinfectant increased about 2%, the same amount as in the Churchill and Maynard exposures. The alteration of composition progressed more rapidly than in the samples exposed at Yuma. After two years the pouch contents were completely dark and liquified. Bacteriological activity was lower than in the Yuma samples, but the performance was nevertheless satisfactory.

It was observed that changes in the appearance of the disinfectant started at points along the seams of the pouches and spread inwardly. The adequacy of the seal was therefore implicated in the deterioration. The dimensions of the pouch (3 1/2 x 5 inches) were such that it was filled to capacity with one ounce of powder causing excess strain to be placed on the seams. For this reason the pouch size has been increased to 5 x 6 inches in current procurement. The larger pouch is thinner when filled, and with less bulk in the body a better seal may be achieved and maintained. With this modification it is expected that the shelf life of the disinfectant in hot environments will be extended.

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13 ABSTRACT

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Fungicides	1		9			
Phenolic	0					
Dry	0					
Determination	4		8			
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