CORE
Metadata, citation and similar papers at core.ac.uk
Provided by NASA Technical Reports Server

COMPARATIVE LEVELS AND SURVIVAL OF NATURALLY OCCURRING MICROORGANISMS DEPOSITED ON SURFACES THROUGH HANDLING AND AERIAL FALLOUT

A Preliminary Report

Services Provided in Support of the Planetary Quarantine Requirements

of the

National Aeronautics and Space Administration

under

Contract R-137

Prepared by: Martin S. Favero

John R. Puleo

James H. Marshall

Gordon S. Oxborrow

Phoenix Field Station, Technology Branch
Communicable Disease Center
Public Health Service
U. S. Department of Health, Education, and Welfare

FACILITY FORM 602
N65-35116 (ACCESSION NUMBER)
88 (PAGES)
CR 62967 (NASA CR OR TMX OR AD NUMBER)
(THRU)
(CODE) 04
(CATEGORY)

REPORT NO. 8

August 9, 1965

GPO PRICE $ _____
CFSTI PRICE(S) $ _____
Hard copy (HC) 1.00
Microfiche (MF) .50
ff 653 July 65

COMPARATIVE LEVELS AND SURVIVAL OF NATURALLY OCCURRING MICROORGANISMS DEPOSITED ON SURFACES THROUGH HANDLING AND AERIAL FALLOUT

ABSTRACT

Studies were performed to determine the death rates of naturally occurring microorganisms deposited on surfaces of stainless steel and electronic components by handling and aerial fallout. Experiments were carried out in both spacecraft assembly areas in operation and in laboratory environments. Stainless steel strips were exposed to industrial clean room and laboratory environments for a period of time and then covered with sterile aluminum foil. Assays were performed immediately before and at intervals up to 8 weeks after covering. The results indicate clearly that the total aerobic mesophilic population decreased by approximately 50 percent after 2 weeks. Non-spore-forming bacteria were reduced by 80 percent after 2 weeks. The aerobic sporeformers, molds, and actinomycetes did not appear to be affected.

In another series of tests sterile stainless steel strips were contaminated by handling, placed in sterile containers and assayed at intervals up to 4 weeks. After 2 weeks, less than 10 percent of the microbial population survived and after 4 weeks, 1 to 2 percent survived.

Handwashing with a hexachlorophene soap reduced slightly the level of microorganisms deposited on surfaces. A 70 percent ethyl alcohol or 70 percent isopropyl alcohol rinse, however, was much more effective. The use of a mild soap usually resulted in an increase in the level of deposited microorganisms.

Author

INTRODUCTION

The National Aeronautics and Space Administration (NASA) requires that spacecraft impacting Mars be dry-heat sterilized prior to launch. The sterilization cycle, as well as all subsequent operations, must insure that the chances for transporting viable terrestrial organisms to Mars be less than one in ten thousand. Because the heat cycle is a relatively low one, the probability of obtaining a sterile spacecraft is enhanced greatly if the levels of microbiological contamination are relatively low prior to the heat treatment.

Consequently, spacecraft required to be sterile must be assembled in areas where the levels of microbiological contamination in the environment can be maintained at an extremely low level. At the present time the NASA is supporting studies to determine whether industrial clean rooms, especially those employing ultra-high efficiency filtration and laminar air flow, can be used reliably for such purposes.

During the course of these studies several groups of investigators, using identical methods, have showed consistently that levels of contamination resulting from the fallout of airborne microorganisms onto collecting surfaces do not increase during relatively long exposures. There appears to be no significant difference in the level of microbiological contamination that accumulates on stainless steel surfaces after exposures up to 21 weeks.[1,2,3,4,5] One study[3] showed that stainless steel strips exposed to the intramural air of an industrial clean room for one week contained the same level of microorganisms as those exposed for 52 weeks. This has been referred to as the "plateau phenomenon." The consensus of these investigators is that the

plateau phenomenon is due to the death of microorganisms and perhaps, to some extent, dislodgment of the organisms from the surfaces.

The objectives of the studies discussed in this report were: (1) to determine the death rate of naturally occurring airborne microorganisms which accumulate on stainless steel surfaces and to obtain an idea of what types of microorganisms tend to survive during storage in a sterile environment; (2) to determine the levels of contamination deposited on stainless steel surfaces resulting from handling and their rate of die-off when exposed to a sterile environment; and (3) to determine the effects of washing with conventional and hexachlorophene soap and rinsing with alcohol on the levels of microorganisms deposited by handling.

MATERIALS AND METHODS

A tray containing 1 x 2 inch stainless steel strips was prepared and sterilized in the manner described previously.[4] The strips were exposed to the intramural air of industrial Clean Room B.[4] This room employs high efficiency filtration[6] and personnel wear full-length lint-free suits, gloves, and plastic booties. At 3-week intervals six strips were retrieved and assayed. These data have been presented, in part, in a previous report.[4] The remaining data will be included in a future report. Only the die-off aspects will be described here. At the end of the regular 21-week period of exposure, the six remaining strips were covered with a sheet of sterile aluminum foil. These strips were assayed after 3 weeks. Identification studies were performed on the series assayed immediately before covering and those which were covered for 3 weeks.

A similar experiment was performed in a laboratory environment. The strips were prepared and exposed as described above. After 3 weeks of exposure six strips were assayed and the remaining strips were covered immediately with sterile aluminum foil. At intervals of 1, 2, 4, 5, 6, and 8 weeks, six strips were assayed. The microorganisms isolated at all intervals were identified.

Experiments designed to determine the death rates of microorganisms deposited on stainless steel strips by handling were set up as follows. Personnel involved handled a series of strips in the same manner, and the strips were placed in individual sterile bottles. Although the strips were handled consecutively, they were not assayed in sequence. For example, when 60 strips were handled consecutively, strips 1, 7, 13, 19, 25, 31, 37, 43, 49, and 55 were assayed at the first interval, and strips 2, 8, 14, 20, 26, 32, 38, 44, 50, and 56 at the second, etc. Every other numbered strip in the series was used for aerobic spore determinations. Assays were made immediately before covering and after 1, 7, 14, 21, and 28 days of storage. At the time of assay, 50 ml of sterile 1 percent peptone water were transferred to each bottle containing a strip. These bottles were shaken mechanically (270 shakes per minute) for 15 minutes and then aseptically poured into 150 x 20 mm sterile petri dishes. Fifty ml of sterile double-strength molten trypticase soy agar were added and the contents mixed thoroughly. For enumerating aerobic spores, the suspensions were heat-shocked for 15 minutes at 80° C prior to plating. Incubation time was 72 hours at 32° C.

Several experiments were conducted to determine the effect of handwashing with a mild soap (Ivory) and one containing hexachlorophene (pHisoHex). Alcohol rinsing also was tested. In all of these studies the subject handled

5 to 10 strips, washed (or rinsed) his hands for 60 seconds, dried them with a sterile towel, and handled another 5 to 10 strips. Assays were made by shaking the strips in 50 ml of 1 percent peptone water and plating with 50 ml double-strength trypticase soy agar, as described above. Aerobic spores were enumerated as described above.

In order to obtain information concerning the level of microbiological contamination deposited by human handling, two series of experiments were performed. One series involved the personnel in Clean Room A.[4] Twelve persons performing actual assembly operations were given, separately, five sterile stainless steel strips to handle. The handling procedure was standardized as well as possible. The strips were then assayed in the usual manner for aerobic mesophilic microorganisms.

A similar experiment was performed in an electronics manufacturing area where personnel were assembling, cleaning, and packaging transistors. Several hundred of one type of transistor were obtained and subsequently cleaned and dry-heat sterilized by the same techniques used to clean and sterilize stainless steel strips described previously.[4] Each of five persons who wore small finger cots during assembly handled 10 sterile transistors. The same procedure was performed by five other persons not wearing finger cots. Transistors to be assayed for aerobic mesophilic microorganisms were placed immediately into bottles containing 50 ml of sterile 1 percent peptone water and processed within 1 hour in the usual manner. Transistors to be assayed for aerobic spores were collected in empty sterile bottles; sterile 1 percent peptone water was added prior to heat-shocking at 80° C for 15 minutes. Cultures were incubated for 72 hours at 32° C.

RESULTS

The results of survival studies performed in Clean Room B are presented in Table 1. Immediately prior to covering, the level of aerobic mesophilic microorganisms was approximately 9,000 per square foot. The majority of these organisms were Staphylococcus spp., Micrococcus spp., and other non-sporeforming bacteria. This qualitative aspect was present throughout the entire study in Clean Room B.[4] The total population of aerobic mesophiles decreased by 51 percent after 3 weeks. Organisms surviving storage were mostly Bacillus spp. (sporeformers) and molds.

Similar results were obtained from experiments in which stainless steel strips were exposed to the intramural air of a laboratory for 3 weeks and then covered. As mentioned previously, microbiological assays were performed immediately prior to covering with sterile aluminum foil and at intervals up to 8 weeks after covering. At each assay period, 30 to 40 colonies were picked randomly and subsequently identified. The results are presented graphically in Figure 1. The population of aerobic mesophilic microorganisms decreased by 50 percent after 2 weeks and then remained at a constant level. Identification studies, however, showed that this seemingly significant decrease was due primarily to the death of non-sporeforming bacteria. Aerobic sporeformers (Bacillus spp.) as well as molds and actinomycetes were hardly affected. The relative humidity and temperature, which were recorded continuously, ranged from 42 to 49 percent and from 71° F to 76° F.

Microorganisms deposited on stainless steel surfaces by handling decreased significantly during sterile storage. Table 2 shows that approximately

80 percent of the initial population failed to survive after 2 weeks of storage. As determined by qualitative studies, the vast majority of microorganisms deposited on these surfaces were non-sporeformers. The levels of aerobic spores deposited by handling were consistently so low that die-off rates could not be measured reliably. Table 3 contains typical results of the latter type of experiment.

The results of hand-washing with a standard hexachlorophene soap and rinsing with 70 percent ethyl alcohol and 70 percent isopropyl alcohol are presented in Table 4. The hexachlorophene soap handwash did not reduce significantly the levels of microbiological contamination deposited on stainless steel surfaces. Both alcohol rinses, especially 70 percent isopropyl alcohol, appeared to reduce deposited contamination levels significantly. Control experiments performed with a mild soap (Ivory) showed that in most cases the level of microbiological contamination increased after washing. Typical results are shown in Table 5.

Table 6 contains the results obtained from the study of levels of microbiological contamination deposited on stainless steel strips by personnel performing actual assembly operations. There was much variation from person to person but the levels from each person were usually consistent, especially in the case of those persons who deposited low levels.

Similar results were obtained from personnel also performing actual assembly operations but half of whom wore finger cots. (Table 7). The use of finger cots reduced the levels of both non-sporeforming organisms and spores deposited on electronic components.

DISCUSSION

The fact that certain types of microorganisms cannot survive in the absence of nutrients and moisture has been known for many years. Most studies have been concerned with food preservation or the preservation of cultures of microorganisms. Few studies concerning the survival of microorganisms, especially naturally occurring microorganisms, on surfaces have been reported. Recently McDade and Hall[7,8] reported the results of definitive studies on the major environmental factors affecting microbial survival on various surfaces. Their studies clearly show that relative humidity has a pronounced effect on the survival of Staphylococcus aureus on surfaces of glass, ceramic tile, stainless steel, asphalt tile, and rubber tile. Death rates of surface-exposed S. aureus increased as the relative humidity increased. Similar results were obtained with five species of gram negative rods.[9]

Temperature is another major environmental factor that governs the death rate of microorganisms. As the temperature level increases, the rate of death also increases.

The data presented in this preliminary report concern the survival of naturally occurring microorganisms on surfaces. It is evident from the results presented in Table 1 and Figure 1 that the death rates of airborne microorganisms that accumulate on stainless steel strips used as collecting surfaces depend to great extent on the type of microorganisms involved. Non-sporeforming bacteria exhibit definite die-off. Aerobic spores, which are of obvious concern, and molds and actinomycetes did not appear to be affected significantly.

Storage of spacecraft components in a sterile or even a "bioclean" environment may cause significant reductions in the levels of non-sporeforming microorganisms on surfaces. However, it appears doubtful that levels of spores would be reduced simultaneously.

Consequently any general conclusions applicable to spacecraft decontamination should be based on future experiments designed to measure die-off of both naturally occurring aerobic and anaerobic spores. Such experiments should be augmented by artificially seeding surfaces with known numbers of aerobic and anaerobic spores and measuring any subsequent die-off in controlled environments. Both temperature and relative humidity should be critically controlled.

The level of microbiological contamination deposited on stainless steel surfaces as the result of handling varied among different subjects. Separating the skin from the surface being touched by a finger cot reduces deposited contaminants. Alcohol rinses appear to be effective in decontaminating hands. Although the use of hexachlorophene soap reduces the level of contamination deposited, it does not appear to be as effective as alcohol rinsing. The use of a conventional soap is practically useless if not undesirable. In fact, a one-minute handwash with a mild soap is used routinely in our laboratory to increase the number of microorganisms deposited on strips to a level high enough to be statistically meaningful. In most instances the level of contamination increases 1 to 2 logs after washing. These results agree with studies concerned with increased shedding of microorganisms after showering.[10,11]

REFERENCES

1. McDade, J. J., A. S. Irons, and V. I. Magistrale. 1965. A microbiological survey of the Hughes Aircraft Company facilities involved in the assembly and/or testing of Surveyor spacecraft. Space Program Summary 3735, vol. 4, April 30, 1965. Jet Propulsion Laboratories, Pasadena, California.

2. Favero, M. S. 1965. Microbiological sampling studies at Hughes Aircraft Company. Report on Contract R-137, National Aeronautics and Space Administration, Washington, D. C., January 26, 1965.

3. Portner, D. M., R. F. Hoffman, H. M. Decker, and C. R. Phillips. 1964. The level of microbial contamination in a clean room during a one year period. Protection Branch Report of Test No. 11-65. Fort Detrick, Frederick, Maryland.

4. Favero, M. S., J. R. Puleo, J. H. Marshall, and G. S. Oxborrow. 1965. Detection and quantitation of microbial contamination to which spacecraft are subjected during manufacture. Report on Contract R-137, National Aeronautics and Space Administration, Washington, D. C., March 18, 1965.

5. Michaelson, G. S. 1965. The bacteriology of clean rooms. Progress report on Contract NSG 643, National Aeronautics and Space Administration, Washington, D. C., March 31, 1965.

6. Decker, H. M., L. Buchanan, L. B. Hall, and K. R. Goddard. 1962. Air filtration of microbial particles. Public Health Service Publication No. 953.

7. McDade, J. J., and L. B. Hall. 1963. An experimental method to measure the influence of environmental factors on the viability and the pathogenicity of Staphylococcus aureus. Amer. Jour. Hygiene 77:98-108.

8. McDade, J. J., and L. B. Hall. 1964. Survival of Staphylococcus aureus in the environment. II. Effect of elevated temperature on surface-exposed staphylococci. Amer. Jour. Hygiene 80:184-191.

9. McDade, J. J., and L. B. Hall. 1964. Survival of gram-negative bacteria in the environment. I. Effect of relative humidity on surface-exposed organisms. Amer. Jour. Hygiene 80:192-204.

10. Speers, R., H. Bernard, F. O'Grady, and R. Shooter. 1965. Increased dispersal of skin bacteria into the air after shower-baths. Lancet 7383:478-480.

11. Bethune, D. W., R. Bowers, and M. Parker. 1965. Dispersal of Staphylococcus aureus by patients and surgical staff. Lancet 7383:480-483.

TABLE 1.

TYPES OF MICROORGANISMS SURVIVING ON STAINLESS STEEL SURFACES INITIALLY EXPOSED TO THE INTRAMURAL AIR OF CLEAN ROOM B FOR 21 WEEKS AND THEN COVERED WITH STERILE ALUMINUM FOIL

Type of microorganisms	Immediately before covering[1]	3 Weeks after covering[2]
	%	%
Staphylococcus aureus	0	0
Staphylococcus epidermidis	61.1	5.2
Micrococcus spp.	11.1	17.9
Bacillus spp. (sporeformers)	13.9	48.7
Misc. gram positive bacilli[3]	5.6	7.7
Gram negative microorganisms	0	0
Yeasts	2.7	0
Molds	5.6	17.9
Actinomycetes and streptomycetes	0	2.6

[1] Level of mesophilic microorganisms was 9,237/ft^2

[2] Level of mesophilic microorganisms was 4,557/ft^2

[3] Predominant genera: Brevibacterium, Corynebacterium, and Lactobacillus

TABLE 2.

SURVIVAL OF MICROORGANISMS DEPOSITED ON STERILE STAINLESS STEEL STRIPS BY HANDLING AND SUBSEQUENTLY STORED IN A STERILE ENVIRONMENT

Subject, average and percent reduction	Number of aerobic, mesophilic microorganisms per strip					
	Immediately after handling	24 hours after storage[1]	7 days after storage	14 days after storage	3 weeks after storage	4 weeks after storage
L-2-W						
Average[2]	69.4	8.6	3.2	2.8	1.0	0.2
Reduction	-	87.6%	95.4%	96.0%	98.6%	99.7%
F-1						
Average[2]	43.3	18.0	2.8	9.2	2.2	0.4
Reduction	-	58.4%	93.5%	78.8%	94.9%	99.1%
F-2-W						
Average[2]	178.4	15.2	3.2	1.4	4.2	1.4
Reduction	-	91.5%	98.2%	99.2%	97.6%	99.2%
P-1						
Average[3]	83.7	25.5	5.5	0.8	0.3	-
Reduction	-	69.5%	93.4%	99.0%	99.6%	-
C						
Average[2]	44.2	20.0	16.5	2.2	1.6	2.2
Reduction	-	54.8%	62.7%	95.0%	96.4%	95.0%
L-1						
Average[2]	6.4	1.4	1.8	1.2	0.8	0.6
Reduction	-	78.1%	71.9%	81.3%	87.5%	90.6%

[1]Average relative humidity throughout test period, 45.5% (39-50%). Average temperature 73° F (70-77° F).

[2]Average of 5 tests.

[3]Average of 4 tests.

TABLE 3.

LEVELS OF AEROBIC SPORES DEPOSITED ON STAINLESS STEEL STRIPS BY HANDLING AND THEIR SURVIVAL IN A STERILE ENVIRONMENT

Subject	Average number[1] of aerobic spores per strip					
	Immediately after handling	Number of days after storage				
		1	7	14	21	28
C	3.4	0	0.2	0.2	0.4	3.8
P	2.3	5.8	0.5	0.8	0	-
O	4.0	1.0	-	2.0	5.0	-
M	0.8	-	0.3	0.5	0.5	-

[1] Average of 5 strips.

TABLE 4.

EFFECT OF WASHING WITH HEXACHLOROPHENE SOAP OR RINSING WITH ALCOHOL ON THE LEVELS OF MICROBIOLOGICAL CONTAMINATION DEPOSITED ON STAINLESS STEEL SURFACES BY HANDLING

Subject	Method	Average[1] percent reduction
A	Hexachlorophene wash[2]	47.7
	70% Ethyl alcohol rinse	93.4
	70% Isopropyl alcohol rinse	100.0
B	Hexachlorophene wash	96.3
	70% Ethyl alcohol rinse	87.8
	70% Isopropyl alcohol rinse	98.5
C	Hexachlorophene wash	10.2
	70% Ethyl alcohol rinse	94.2
	70% Isopropyl alcohol rinse	98.9
D	Hexachlorophene wash	2.3
	70% Ethyl alcohol rinse	50.5
	70% Isopropyl alcohol	81.0

1 Average of ten determinations except 70% ethyl alcohol rinse which had 20 determinations.

2 pHisoHex.

TABLE 5.

EFFECT OF WASHING ON THE LEVEL OF MICROBIOLOGICAL CONTAMINATION DEPOSITED ON STAINLESS STEEL STRIPS BY HANDLING

Subject	Condition	Average number of aerobic mesophilic microorganisms per strip[1]	Range
L	Before washing	25.4	15 - 42
	After washing[2]	1796.0	252 - 4020
T	Before washing	9.7	1 - 51
	After washing	256.4	58 - 796
P	Before washing	67.9	6 - 396
	After washing	225.3	131 - 384
O	Before washing	276.0	12 - 648
	After washing	193.4	38 - 416
J	Before washing	171.4	0 - 504
	After washing	25.0	4 - 74

[1] Average of ten tests.

[2] Ivory soap.

TABLE 6.

LEVELS OF MICROBIOLOGICAL CONTAMINATION DEPOSITED ON STAINLESS STEEL STRIPS BY PERSONNEL PERFORMING ASSEMBLY OPERATIONS IN CLEAN ROOM A

Subject	Aerobic mesophilic microorganisms	
	Average Number per strip[1]	Range
A	0.8	0 - 2
B	23.8	1 - 77
C	0.8	0 - 2
D	34.8	18 - 69
E	0.8	0 - 1
F	10.1	6 - 16
G	1.2	0 - 3
H	104.0	41 - 207
I	2.0	0 - 4
J	1.4	0 - 3
K	12.2	2 - 24
L	0.3	0 - 1

[1] Average of 5 samples.

TABLE 7.

LEVELS OF MICROBIOLOGICAL CONTAMINATION DEPOSITED ON TRANSISTORS BY PERSONNEL PERFORMING ASSEMBLY OPERATIONS

Subject	Aerobic microorganisms		Aerobic spores	
	Average[1] No./component	Range	Average[1] No./component	Range
	Ungloved personnel			
A	15.8	5 - 21	13.2	0 - 21
B	87.8	31 - 269	5.2	1 - 12
C	51.4	3 - 223	0.2	0 - 1
D	1.2	0 - 4	10.8	2 - 26
E	54.2	18 - 122	33.6	2 - 152
	Personnel wearing finger cots			
F	11.2	2 - 22	2.2	0 - 6
G	4.7	0 - 19	0.4	0 - 2
H	13.2	6 - 16	0.8	0 - 2
I	13.7	8 - 27	0.4	0 - 1
J	15.8	12 - 25	1.6	0 - 4

[1] Average of 5 samples.

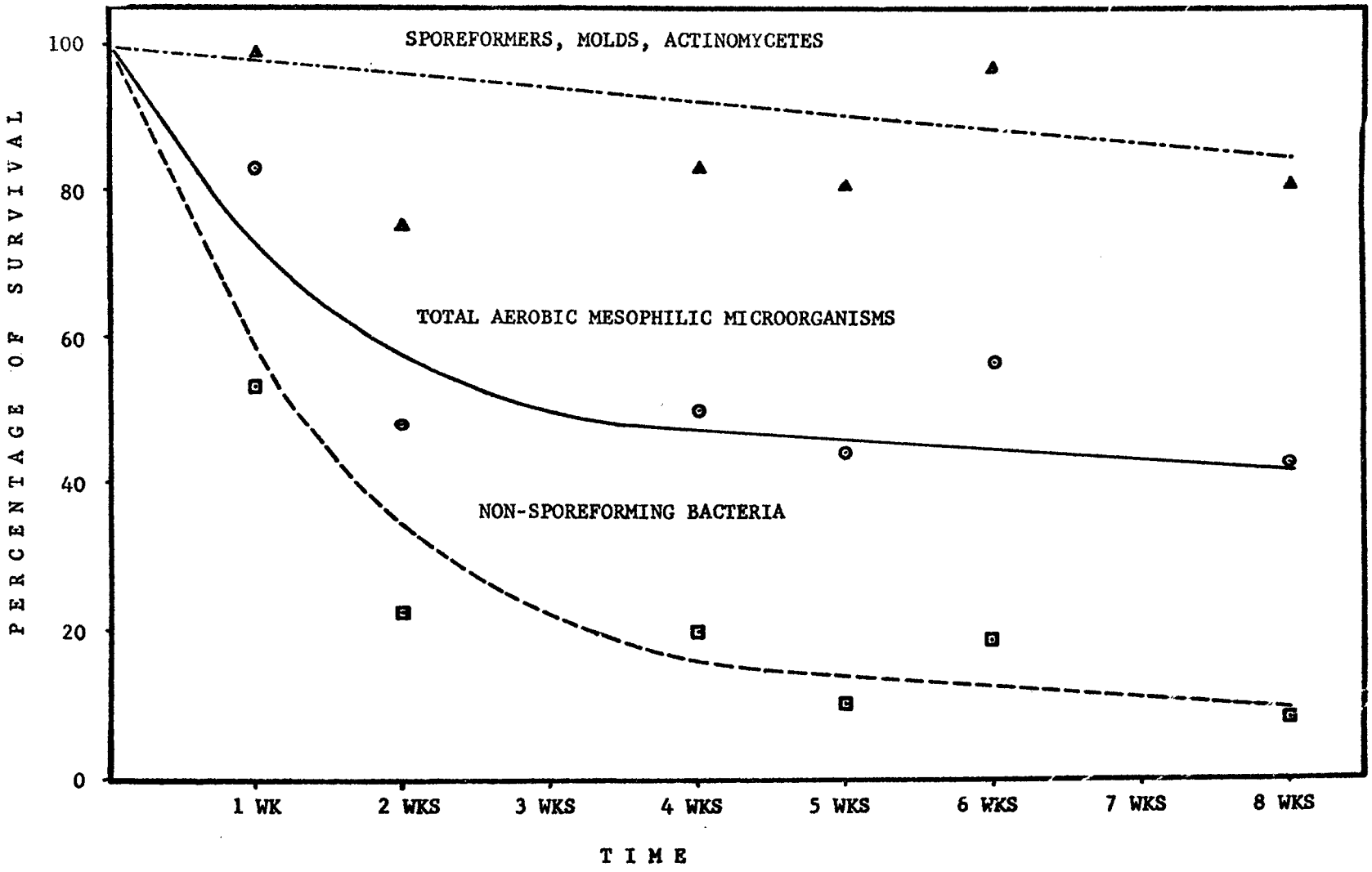

Figure 1: Survival of airborne naturally occurring microorganisms on stainless steel surfaces