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3,301,752

## VAPORIZABLE BIOCIDAL COMPOSITIONS

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No Drawing. Filed Sept. 3, 1965, Ser. No. 485,124

2 Claims. (Cl. 167-39)

This application is a continuation-in-part of prior co-pending application Serial No. 97,157, filed March 21, 1961, now abandoned.

This invention relates to improvements in disinfection and sterilization, and more particularly to an improved vaporizable composition which displays marked biocidal effects and growth inhibition on a broad spectrum of organisms.

The invention also comprehends improved methods of utilizing the improved products of the invention in disinfection and sterilization of infected and/or potentially infected areas and articles or materials to be protected.

In a more particular aspect, the invention contemplates the provision and use of a dry disinfecting and sterilizing composition embodying balanced, synergistically effective amounts of solid germicidal and bactericidal components which are rapidly transformed from solid to vapor phase in selected enclosed areas under ambient temperatures to insure a rapid kill of undesirable micro and macro organisms within such areas or on such articles and/or to effectively inhibit growth of such organisms for a prolonged period.

In the past, generally considered, the disinfection of structures such as farm, poultry and dairy installations; articles or instruments designed for or involved in topical use, such as surgical and tonsorial instruments; hotel, hospital, and laboratory equipment, linens, glassware, and the like, has been largely accomplished by aqueous germicidal and bactericidal solutions or emulsions by such methods as spraying, brushing, dipping, saturation, and the like. A disadvantage of such approach is the rather considerable time expended in the preparation of such solutions and their applications to the sites to be disinfected and protected against subsequent infestations of the suspect organisms. A general drawback of such methods of solution application in the case of structures is that it is difficult to insure coverage and contact of the disinfectant over the entire area to be protected. By invoking the concept of application of the disinfectant in the vapor state complete penetration into pervious material and complete contact with infected impervious surfaces is achieved and without the necessity of providing any equipment other than an existing or applied vapor-impermeable enclosure.

As a result of considerable experimentation and research, it has been found that unexpectedly effective disinfecting and sterilizing compositions in dry form may be produced by formulating balanced amounts of certain vaporizable disinfecting compounds with suitable binders to provide a superior sterilizing composition. Such composition may be produced and used as a dusting powder or may be compacted into pellets or tablets of various shapes, such as balls, cylinders, flakes, and the like. The compound can be used without binders or fillers.

Considered specifically, the active ingredients of the preferred composition of the invention embodies an intimate mixture of a preponderant amount of paraformaldehyde and minor but effective amounts of phenol

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crystals and iodine. These are extended and consolidated into powder or tablet form by means of a suitable inert filler and a compatible binder. The filler may be any inert organic material; because of its ready availability and low-cost, anhydrous magnesium silicates, such as talc, are preferred. The binder preferably comprises an oleaginous material capable of wetting and binding the active ingredients; suitable materials for this purpose are vegetable oils, such as corn, cottonseed, safflower, or soybean oil, or mixtures of these. To prevent rancidification of the dry compound in storage, effective amounts of a suitable antioxidant may be incorporated in the binder oil.

While the relative percentages of the active ingredients are not essentially critical it has been found, as substantiated by numerous tests, that the following composition of Example I is highly effective for the purposes of the invention and constitutes the preferred embodiment:

### Example I

|                                           |                |
|-------------------------------------------|----------------|
| Active ingredients 88% by wt.:            | Percent by wt. |
| Paraformaldehyde                          | 85.5           |
| Phenol crystals                           | 2.0            |
| Iodine                                    | 0.5            |
| Inactive ingredients 12% by wt.:          |                |
| Binder, cottonseed oil                    | 5.0            |
| Filler, hydrous magnesium silicate (talc) | 7.0            |

It will be understood that if desired, the talc or equivalent inorganic silicate may itself comprise the binding material, particularly when the product is made up as a compacted or compressed tablet.

The composition of Example I may be modified for some purposes by omitting the phenol component and/or by adding an effective amount of thymol to the composition.

In use in structures of the type described, it is necessary only to place the requisite quantity of the compound within the structure after proper sealing, and permit the active ingredients to simultaneously volatilize and permeate the confined area of the structure to effect a kill of organisms in the confined atmosphere and those on the internal surfaces of the structure. The quantity of the compound employed for such purpose to insure adequate concentration of the biocidal vapors is of course a direct function of the enclosed volume of such structure.

The following chart illustrates the use of the composition of Example I in fumigating areas, such as in incubators, of various volume in cubic feet:

| Incubator size in cubic feet | Grams of Dry Sterilizer employed in empty machine | Grams of Dry Sterilizer for fumigating eggs preincubated |
|------------------------------|---------------------------------------------------|----------------------------------------------------------|
| 28                           | 3                                                 | 9                                                        |
| 40                           | 4                                                 | 12                                                       |
| 45                           | 4                                                 | 13                                                       |
| 47                           | 5                                                 | 15                                                       |
| 51                           | 5                                                 | 15                                                       |
| 58                           | 6                                                 | 17                                                       |
| 59                           | 6                                                 | 18                                                       |
| 60                           | 6                                                 | 18                                                       |
| 71                           | 7                                                 | 21                                                       |
| 86                           | 8                                                 | 25                                                       |
| 92                           | 9                                                 | 27                                                       |
| 105                          | 10                                                | 31                                                       |
| 107                          | 11                                                | 33                                                       |
| 118                          | 12                                                | 36                                                       |
| 136                          | 13                                                | 40                                                       |
| 153                          | 15                                                | 45                                                       |
| 164                          | 15                                                | 46                                                       |

| Incubator size in cubic feet | Grams of Dry Sterilizer employed in empty machine | Grams of Dry Sterilizer for fumigating eggs preincubated |
|------------------------------|---------------------------------------------------|----------------------------------------------------------|
| 169                          | 17                                                | 51                                                       |
| 196                          | 19                                                | 58                                                       |
| 230                          | 23                                                | 69                                                       |
| 235                          | 23                                                | 70                                                       |
| 241                          | 24                                                | 72                                                       |
| 248                          | 24                                                | 78                                                       |
| 272                          | 27                                                | 72                                                       |
| 282                          | 28                                                | 82                                                       |
| 306                          | 30                                                | 91                                                       |
| 313                          | 31                                                | 94                                                       |
| 330                          | 33                                                | 99                                                       |
| 343                          | 34                                                | 103                                                      |
| 356                          | 33                                                | 99                                                       |
| 465                          | 46                                                | 139                                                      |
| 482                          | 48                                                | 144                                                      |
| 515                          | 51                                                | 154                                                      |
| 564                          | 56                                                | 156                                                      |
| 670                          | 67                                                | 201                                                      |
| 735                          | 73                                                | 220                                                      |
| 906                          | 90                                                | 271                                                      |
| 1,275                        | 127                                               | 382                                                      |
| 1,577                        | 157                                               | 472                                                      |
| 1,880                        | 188                                               | 564                                                      |
| 1,912                        | 191                                               | 573                                                      |
| 1,482                        | 148                                               | 444                                                      |

It has been found that satisfactory vaporizable solid disinfectants having sterilizing action may be produced, incorporating a preponderant amount of paraformaldehyde and a minor amount of iodine with a binder comprised of corn oil and talc. As will be seen from the results of tests to be described subsequently, the composition of the type described in Example I has been found to be highly effective for disinfection and sterilization and constitutes the preferred, although not the exclusive, embodiment of the invention.

As indicated, the invention contemplates a method of sterilization and disinfection utilizing a solid composition containing vaporizable germicidal and bactericidal components and evolving vapors of such components in a confined area to contact and sterilize the area and articles or materials within such area. This method is to be distinguished from certain prior methods in which disinfecting compositions have been incorporated in plastics and other solid materials in an attempt to sterilize such materials. Typical of such suggestions is the disclosure of U.S. Patent 2,075,768 in which small amounts of germicidal agents such as phenol, paraformaldehyde and iodine are homogeneously distributed through the mass of a molded fiber product. Such product is vapor impermeable and the incorporated germicidal agent would be available for biocidal action only in solid form on the surface of the composition.

To illustrate the essential difference in the concept and mechanism of the present invention and the respective results obtained by the procedure of the patent and that comprehended herein, microbiological tests were conducted to compare the bactericidal effects secured in the product of the patent and those achieved by the method of the invention. A molded cylinder (1" x 4"), simulating a screwdriver handle, was prepared according to the formula of the patent. Such cylinder was held in the palm of the hand and enclosing fingers for a period of 30 minutes. The cylinder was then rubbed over the surface of a standard bacteriological medium and incubated for 24 hours at 37° C. After incubation, the organisms were found growing on the medium showing that the screwdriver handle had no bacteriological action. A parallel test was conducted in which bacteria normally present on the hand were isolated and cultured for 24 hours at 37° C. and then subjected to the vapors evolved from the composition of Example I. In such tests, controls were maintained without exposure to either the cylinder or to evolve vapors from the composition of the invention. The tests were performed at room temperature after the described incu-

bation period. The conditions of the tests and results obtained are given in the following table:

TABLE A

|    |                                                | 24 hour control cultures | Minutes                                  | Composition of Example 1           | Composition of Pat. 2,075,768          |
|----|------------------------------------------------|--------------------------|------------------------------------------|------------------------------------|----------------------------------------|
| 5  |                                                |                          |                                          |                                    |                                        |
| 10 | <i>Shigella sonnei</i> -----                   | +                        | 10<br>20<br>30<br>60<br>90<br>120<br>124 | 4+<br>3+<br>2+<br>-<br>-<br>-<br>- | 4+<br>4+<br>4+<br>4+<br>4+<br>4+<br>4+ |
| 15 | <i>Salmonella typhimurium</i> -----            | +                        | 10<br>20<br>30<br>60<br>90<br>120<br>124 | 4+<br>4+<br>3+<br>-<br>-<br>-<br>- | 4+<br>4+<br>4+<br>4+<br>4+<br>4+<br>4+ |
| 20 | <i>Escherichia coli</i> -----                  | +                        | 10<br>20<br>30<br>60<br>90<br>120<br>124 | 3+<br>2+<br>1+<br>-<br>-<br>-<br>- | 4+<br>4+<br>4+<br>4+<br>4+<br>4+<br>4+ |
| 25 | <i>Staphylococcus aureus</i> (Pathogenic)----- | +                        | 10<br>20<br>30<br>60<br>90<br>120<br>124 | 4+<br>4+<br>3+<br>-<br>-<br>-<br>- | 4+<br>4+<br>4+<br>4+<br>4+<br>4+<br>4+ |
| 30 | <i>Pasteurella multocida</i> ---               | +                        | 10<br>20<br>30<br>60<br>90<br>120<br>124 | 4+<br>3+<br>2+<br>-<br>-<br>-<br>- | 4+<br>4+<br>4+<br>4+<br>4+<br>4+<br>4+ |
| 35 |                                                |                          |                                          |                                    |                                        |

<sup>1</sup> Hours.  
(+) Indicates a quantitative rating of bacterial growth after exposure to each system.  
(-) No growth.

It will be seen from the results recorded in Table A that the composition of Patent 2,075,768 displayed no effective bactericidal action on any of the test organisms over a 24-hour period, whereas applicant's treatment of subjecting such organisms to the action of vapors from the composition completely inhibited bacterial growth after a 60-minute period.

The sterilizing composition of Example 1 has been tested and found to be a very effective disinfectant against a broad spectrum of bacteria, fungi and yeast. Specifically, such composition has been employed and found to be an effective sterilizing medium against the following organisms:

## Bacteria:

- Salmonella typhimurium*
- Salmonella pullorum*
- Salmonella californica*
- Salmonella arizona*
- Salmonella kentucky*
- Salmonella typhosa*
- Staphylococcus citreus*
- Staphylococcus aureus*
- Pasteurella avicida*
- Pasteurella multocida*
- Escherichia coli* Strain 017 or 018
- Escherichia coli* Strain 078
- Escherichia coli* Atypical (rough)
- Bacillus subtilis*<sup>1</sup>
- Bacillus stearothermophilus* (spores & vegetative cells)
- Listeria*
- Proteus vulgaris*<sup>1</sup>
- Erysipelas rhusiopathae*
- Streptococcus* B (hemolytic pyogenic)
- Streptococcus* D Lancefield (fecalis)
- Streptococcus* HWD.

<sup>1</sup> Isolated from hatchery air samples.

Fungi and yeasts:

- Candida albicans*
- Mucor
- Aspergillus fumigatus*
- Rhizopus
- Streptomyces
- Penicillium
- Pullularia
- Hormodendrum
- Scopulariopsis
- Helmintosporium
- Botrytis
- Microsporium gypseum*
- Trichophyton gallinae*
- Alternaria
- Microsporium nanum*

Bacteria:

- Sarcina lutea*<sup>1</sup>
- Bacillus cereus*<sup>1</sup>
- Prodigiosum*<sup>1</sup>
- Staphylococcus albus*<sup>1</sup>
- Pseudomonas aeruginosa*<sup>1</sup>
- Aerobacter*<sup>1</sup>

<sup>1</sup> Isolated from hatchery air samples.

As will be observed from the results recorded in this table, organisms were found to be present on all ten of the control plates (1-10) at each of the indicated 24-, 48- and 72-hour periods. All the test plates (11-20) on macroscopic examination were found to be free of live organisms at the end of each of these three periods, showing that the novel composition effectively killed contaminants within a 24-hour period and inhibited growth within a 72-hour period.

In this test no attempt was made to isolate and identify all the organisms growing on the control plates but numerous typical organisms were isolated from such control plates among which were *E. coli*, *Staphylococcus albus*, *Pseudomonas aeruginosa*, *Aspergillus fumigatus*, and *Candida albicans*.

A similar test was conducted to determine the biocidal effect of the composition of Example I on a number of characteristic contaminating organisms. In this test, three plates of veal infusion agar were inoculated with each of the various organisms listed in the following table:

TABLE II

| Test Organisms                 | Control Plate |         | Test Plates |         |       |         |         |             |         |   |
|--------------------------------|---------------|---------|-------------|---------|-------|---------|---------|-------------|---------|---|
|                                | 1             |         | 2           |         |       | 3       |         | Subcultured |         | 4 |
|                                | 24 hrs.       | 48 hrs. | 24 hrs.     | 48 hrs. |       | 24 hrs. | 48 hrs. | 24 hrs.     | 48 hrs. |   |
| <i>Salmonella typhimurium</i>  | +             | +       | -           | -       | -     | +       | -       | -           | -       | - |
| <i>Salmonella pullorum</i>     | +             | +       | -           | -       | -     | +       | -       | -           | -       | - |
| <i>Salmonella typhosa</i>      | +             | +       | -           | -       | -     | +       | -       | -           | -       | - |
| <i>Escherichia coli</i>        | +             | +       | -           | -       | -     | +       | -       | -           | -       | - |
| <i>Staphylococcus</i>          | +             | +       | -           | -       | -     | +       | -       | -           | -       | - |
| <i>Staphylococcus aureus</i>   | +             | +       | -           | -       | -     | +       | -       | -           | -       | - |
| <i>Bacillus subtilis</i>       | +             | +       | -           | -       | -     | +       | -       | -           | -       | - |
| <i>Pasteurella multocida</i>   | +             | +       | -           | -       | -     | +       | -       | -           | -       | - |
| <i>Listeria</i>                | +             | +       | -           | -       | -     | +       | -       | -           | -       | - |
| <i>Proteus vulgaris</i>        | +             | +       | -           | -       | -     | +       | -       | -           | -       | - |
| <i>Erysipelas rhytopathiae</i> | +             | +       | -           | -       | -     | +       | -       | -           | -       | - |
| <i>Aspergillus fumigatus</i>   | +             | +       | -           | -       | -     | +       | -       | -           | -       | - |
| Mucor sp.                      | +             | +       | -           | -       | -     | +       | -       | -           | -       | - |
| <i>Candida albicans</i>        | +             | +       | -           | -       | -     | +       | -       | -           | -       | - |
| Fungi                          | 48 hrs.       | 72 hrs. | 48 hrs.     | 72 hrs. | 1 wk. |         |         | 48 hrs.     | 72 hrs. |   |
| <i>Trichophyton gallinae</i>   | +             | +       | -           | -       | -     | +       | -       | -           | -       | - |
| <i>Microsporium gypseum</i>    | +             | +       | -           | -       | -     | +       | -       | -           | -       | - |
| <i>Microsporium nanum</i>      | +             | +       | -           | -       | -     | +       | -       | -           | -       | - |

The improved composition of the invention has been found to be markedly effective in the disinfection of poultry houses as is illustrated by the following test.

Twenty culture plates of veal infusion agar media were exposed for one-half hour to dust at various places in the halls and pens of a turkey breeding building. All such plates were then incubated for 24 hours at 37° C. Ten of the plates were used as controls and the remaining ten were subjected to the action of the vapors from the composition of Example I for the periods indicated in the following table:

TABLE I

|                |    |    |    |    |    |    |    |    |    |    |
|----------------|----|----|----|----|----|----|----|----|----|----|
| Control plates | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 |
| 24 hours       | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| 48 hours       | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| 72 hours       | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| Test Plates    | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| 24 hours       | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |
| 48 hours       | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |
| 72 hours       | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |

(+) Indicates presence of live organisms.  
(-) Indicates absence of live organisms.

Plates listed in column 1 of the table represent the control plates in each test. In the plates listed in column 2, one gram of the novel composition was placed on the lid of each Petri dish containing the test organism culture and was then incubated at 37° C. for 24 hours. As shown in the table, effective kill of the organisms was obtained in the 24-hour test period.

In the test recorded in column 3, the particular cultured organism on the listed plate was incubated at 37° C. for 24 hours. After such period of growth of the organism, it was subjected at ambient temperature, for a period of 24 hours, to an atmosphere of the vapors derived from one gram of the novel composition of Example I placed on the lid of each Petri dish, resulting in effective kill of the organisms within such contact period.

After this period, specimens of the organisms were subcultured to determine the persistent effect of the previous sterilizing by the novel composition, and as indicated in the results recorded in column 4, after periods of 24 hours and 48 hours, no organism growth occurred. As will be observed from the recorded results by subjecting the cultured organisms to the effect of the vapors of the test disinfecting composition, said listed bacterial organisms were effectively killed within a 24 hour vapor contact period and growth was inhibited for at least an additional 24

hours. In the case of the tested fungi, the disinfecting composition effected a kill within a 48-hour period.

The efficacy of the improved disinfecting composition and its marked germicidal effectiveness over a broad spectrum of bacteria is further illustrated by the following typical test. In this test, two tryptose plates (one for control and one for test) were inoculated with the various enumerated bacteria. The plates were placed in a desiccator with the lids of the Petri dishes intact. In the test-plate, a measured amount of the sterilizing composition was first inserted. The two plates were incubated for 24 hours at 37° C., the control plates being in an atmosphere of normal air, and the test plate in an atmosphere of vapors evolved from the composition of Example I. The plates were examined by the conventional technique to determine the presence of viable organisms. The test plates were examined after the 24-hour incubation period and after an additional interval of 24 hours. The results are tabulated below:

TABLE III

| Bacteria                              | Test Plates |         | Control Plates, 24 hrs. |
|---------------------------------------|-------------|---------|-------------------------|
|                                       | 24 hrs.     | 48 hrs. |                         |
| <i>Salmonella typhimurium</i> .....   | —           | —       | +                       |
| <i>Salmonella pullorum</i> .....      | —           | —       | +                       |
| <i>Staphylococcus</i> .....           | —           | —       | +                       |
| <i>Salmonella californica</i> .....   | —           | —       | +                       |
| <i>Salmonella arizona</i> .....       | —           | —       | +                       |
| <i>Prodigiosum</i> .....              | —           | —       | +                       |
| <i>Staphylococcus albus</i> .....     | —           | —       | +                       |
| <i>E. coli</i> strain 017 or 018..... | —           | —       | +                       |
| <i>E. coli</i> strain 078.....        | —           | —       | +                       |
| <i>Bacillus subtilis</i> .....        | —           | —       | +                       |
| <i>Staphylococcus aureus</i> .....    | —           | —       | +                       |
| <i>Listeria</i> .....                 | —           | —       | +                       |
| <i>E. coli</i> atypical.....          | —           | —       | +                       |
| <i>Erysipelae</i> .....               | —           | —       | +                       |
| <i>Sarcina lutea</i> .....            | —           | —       | +                       |
| <i>Pseudomonas</i> .....              | —           | —       | +                       |
| <i>Staphylococcus</i> .....           | —           | —       | +                       |

The control plate was incubated in a closed petri dish in normal air for 24 hours at 37° C. A gram of the composition of Example I was introduced into the petri dish of the test plate, the lid was closed and the plate was incubated for a 24-hour period at 37° C. The control and test plates were examined after the 24-hour incubation period and also at the indicated 48- and 72-hour intervals. In the case of *Penicillium* and *Pullularia*, the control plates were examined after 96 hours from incubation.

As will be seen from the results recorded in Table IV, the inoculated test plates after a 24-hour incubation period in an atmosphere of the vapors from the novel disinfecting composition displayed no viable fungi and such inactivation persisted for the 72-hour limit of the examination. While certain of the control plates indicated no positive growth within a 48- or 72-hour period, notably *Penicillium* and *Pullularia* developed positive growth in 96 hours. In the test plates, in every instance, the particular fungus was effectively destroyed within the 24-hour incubation period and such plates were sterile for the 72-hour extent of the examination.

A particularly valuable function of the improved disinfecting and sterilizing composition is its marked bactericidal effect on thermophilic spore formers. To establish this, a series of tests were conducted to determine the effect of the composition of the invention on *Bacillus stearothermophilus* (B.s.).

In an initial test, a 36-hour culture of Bs was exposed in a desiccator, for various lengths of time, to the action of the vapors evolved from the composition of Example I. The test organisms were seeded at about 10 million/sq. in., grown on tryptose agar and were incubated at 57°–59° C. At all times during the test, the test organism was kept at this temperature to insure its continued growth. During the test, the test specimen was examined at intervals to determine the rapidity and duration of the bactericidal effect. The results are recorded in the following table:

TABLE V

| Exposure time in minutes | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 | 110 | 120 | 130 | 140 | 150 |
|--------------------------|----|----|----|----|----|----|----|----|----|-----|-----|-----|-----|-----|-----|
| No. of colonies:         |    |    |    |    |    |    |    |    |    |     |     |     |     |     |     |
| 24 hours.....            | 70 | 43 | 7  | 7  | —  | —  | —  | —  | —  | —   | —   | —   | —   | —   | —   |
| 48 hours.....            | 70 | 43 | 7  | 7  | —  | —  | —  | —  | —  | —   | —   | —   | —   | —   | —   |
| 72 hours.....            | 70 | 43 | 7  | 7  | —  | —  | —  | —  | —  | —   | —   | —   | —   | —   | —   |

As will be seen from the recorded results, the several tested bacteria were completely inactivated within the 24-hour incubation period which sterilization persisted for the 48-hour period of the test.

The composition of the invention is similarly effective as a general fungicidal and fungistatic agent as is indicated by the following test. In this test, a series of two BHIA (Beef Heart Infusion Agar) plates were inoculated with the various fungi listed in the following table:

TABLE IV

|                                          | Test Plates |         |         | Control Plates |         |         |         |
|------------------------------------------|-------------|---------|---------|----------------|---------|---------|---------|
|                                          | 24 hrs.     | 48 hrs. | 72 hrs. | 24 hrs.        | 48 hrs. | 72 hrs. | 96 hrs. |
| <i>Rhizopus</i> .....                    | —           | —       | —       | +              | +       | +       | —       |
| <i>Aspergillus</i> .....                 | —           | —       | —       | +              | +       | +       | —       |
| <i>Streptomyces</i> .....                | —           | —       | —       | +              | +       | +       | —       |
| <i>Alternaria</i> .....                  | —           | —       | —       | —              | —       | +       | —       |
| <i>Penicillium</i> .....                 | —           | —       | —       | —              | —       | —       | +       |
| <i>Pullularia</i> .....                  | —           | —       | —       | —              | —       | —       | +       |
| <i>Hormodendrum</i> .....                | —           | —       | —       | —              | —       | +       | —       |
| <i>Mucor</i> .....                       | —           | —       | —       | —              | —       | +       | —       |
| <i>Scopularopsis</i> .....               | —           | —       | —       | —              | +       | +       | —       |
| <i>Helmintosporium</i> .....             | —           | —       | —       | —              | —       | +       | —       |
| <i>Botrytis</i> .....                    | —           | —       | —       | —              | —       | +       | —       |
| <i>Microsporium gypseum</i> .....        | —           | —       | —       | —              | —       | +       | —       |
| <i>Trichophyton gallinae</i> .....       | —           | —       | —       | —              | +       | +       | —       |
| <i>Candida albicans</i> .....            | —           | —       | —       | +              | +       | +       | —       |
| <i>Bacillus stearothermophiles</i> ..... | —           | —       | —       | —              | —       | +       | —       |

As will be seen from the results tabulated in the above table, the number of bacterial colonies grown in the culture medium diminished with increased time of exposure to the biocidal vapors and such growth whether in 24-, 48- or 72-hour periods was completely inhibited after a 50-minute exposure to the described biocidal vapors.

Another test was conducted to determine the approximate quantity of the disinfectant necessary to secure effective bactericidal action on Bs. In such test, a 48-hour cul-

ture of Bs was transferred to tryptose agar plates. One plate was used as a control. One gram of the composition of Example I was added to one test plate. 0.25 gram of such composition was added to a second test plate, and a third test plate contained no added composition.

The three test plates were stacked and sealed in a vapor-impermeable plastic bag, the plate having 1 gram of the disinfecting composition being on the bottom, the plate containing 0.25 gram of the test disinfecting composition being in the middle and the test plate with no added disinfecting composition being on top of the stack. The three test plates within the bags and the control plates were incubated at 57°-59° C. for the time intervals indicated in the following table showing the results of the test:

TABLE VI

| Incubation           | 24 | 48 | 72 hours at 57°-59° C. |
|----------------------|----|----|------------------------|
| 1 gram plate.....    | -  | -  | -                      |
| 0.25 gram plate..... | -  | -  | -                      |
| None plate.....      | -  | -  | -                      |
| Controls.....        | +  | +  | +                      |

As will be seen from the data in the above table, after a 24-hour incubation period, and after 48 and 72 hours, each of the three test plates showed no bacterial colony growth whereas the control plates showed positive growth. This test illustrated uniform permeation and the marked penetrating effect of the sterilizing vapors within a confined area and the relatively small quantity of the composition required to effect sterilization.

The effective fungicidal action of the composition of the invention establishes it as a valuable product for the disinfection and sterilization of fungal contaminated products, particularly leather products, the protein composition of which provides a substrate for active growth of fungi. This effectiveness has been confirmed by a number of tests. In a typical such test, each glove of a pair was inoculated with a pathogenic fungus *Aspergillus fumigatus*. Each glove was placed in a separate plastic bag. Less than a gram of the composition of Example I was introduced into one bag which was then tied to provide a vapor-retaining enclosure while the other inoculated glove was subjected to normal ambient atmosphere and served as a control.

Within a short time, the inoculated glove submitted to the action of the vaporized disinfectant displayed no growth of the fungus and retained its original pliant, soft condition or structure. The test fungus grew copiously on the control glove resulting in shrinkage and embrittlement of contaminated surfaces of the glove.

Similarly, the composition of the invention has been found to be most effective as a fungicidal agent for practical application in disinfecting shoes and reducing the incidence of topical fungal infestations such, for example, as Epidermophytosis or "athlete's foot." Other tests, similar to the described tests on gloves were conducted on shoes. A pair of shoes were selected which had visually-observable fungus growth therein. One of the shoes was placed in a plastic bag with a quantity of the composition of Example I and the bag was tied to form a vapor retaining enclosure. After a relatively short time, it was found on inspection of the treated shoe that the fungal growth was eliminated whereas the untreated shoe remained contaminated.

It will now be appreciated that the composition of the invention has a wide potential application for disinfection and sterilization. As will be understood in marketing and distributing the volatile composition, it is necessary to package it in air-tight containers. For some uses as for example in disinfection of relatively large enclosed areas the product may be packaged in powdered or pellet form in relatively large air-tight containers from which

the calculated requisite amount of the composition is withdrawn for treatment of a given enclosed area. For other uses such as for example for disinfection of leather articles, linens, glassware, instruments and the like, the composition may be packaged in perforate paper or plastic bags, which containers may be enclosed within a larger air-tight container from which one or more of such perforate bags or envelopes may be withdrawn as desired for a particular disinfecting use. The quantity of the composition in such bags may be varied or adjusted to conform it to the vapor concentration required for the treatment of the particular areas or to sizes of the vapor-impermeable bags used in treating articles of apparel, instruments and the like. As noted previously, for the disinfection of portable articles; e.g., linens, instruments, shoes and the like, the vapor-containing enclosure for such articles comprises preferably a relatively thin plastic bag such for example as a polyethylene envelope or bag of 1 to 2 or more mils thickness. For recurrent sterilization of particular items such as linens, cutlery, instruments, and the like, such bags may be of a series of standard sizes requisite for the particular sterilizing operation and may comprise a stock reusable item.

It is also within the contemplation of the present invention to employ the improved composition for disinfection of topical areas. For example, infestation of "athlete's foot" may be treated by introducing the composition of the invention into a plastic bag of suitable size, drawing this over the pedal extremity and securing the open end tightly about the leg and retaining the bag on the foot for a relatively short time.

While a preferred embodiment of the composition and particular useful applications thereof have been described, it is to be understood that these are given to exemplify the underlying principles of the invention and not as limiting it to such illustrative embodiments except as such limitations are clearly imposed by the appended claims.

I claim:

1. A substantially dry vaporizable disinfecting composition effective against a broad spectrum of bacteria, fungi and yeast consisting essentially of about 85.5% by weight of paraformaldehyde, about 2.0% by weight of phenol, about 0.5% by weight of iodine, about 5.0% by weight of a vegetable oil binding agent and about 7.0% by weight of a filler.

2. A dry vaporizable disinfecting and sterilizing composition effective against a broad spectrum of bacteria, fungi and yeast consisting essentially of a powdered mixture of a preponderant amount of paraformaldehyde together with minor germicidally effective amounts of phenol and iodine the amount of phenol being substantially four times the amount of iodine by weight and the amount of paraformaldehyde by weight being in excess of thirty times the combined amounts of the iodine and phenol.

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