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Protection Branch Report of Test No. 13-68

Survival of Microorganisms on Covered Stainless Steel Initially Contaminated by Handling and by Aerial Fallout During a $2\frac{1}{2}$ Year Study

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As part of the overall investigation of spacecraft sterilization, this study was undertaken to determine the death rate of naturally accumulated microorganisms on stainless steel strips. The probability of sterilizing a spacecraft by any method is enhanced when the initial microbial contamination is minimal. It was first observed in this laboratory (1) that fallout and accumulation of airborne microorganisms on stainless steel surfaces rapidly reached a level that did not increase significantly thereafter, even during a long exposure period. In studies conducted in clean rooms (2,3,4), this "plateau" phenomenon was also shown to occur but at a lower level than that in adjacent areas. The "plateau" level is a function of the amount of airborne contamination in the area. Thus, if a spacecraft is constructed and assembled in a clean room, the level of contamination collecting on it from aerial fallout will be less.

The "plateau" phenomenon indicates that since there is a continual accumulation of microorganisms on a surface from aerial "fallout", there must be an equivalent "die-off" rate, thus, theoretically, if the contaminated surface was covered to prevent additional contamination, sterility would result in time. Favero et al. (5) determined the "die-off" rate for organisms on stainless steel, accumulated from natural "fall-out" and from handling. Three weeks after covering, they found a reduction of 50% in viable organisms accumulated from fallout and 90% in viable cells deposited by handling. The extensive study reported here shows the rate of decrease ov viable microorganisms on stainless steel strips initially contaminated by handling and by aerial fallout and then recovered and stored for 2 weeks to $2\frac{1}{2}$ years.

MATERIALS AND METHODS

To minimize seasonal variation, the six tests in this study were staggered at intervals over a 4-month period. A total of 480 sterile stainless steel strips (1 x 2 inches) were contaminated, 80 for each test. First a tray of 40 strips, placed on a shelf in the laboratory, were exposed to aerial fallout for 22 days. Then the other 40 sterile strips were each well-fingered for a few seconds by five persons. Five strips contaminated by aerial fallout and five by handling were then assayed to obtain the initial contamination level. The remainder of the 80 strips were placed in sterile petri dishes and stored in a cupboard from 2 weeks to about $2\frac{1}{2}$ years before being assayed. By staggering the intervals for assaying the strips, the periods of storage were the same for all tests. The final assay, however, was not similarly staggered because after such an extended storage period the differences in the length of storage among the tests seemed negligible. The final storage period varied from 129 to 143 weeks.

The remaining viable microbial population on the steel after each storage period was determined for five strips contaminated by aerial fallout and for five contaminated by handling. A steel strip was placed in a bottle containing 50 ml of 0.05% Tween 20 solution and shaken mechanically for five minutes. Then 25 ml ($\frac{1}{2}$ of the sample) were placed in petri dishes and pour plates prepared with tryptose agar. Plate counts were made after 72 hours incubation at 98 F. For the final storage period (129 to 143 weeks), the entire 50 ml sample and the strip were placed in petri dishes and then pour plates prepared in order to substantiate a sterile sample.

RESULTS AND DISCUSSION

The number of microorganisms recovered from 30 stainless steel strips contaminated by handling or aerial fallout, is given for each storage period in Table I. The 30 steel strips represent the total number of strips used for the six tests for one storage period. The total rather than the average recovery of six tests per storage period is given because it seemed more meaningful to express the data in whole numbers rather than fractions. The strips were not uniformly contaminated initially by either method. With the maximum recoveries of only a few hundred microorganisms per strip, the variation among the strips contaminated by aerial fallout was ten-fold while a hundred-fold variation was observed among the strips that were handled. No microorganisms were recovered from two of the control strips even though they were assayed within a short period after handling. After storage, no microbial recovery was obtained for many of the strips assayed. The frequency of this occurrence is also given in Table I.

Approximately the same initial level of contamination was obtained by both handling and aerial fallout, but the survival after storage was markedly different. After two weeks storage, the contamination level from handling was reduced 94% while the reduction in the contamination level from aerial fallout was only about 40%. Yet 7% of the viable organisms initially accumulated from aerial fallout survived $2\frac{1}{2}$ years storage. Twenty-six of the 30 strips initially contaminated by handling were sterile after $2\frac{1}{2}$ years, but the fact that four strips were not sterile indicate that a surface covered after handling would not necessarily be sterile in a reasonable time.

No attempt was made to identify any of the microorganisms recovered. However, since the contamination level decreased rapidly on the steel that was handled, the population probably was mainly non-sporeforming bacteria. Favero, et al. (5) identified the microorganisms from handling steel strips to be mainly non-sporeformers. They reported an 80% reduction on handled steel strips after two weeks storage at a fairly constant temperature and relative humidity. The results given here also show the same magnitude of reduction after two weeks storage even though there was a fluctuation in temperature and relative humidity. During contamination and subsequent storage, the steel strips were subjected to seasonal environmental changes in temperature (60 to 90 F) and relative humidity (20 to 80%). The effect of ambient temperature and relative humidity upon the survival of a hetergenous population on a surface contaminated by handling or aerial fallout would not necessarily be too meaningful because the type and/or the number of microorganisms composing the hetergeneous population would differ greatly from one location to another and from one time to the next. Furthermore, the protection afforded to microorganisms by oils or other materials from the hands, dust, etc., will vary tremendously with people and time and thus would not be too meaningful.

The results indicate that the apparent plateau is not a true plateau of numbers of viable organisms accumulated on surfaces as it appeared to be in the samples exposed to aerial fallout for several weeks up to a year. There must be a gradual increase in viable count over a much longer time due to the accumulation of hardy sporeformers. But even they have a death rate so at sometime a true plateau must be reached. The reason for an apparent plateau occurring within a few weeks is that microorganisms accumulate quite readily on exposed surfaces but a great percentage of these are cells that die rapidly. Within a short time the die-off rate approaches the collection rate but even so a gradual upward trend will continue due to the accumulation of slow dying sporeformers. This is a gradual trend that is hidden by the great variation in numbers of organisms that accumulate concurrently on surfaces even next to one another. Previous studies in this laboratory showed this variation to be almost a 100-fold.

In this study, after $2\frac{1}{2}$ years storage a 15-fold decrease in population occurred on steel initially contaminated by aerial fallout. Most, but not all of the population died on steel contaminated by handling. The results indicate that the "die-off" of naturally accumulated microorganisms on surfaces is insufficient to produce a sterile spacecraft in a reasonable time by merely covering the constructed craft with a shroud and allowing it to stand.

This study also showed that there was an initial rapid death rate of microorganisms on surfaces contaminated by aerial fallout or handling, which was followed by a very slow death rate of the remaining few. The latter rate was so slow it was not obvious over a one year period but was in $2\frac{1}{2}$ years.

Table I.

Microbial Recovery from Stainless Steel Initially Contaminated by Handling or Fallout and Then Covered and Stored for Various Periods

Storage (weeks)	Handling[a] Total Number Organisms Recovered[c]	Handling[a] No. Samples Plated Showing No Organisms[c]	Fallout[b] Total Number Organisms Recovered[c]	Fallout[b] No. Samples Plated Show No Organism
0	1,640	2	1,946	0
2	92	16	1,162	0
8	46	20	656	0
16	30	19	526	0
24	24	21	522	1
32	18	24	426	0
52	26	22	298	1
129-143	6	26	132	3

a All samples handled by five persons.

b Samples exposed to aerial fallout for 22 days.

c Based on 30 determinations.

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