Protection Branch Report of Test No. 8-69

The Effect of Dimethyl Sulfoxide on the Sporicidal Activity of Ethylene Oxide Gas

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At the request of the NASA the following tests were conducted to obtain a quick evaluation of whether there is an enhancement of the sporicidal activity of ethylene oxide (EtO) gas by incorporating dimethyl sulfoxide (DMSO).

Previous screening tests, in which a cloth patch contaminated with B. subtilis var niger (BG) spores was suspended for 24 hours in a liter bottle over 0.1 ml of DMSO revealed no measurable sporicidal activity, but a similar test with Staphylococcus aureus cells killed 100%, showing that DMSO is antibacterial.

Similar 4-hour vapor tests were conducted using five chemicals, with and without 0.1 ml DMSO contained in a small glass weighing cup. A 0.1 ml amount of the other chemical was placed on the bottom of the bottle to volatilize. As shown in Table I, the activity of four of the chemicals was depressed by the presence of DMSO. One chemical's activity was not affected. Vapor pressure is probably an important factor here. It was noted that little of the DMSO had volatilized and the liquid could be absorbing some of the other vapor. The vapor pressure of pure DMSO at 25° C is 0.6 mm Hg, which is equivalent to approximately 1.6 mg/liter. At this point work with DMSO discontinued, to be started again only when the NASA requested more information.

PROCEDURE

The present tests may be grouped into four parts: (a) exposure of BG spores to DMSO vapor (DMSO controls), (b) exposure of spores to EtO gas (EtO controls), (c) exposure of spores to a gaseous mixture of EtO-DMSO, and (d) exposure of spores to the vapor emitted from a solution of EtO gas dissolved in liquid DMSO.

Tests were conducted at 25° C in a large desiccator fitted with two stopcocks, except for the test (Table II) in which a liter bottle was used. The cloth patches contained about 2×10^5 spores and were stored at 33% RH prior to use. Saturated DMSO vapor was obtained by flushing ambient air through 40 ml DMSO in a Vigreaux bubbler and into the desiccator. Ambient air RH varied, but was not under 33%.

A 2-hour exposure was chosen for the DMSO control because the test samples were exposed for one hour to a DMSO vapor flush followed by a 1-hour exposure to EtO (100 mg/l) in the presence of DMSO vapor. The EtO gas was introduced by reducing the desiccator pressure by 42 mm Hg and replacing with EtO gas.

A variation of the technique involved placing a solution of 1 part EtO in 2 parts liquid DMSO on the bottom of a desiccator to volatilize. Enough of the solution was used to furnish 100 mg of EtO gas per liter of desiccator volume. A week old and a freshly mixed solution were tested. The results are shown in Table III.

After exposure to the gases, the cloth patches were placed in 10 ml distilled water, shaken vigorously to dislodge the spores, and serial dilutions plated in Tryptose agar. Colony counts of viable organisms were made after 48 hours incubation at 37° C.

The affinity of DMSO for EtO was illustrated by placing 10 ml of DMSO on the bottom of a large desiccator and introducing half an atmosphere of EtO gas (900 mg/L) by replacing an equivalent vacuum (380 mm Hg). Within an hour a vacuum of 32 mm Hg occurred which increased over 2-$\frac{1}{2}$ days to 48 mm Hg (Table IV). So in only one hour 10 ml of DMSO absorbed 732 mg EtO and in 2-$\frac{1}{2}$ days 1,094 mg EtO.

When 25 ml of chilled liquid EtO is placed within a 25 liter pressure container at room temperature, its vapor pressure rises steadily until it reaches the theoretical 380 mm in about two hours (Fig. 1). When the same experiment is repeated however in the presence of 50 ml DMSO the rise is slower and the pressure levels off at a little over 200 mg Hg, revealing that almost half of the EtO is adsorbed in the DMSO. Thus, the DMSO is acting as a reservoir (or storage) holding the EtO vapor and capable of slowly releasing it.

CONCLUSIONS

The sporicidal activity of DMSO vapor appears measurable at saturation concentration, but its low volatility makes the rate slow and difficult to measure. Exposure to EtO in the presence of DMSO vapor

seemed to cause an increase in the number of spores killed (Test IIIB, Table II), as did exposure to the vapor from a solution of one part EtO in two parts DMSO (Table III). However, too few data were collected to prove statistically that the addition of DMSO vapor significantly enhanced the sporicidal activity of EtO. It is difficult to judge whether the effect is additive or synergistic, since Test IIIA, Table II, suggests that DMSO does not precondition the spores for faster EtO activity, yet the vapor pressure test showed that EtO is absorbed by DMSO, i.e., DMSO on the cloth patches may concentrate EtO gas.

Table I.

Gaseous Fumigant Test with Addition of 0.1 ml Dimethyl Sulfoxide

Test Organism: Bacillus subtilis var niger
1.6×10^6 spores/patch

Exposure : 4 hours

Chemical	Per Cent Reduction	
	Without DMSO	With DMSO
2,2,4-Trimethyl-3-hydroxy-pentenoic acid, B-Lactone	72	None
Ethylaziridinyl Formate	90	None
Alpha-bromocyclobutanone	56	None
2-Methyl-2-oxazoline	60	None
Trimethylene oxide	14	12

Table II.

Effect of Dimethyl Sulfoxide on Bactericidal Activity of Ethylene Oxide Gas

Results:

Cloth patch contamination averaged $\sim 2 \times 10^5$ B. subtilis var niger spores. Tests conducted at 25° C, inside desiccators.

Test I. DMSO Controls	Exposure (hrs)	% Reduction	No. of Patches
A. Saturated DMSO vapor	2	23.3	3
B. Saturated DMSO vapor	2	14.0	6
C. 0.1 ml DMSO in a 1 liter bottle	2	0	3
Test II. EtO Controls			
A. 100 mg EtO/L	1	68.0	3
B. 100 mg EtO/L	1	42.7	4
C. 100 mg EtO/L	1	81.6	3
D. 100 mg EtO/L	2	67.0	4
Test III. EtO-DMSO Gaseous Mix			
A. One hr exposure to saturated DMSO vapor, remove and expose one hour to 100 mg EtO/L		71.4	3
B. One hour exposure to saturated DMSO vapor followed by one hour EtO (100 mg EtO/L) still in presence of DMSO vapor.		98.5	3
C. Like B, except increase EtO exposure to two hours. Compare to IID.		99.6	4

Table III.

Exposure of Bacillus subtilis var niger Spores
to Vapor from EtO-DMSO Solution

DMSO-EtO Solution:

2 parts DMSO + 1 part EtO

3.4 ml above solution in 9.5 liter desiccator (equivalent to 100 mg EtO/L volume)

	Exposure (hrs)	% Reduction*	No. of Patches
A. Freshly mixed	1	95.5	3
B. One week old	1	98.05	3

* Bacillus subtilis var niger spores

Table IV.

Absorption of Ethylene Oxide Gas by Liquid DMSO

Time (hrs)	Manometer Pressure (mm Hg)	Amount of EtO Absorbed by the DMSO (mg)
0	0	0
1	-32	732
2	-34	775
60	-48	1094

Note:

Chamber = 9.5 liter
Amount DMSO = 10 ml
Amount EtO = 900 mg/liter or 8,550 mg/9.5 liters

THE VAPOR PRESSURE OF ETHYLENE OXIDE WITH AND WITHOUT THE PRESENCE OF DIMETHYL SULFOXIDE