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REPORT ON A

"STUDY OF CHEMICAL GERMICIDES"

15 April 1967
Task 5.1
JPL CONTRACT 951624

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for

JET PROPULSION LABORATORY
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

-NOTE-

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ABBREVIATIONS

ml	Milliliter
B. B. L.	Baltimore Biological Laboratory
°C	Degree Centigrade
mg	Milligram

I. INTRODUCTION

This study was performed to determine the effectiveness of several types of chemical germicides as decontaminating agents. These compounds kill or inhibit the growth of microorganisms by various means. For example, quarternary ammonium compounds (cationic surfactants) may kill by the lysing of the bacterial cell or by denaturation of cellular or enzyme protein. The phenol derivatives may kill by protein denaturation, metabolic blocking, or surfactant effects.

The prime concern of this study was the effectiveness of kill, not the mechanism. The reduction of biological burden on surfaces, parts, tools, instruments, and supplies in low-burden areas such as EASL will depend in part on the germicide used as a decontaminant. A cationic surfactant in aqueous and alcoholic solution, a phenolic compound, and a chlorinated phenol were chosen to be studies for their efficacy as germicides against Escherichia coli, Staphylococcus epidermidis, Proteus vulgaris, and Bacillus globigii in the vegetative and spore state.

II. MATERIALS AND METHODS

A. GERMICIDES

Hyamine 3500 80-percent concentrate in ethanol is a blend of alkyl dimethyl benzyl ammonium chlorides.[1] This is a surfactant particularly effective in hard waters, and may be used as an aqueous or alcoholic solution. For this study Hyamine 3500 was prepared in a 1:1000 concentration in distilled water and in 70-percent isoproponal, by adding 1.25 parts of Hyamine 3500 to the appropriate quantity of solvent. This proportion of Hyamine 3500 was necessary to correct for the concentration in the undiluted solution (80 percent).

Dowicide A[2] is the sodium salt of 0-phenyl phenol. Dowicide B[2] is the sodium salt of 2, 4, 5 trichlorophenol. These compounds were prepared by adding 1 gram of material to 1 liter of distilled water, a 1:1000 concentration. Both Dowicides were soluble in water, as was the Hyamine 3500.

B. ORGANISMS

As stated previously, the organisms to be subjected to the germicidal activity were E. coli, S. epidermidis, P. vulgaris, and B. globigii (spores and vegetative cells). All cells were prepared as follows. An inoculum from an agar slant was placed in 50 ml of trypticase soy broth (B. B. L.) and incubated at 32° C for 18 – 24 hours. Harvesting was done in a refrigerated centrifuge at 0° C, using three successive washings with cold, 1 percent peptone water.

A final suspension of the cells in 10 ml of cold peptone water was prepared. Peptone water was chosen as the washing and suspending medium because it was found that the vegetative cells lysed when suspended in distilled water. The B. globigii spore crop was grown by successive inoculation into increasing amounts of trypticase soy broth (B. B. L.) for three days, followed by plating on trypticase soy agar (B. B. L.) supplimented with 40 mg $CaCl_2$ and 10 mg $MnSo_4$ per liter of agar. One ml of broth suspension was spread over the surface of the agar. The plates were incubated until they contained 80—90 percent spores. Harvesting was done by scraping the growth off the plates (using bent glass rods) into distilled water. The spores were centrifuged at 0°C and washed 3 times and finally suspended in distilled water. The organisms were then diluted and plated to determine their viable counts before each assay. Both spores and vegetative cells were diluted for use to approximately 10^7 cells/0.01 ml.

C. SURFACES UTILIZED

To properly evaluate the germicide, materials were chosen which would simulate surfaces in low-burden areas. Strips 2 x 3/4 x 0.02 inches were fabricated of stainless steel and Formica. Half of the stainless steel strips were painted with an epoxy paint (Sinclair semi-flat). These strips were steam sterilized in groups of 60 on aluminum-foil-covered flat trays with an outer wrapping of kraft paper. The test organisms were inoculated with 0.01 ml of the cell suspension, onto the surface of each strip in order to cover an area approximetely 0.6 in^2. The inoculated strips were dried at 32°C before assay. Five strips of each material were used with each germicide and each organism.

D. RECOVERY MEDIUM

Trypticase soy agar with neutralizers was used for plating to recover possible survivors after exposure to the germicides. The neutralizers were needed to counteract the effects of germicides that might have been carried over to the plating medium. A combination of Tween 80 and lecithin was used as the neutralizers. The TSA was prepared containing 0.7 gms of lecithin and 5 gm of Tween 80 per liter of agar.[3]

E. ASSAY METHOD

In a laminar flow bench, each strip was dipped into germicide for 2 seconds, removed and placed upright in a sterile rack to dry. This procedure most closely approximates a wipedown of a surface. The dried strips were then placed into Bussey bottles containing 10 ml of 1 percent peptone water. The strips were sonicated face down at 25 kc/second for 12 minutes, then plated as follows: The strip was placed onto a layer of TSA and covered by another layer of melted cooled TSA. In addition 1 ml of the peptone water rinse (undiluted) was plated, as well as 1 ml aliquots of the $1/10^2$ and $1/10^3$ dilutions. All plates were incubated aerobically at 32°C for 72 hours. Colony counts were made at 24, 48, and 72 hours.

The counts were recorded from each of the five plates for each strip. Since five replicas were used, the colony counts for each, plus the average of the five, were reported.

III. RESULTS

The only organism that survived in numbers greater than 10 percent of its initial population with all four germicides was the spore of B. globigii. The percent survival ranged from 10 percent on Formica strips when treated with aqueous Hyamine 3500 to 57 percent on the epoxy paint strips when treated with Dowicide B. The results are recorded in Figures 1 and 2. Following immersion in the two Dowicides, S. epidermidis on epoxy paint strips survived in greater numbers than on stainless steel and Formica. S. epidermidis exposed to the aqueous and tincture of Hyamine 3500 had less than 1 percent survivors or no survivors. These results are in Figure 3.

Figures 4 and 5 show the survival of E. coli and P. vulgaris respectively. There were no observable colonies in 72 hours with P. vulgaris, and less than 1 percent survival of E. coli on all materials after exposure to the germicides tested. In all cases, the two Hyamine 3500 formulations were more effective than the Dowicides in the concentrations used.

IV. DISCUSSION

The four germicides in recommended use dilutions and for the exposure times of this experiment were shown to be 99-percent effective in reducing the populations of such vegetative cells as E. coli, P. vulgaris, and B. globigii on the three surfaces used. (See Table I.) They were at least 95-percent effective against S. epidermidis. Hyamine 3500 in aqueous solution was the only germicide to reduce (kill) as much as 90-percent of the B. globigii spore population, and this was for the stainless steel surface. The alcoholic Hyamine 3500 killed approximetely 75 percent of the spores. The results with the Staphylococcus and the spore populations seem to indicate that the epoxy-painted surface may have a protective effect on the organisms deposited on this type of surface. This is perhpas a protective effect due to the paint surface. The number of tests done in this study is insufficient to illustrate this phenomenon as more than a possibility. For practical purposes of use in decontaminating the surfaces of tables, walls, tools, and other equipment found in low-burden areas, especially where the microbiological population consists mainly of spore formers, the quaternary ammonium compounds appear to be most effective. Where the microbial flora is largely composed of vegetable cells, both the quaternary ammonium and phenol derivatives are approximately equally effective.

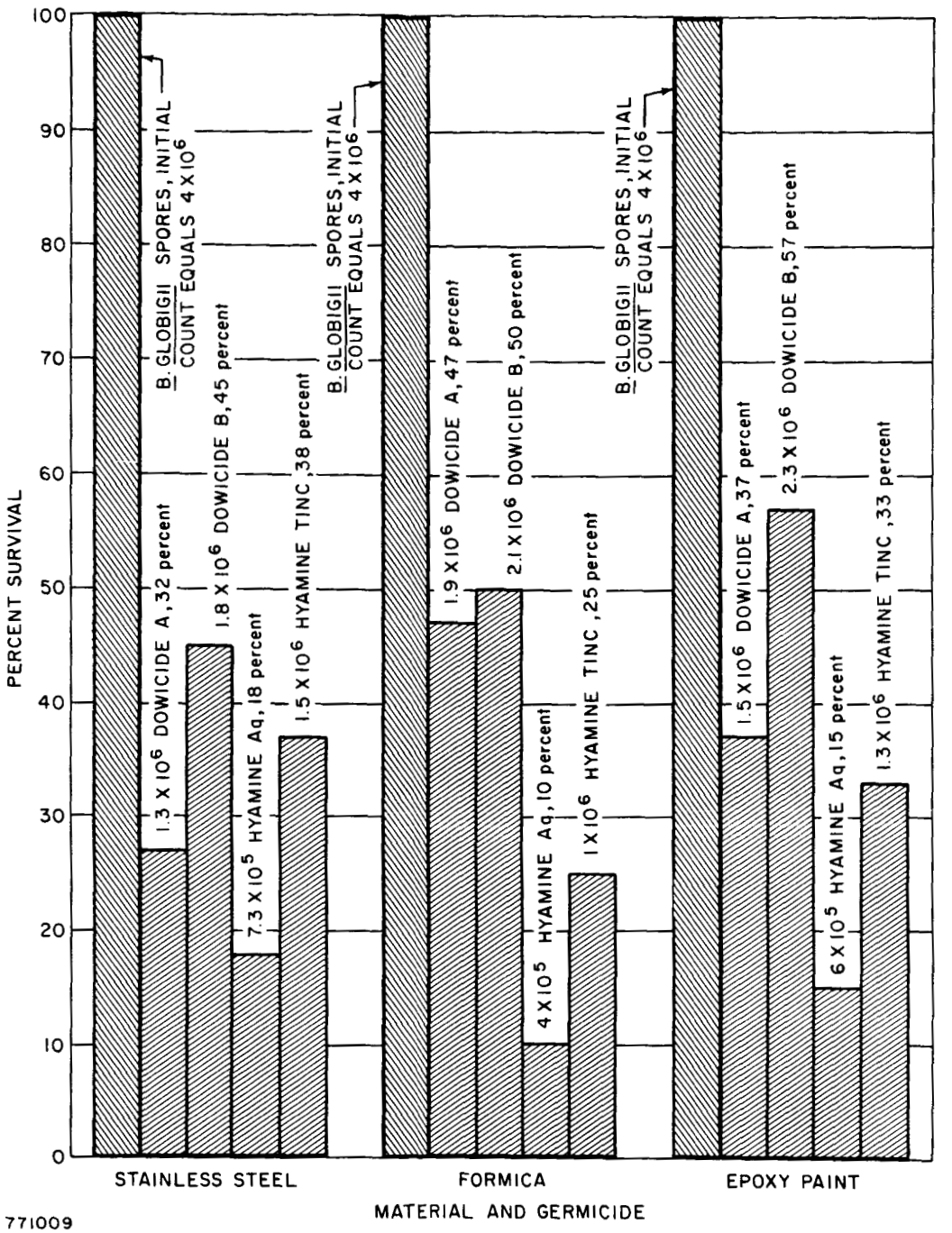

Figure 1. SURVIVAL OF B. GLOBIGII SPORES ON THREE TYPES OF SURFACES EXPOSED TO FOUR GERMICIDES

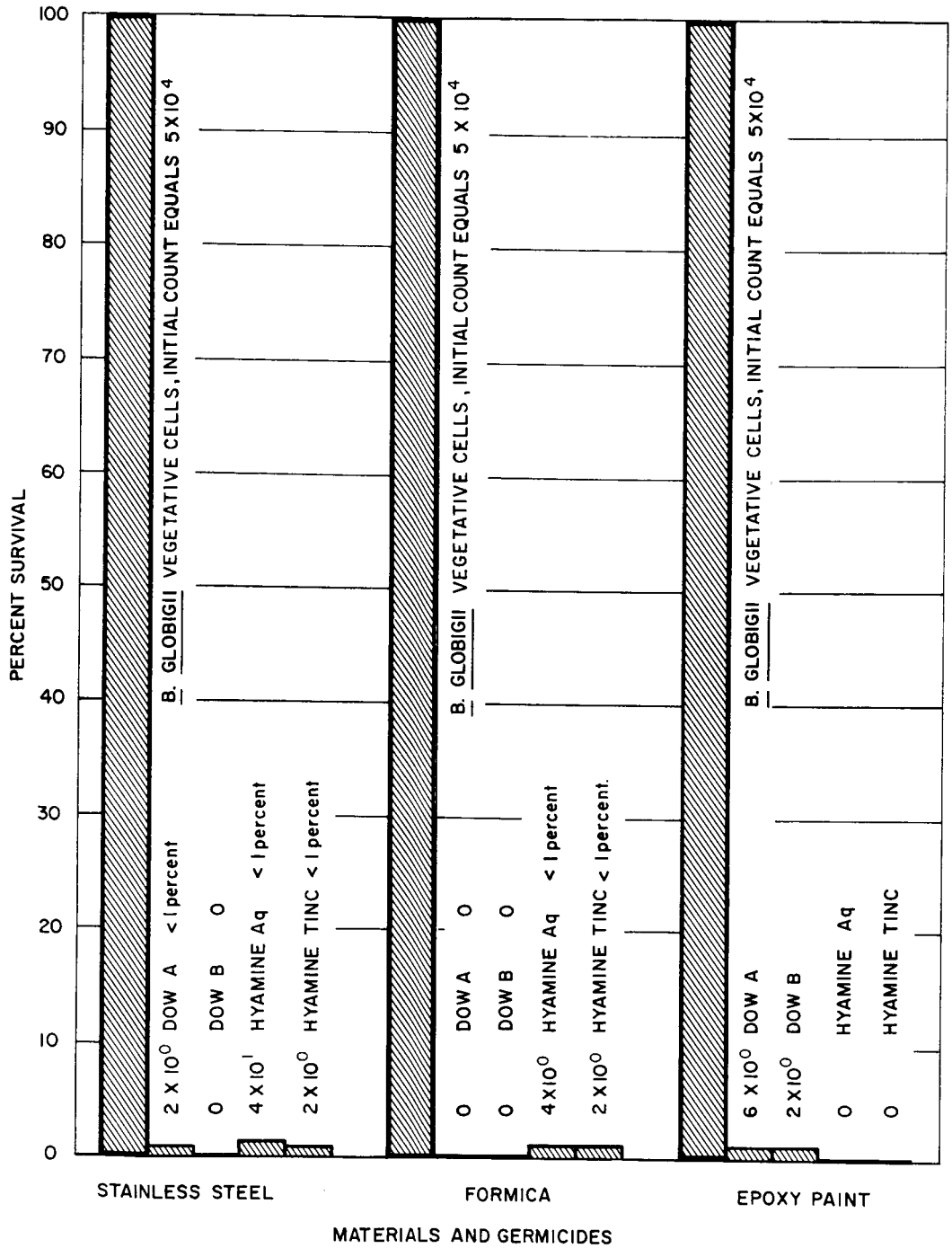

Figure 2. SURVIVAL OF B. GLOBIGII VEGETATIVE CELLS ON THREE TYPES OF SURFACES EXPOSED TO FOUR GERMICIDES

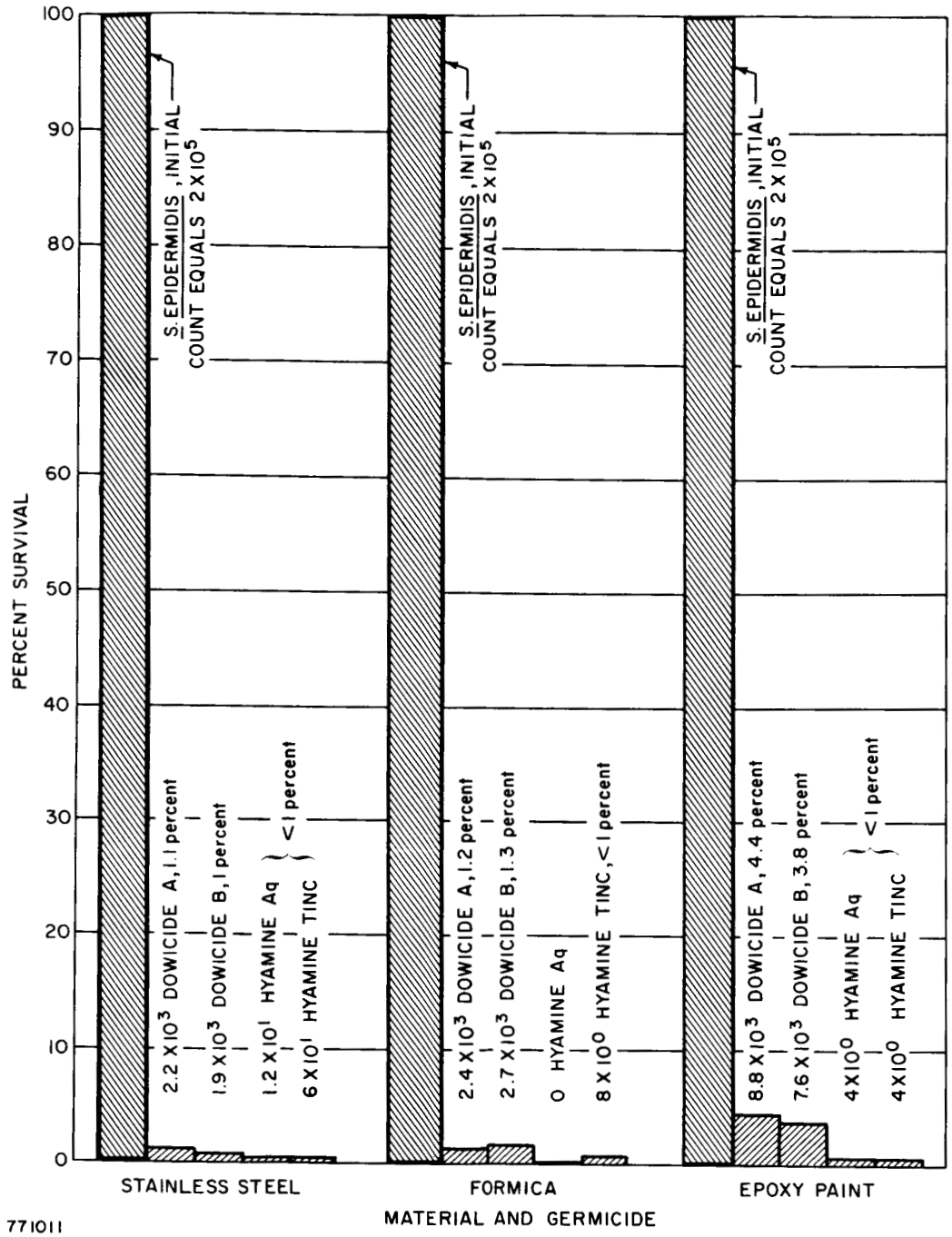

Figure 3. SURVIVAL OF S. EPIDERMIDIS ON THREE TYPES OF SURFACES EXPOSED TO FOUR GERMICIDES

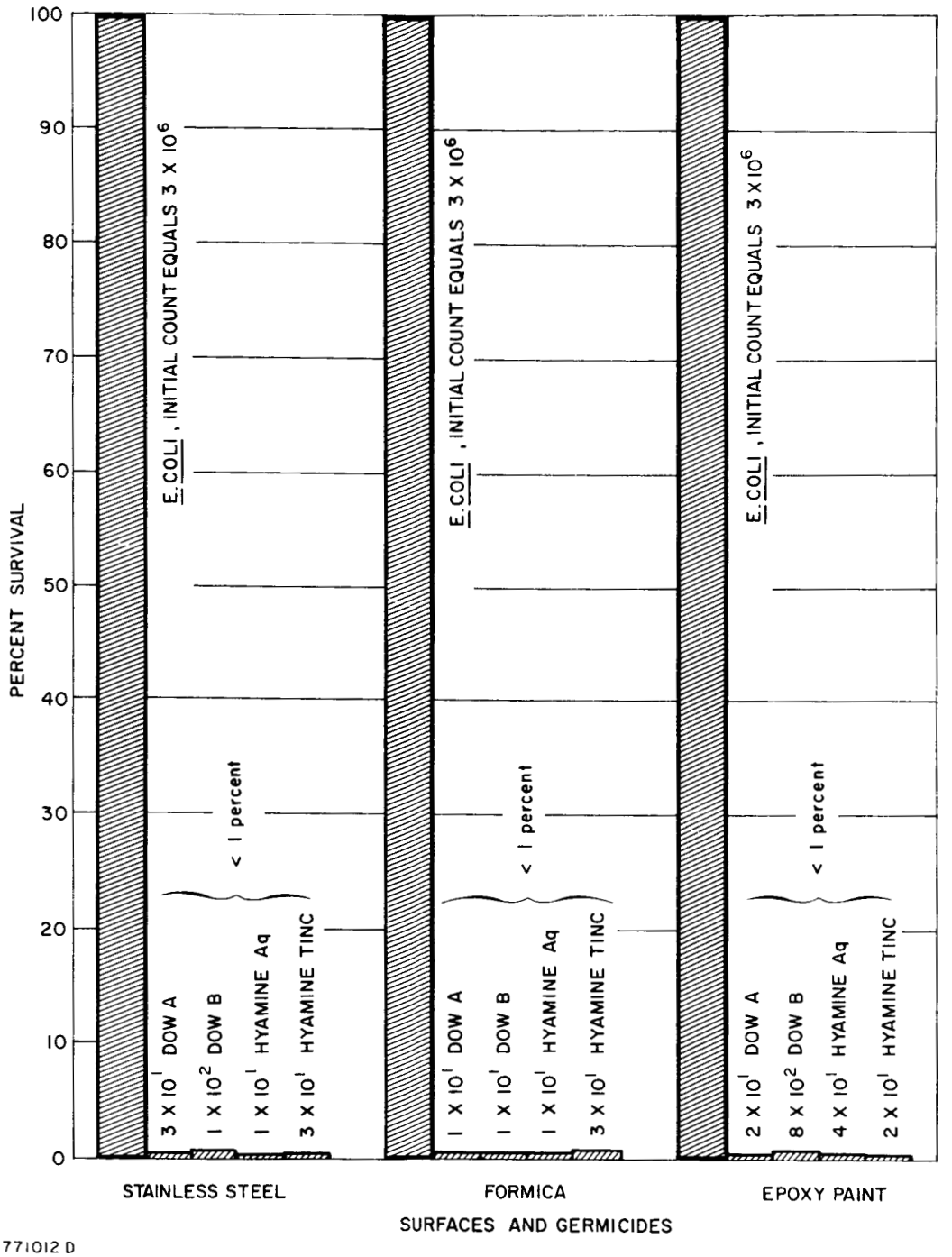

Figure 4. SURVIVAL OF E. COLI ON THREE TYPES OF SURFACES EXPOSED TO FOUR GERMICIDES

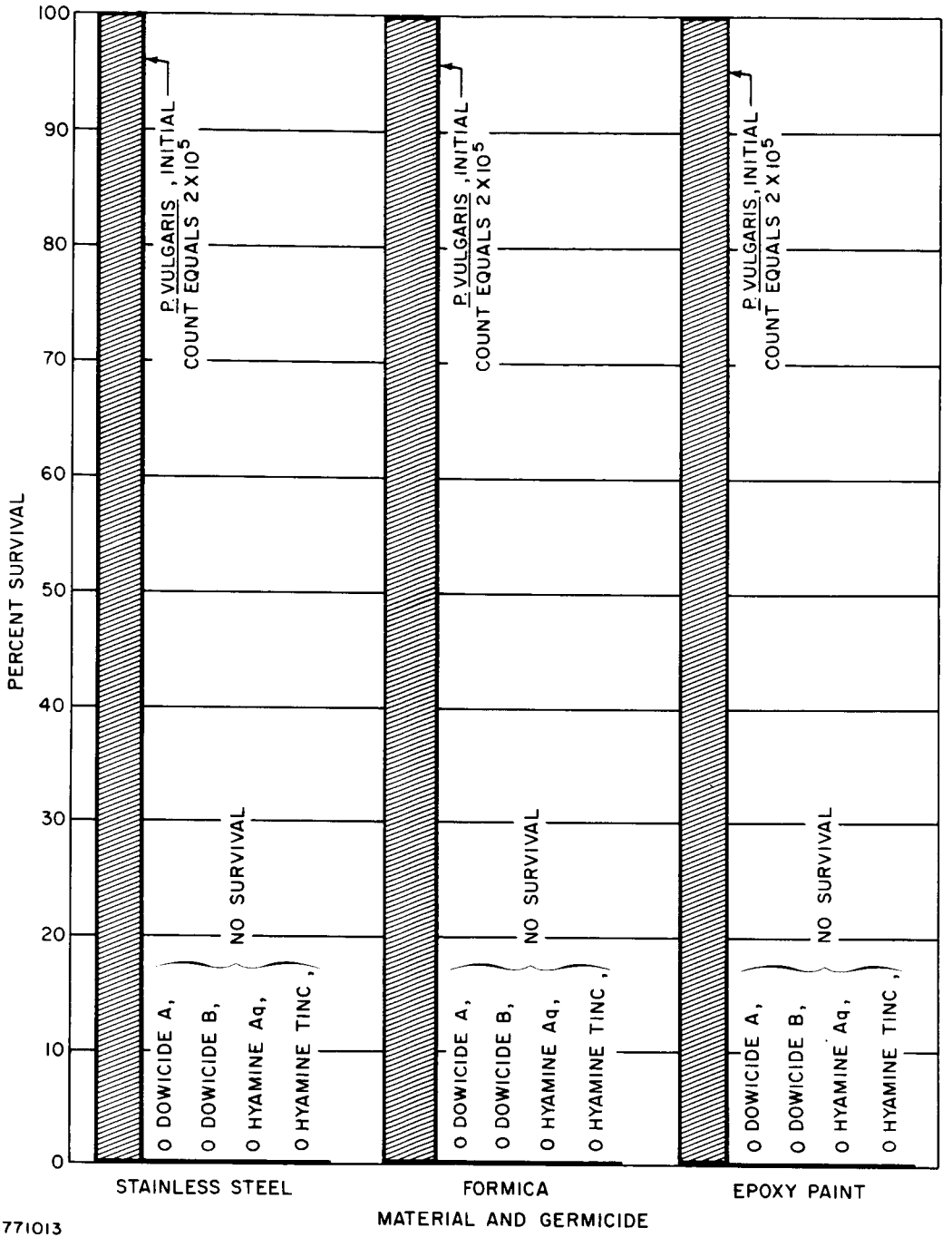

Figure 5. SURVIVAL OF P. VULGARIS ON THREE TYPES OF SURFACES EXPOSED TO FOUR GERMICIDES

TABLE I

KILL EFFECTIVENESS OF HYAMINE 3500 AND DOWICIDE PREPARATIONS

GERMICIDE STUDY RESULTS

Organism	Material		Dowicide A	Dovicide B	Hyamine AQ.	Hyamine Tincture
B. globigii Spores Inoculum: 1.5×10^8/ml	Stainless Steel	Initial Count	4×10^6	4×10^6	4×10^6	4×10^6
		Survivors	1.3×10^6	1.8×10^6	7.3×10^5	1.6×10^6
		Percent Survival	33	45	18	37
	Formica	Initial Count	4×10^6	4×10^6	4×10^6	4×10^6
		Survivors	1.9×10^6	2.1×10^6	4×10^5	1.0×10^6
		Percent Survival	47	50	10	25
	Epoxy Paint	Initial Count	4×10^6	4×10^6	4×10^6	4×10^6
		Survivors	1.5×10^6	2.3×10^6	6×10^5	1.3×10^6
		Percent Survival	37	57	15	33
B. globigii veg. cells Inoculum: 7×10^6/ml	Stainless Steel	Initial Count	5×10^4	5×10^4	5×10^4	5×10^4
		Survivors	2×10^0	0	4×10^1	2×10^0
		Percent Survival	<1	0	<1	<1
	Formica	Initial Count	5×10^4	5×10^4	5×10^4	5×10^4
		Survivors	0	0	4×10^0	2×10^0
		Percent Survival	0	0	<1	<1
	Epoxy Paint	Initial Count	5×10^4	5×10^4	5×10^4	5×10^4
		Survivors	6×10^0	2×10^0	0	0
		Percent Survival	<1	<1	0	0
E. coli Inoculum: 3×10^8/ml	Stainless Steel	Initial Count	3×10^6	3×10^6	3×10^6	3×10^6
		Survivors	3×10^1	1×10^2	1×10^1	3×10^1
		Percent Survival	<1	1	<1	<1
	Formica	Initial Count	3×10^6	3×10^6	3×10^6	3×10^6
		Survivors	1×10^1	1×10^1	1×10^1	3×10^1
		Percent Survival	1	1	<1	<1
	Epoxy Paint	Initial Count	3×10^6	3×10^6	3×10^6	3×10^6
		Survivors	2×10^1	8×10^2	4×10^1	2×10^1
		Percent Survival	<1	<1	<1	<1
S. epidermidis Inoculum: 2×10^7/ml	Stainless Steel	Initial Count	2×10^5	2×10^5	2×10^5	2×10^5
		Survivors	2.2×10^3	1.9×10^3	1.2×10^1	6×10^1
		Percent Survival	1.1	1	<1	<1
	Formica	Initial Count	2×10^5	2×10^5	2×10^5	2×10^5
		Survivors	2.4×10^3	2.7×10^3	0	8×10^0
		Percent Survival	1.2	1.3	0	<1
	Epoxy Paint	Initial Count	2×10^5	2×10^5	2×10^5	2×10^5
		Survivors	8.8×10^3	7.6×10^3	4×10^0	4×10^0
		Percent Survival	4.4	3.8	<1	<1
P. vulgaris Inoculum 2×10^7/ml	Stainless Steel	Initial Count	2×10^5	2×10^5	2×10^5	2×10^5
		Survivors	0	0	0	0
		Percent Survival	0	0	0	0
	Formica	Initial Count	2×10^5	2×10^5	2×10^5	2×10^5
		Survivors	0	0	0	0
		Percent Survival	0	0	0	0
	Epoxy Paint	Initial Count	2×10^5	2×10^5	2×10^5	2×10^5
		Survivors	0	0	0	0
		Percent Survival	0	0	0	0

V. CONCLUSIONS

Aqueous Hyamine 3500 and the tincture of Hyamine 3500 were most effective against the spore population, in the order cited.

An epoxy-painted surface may afford some protection to the microbial flora against the action of a chemical germicide.

Either type of Hyamine 3500 is the germicide most effective against the vegetative cells.

It may be possible to effectively use the germicides studied in higher concentration or for longer exposure times against spores.

VI. RECOMMENDATIONS

It is recommended that further studies be done in determining the effectiveness of germicides against spore populations.

It is recommended that further studies be performed to elucidate the protective phenomenon associated with epoxy-painted surfaces.

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