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BACTERIAL AND FUNGAL METHODS

Lester A. Brooks and Robert T. O'Shaughnessy, East Norwalk, Conn., assignors to R. T. Vanderbilt Company, Inc., New York, N.Y., a corporation of New York

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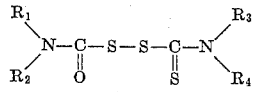
This application is a continuation-in-part of our copending application Ser. No. 250,742, filed Jan. 11, 1963, which, in turn, is a continuation-in-part of our application Ser. No. 133,552, filed Aug. 24, 1961 (both of which are now abandoned).

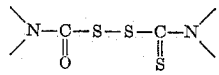
The present invention pertains to organic disulfides and, more particularly, to carbamoyl thiocarbamoyl disulfides, to a process for producing such compounds and to novel uses thereof.

Carbamoyl thiocarbamoyl disulfides hereinafter referred to as "oxydisulfides," have the following structural formula:



R_1, R_2, R_3 and R_4 are saturated hydrocarbon radicals or other inert saturated organic radicals having from 1 to 10 carbon atoms and can be the same or different radicals. R_1 plus R_2 and/or R_3 plus R_4 together with the adjoining nitrogen atom can form a heterocyclic ring. Typical of the hydrocarbon radicals are the alkyl radicals having from 1 to 10 carbon atoms, e.g., methyl, ethyl, propyl, butyl, amyl, hexyl, etc.; the cycloalkyl radicals having from 5 to 7 carbon atoms, e.g. cyclopentyl, cyclohexyl and cycloheptyl; the aralkyl radicals having from 7 to 10 carbon atoms, e.g., benzyl and phenyl ethyl. Typical of the heterocyclic radicals are the morpholyl, piperidyl and pyrrolidyl radicals.

It is believed that the activity and utility (hereinafter described more fully) of the oxydisulfides, and the operativeness of the method of preparing them, depends not so much on the nature of the groups "R," but on the



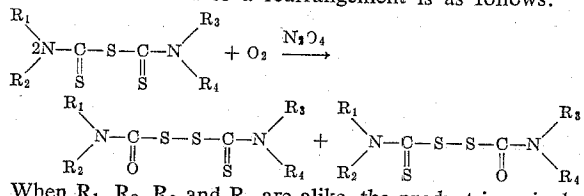
nucleus. It is true of course that not all compounds within the scope of the invention have the same activity. This is believed to be due to the effect exerted by the group "R" on the inherent activity of the described nucleus. Accordingly, modifications are permissible in respect to the group "R."

Exemplary carbamoyl thiocarbamoyl disulfides or oxydisulfides of the invention include dimethylcarbamoyl dimethylthiocarbamoyl disulfide, diethylcarbamoyl diethylthiocarbamoyl disulfide, dibutylcarbamoyl dibutylthiocarbamoyl disulfide, dimethylcarbamoyl dibutylthiocarbamoyl disulfide and its position insomer dibutylcarbamoyl dimethylthiocarbamoyl disulfide, dimethylcarbamoyl diethylthiocarbamoyl disulfide and its position isomer diethylcarbamoyl dimethylthiocarbamoyl disulfide, dimethylcarbamoyl dihexylthiocarbamoyl disulfide and its position isomer dihexylcarbamoyl dimethylthiocarbamoyl disulfide, dimethylcarbamoyl dibenzylthiocarbamoyl disulfide and its position isomer dibenzylcarbamoyl dimethylthiocarbamoyl disulfide, diethylcarbamoyl tetramethylenethiocarbamoyl disulfide and its position isomer tetramethylenecarbamoyl diethylthiocarbamoyl disulfide, dimethylcarbamoyl pentamethylene thiocarbamoyl disulfide and its position isomer pentamethylenecarbamoyl dimethylthiocarbamoyl disulfide, and cyclohexylmethylcarbamoyl diethylthiocarbamoyl disulfide and its position

isomer diethylcarbamoyl cyclohexylmethylthiocarbamoyl disulfide.

The oxydisulfides are suitable for a variety of uses, such as bacteriostats, fungistats, agricultural fungicides, and antifouling agents.

The oxydisulfides are prepared in accordance with the process of the present invention by passing a free oxygen-containing gas, such as oxygen or air, as an oxidizing agent and nitrogen tetroxide as a catalyst into a thiocarbamoyl monosulfide, commonly called a thiuram monosulfide, and separating the resulting oxydisulfides from the reaction mixture. The equation for this reaction which involves a rearrangement is as follows:



When R_1, R_2, R_3 and R_4 are alike, the product is a single oxydisulfide, but when any of these four radicals differ from one another, the product is a mixture of position isomers.

The amount of free oxygen-containing gas which is employed is preferably at least about a stoichiometric amount since smaller amounts lower the yield appreciably. The nitrogen tetroxide catalyst is used in an amount to promote the reaction of the thiuram monosulfide to the oxydisulfides. Significant catalysis is observed as low as about 0.1 mole of nitrogen tetroxide (N_2O_4, equivalent to 0.2 mole of NO_2) and even less in some instances (i.e., 0.01 mole) per mole of thiuram monosulfide and amounts as high as about 0.5 mole of nitrogen tetroxide (equivalent to 1.0 mole of NO_2) and more may be used. The larger quantities are generally applicable where aqueous solvent is used, and the smaller quantities are useful when organic solvents are used.

The reaction is normally conducted at a temperature from about 0° C. to about 35° C. At higher temperatures, for example, above about 60° C., decomposition of the product sets in with the corresponding decrease in yields. However, temperatures lower than 0° C. may be maintained in non-aqueous systems.

The medium for the reaction can be water. An inert solvent system, such as toluene, benzene or chloroform, is used where anhydrous conditions are desirable. No solvents are required where the thiuram monosulfide is a liquid. The reaction mixture is normally agitated during the reaction to insure intimate contact between the reactants.

The lower molecular weight oxydisulfides which are solids can be separated from the reaction mixture by filtration or centrifugation, while the higher molecular weight oily oxydisulfides can be separated from the reaction mixture by evaporation of the solvent under reduced pressure. In the case where no solvent has been used, only an alkali wash is required.

The oyxdisulfides of the invention can also be prepared by the action on thiuram monosulfides of oxidizing agents other than nitrogen tetroxide. A good yield of oxydisulfide is obtained when thiuram monosulfides are oxidized with a nitrite salt such as sodium nitrite in the presence of an acid such as hydrochloric acid, provided the reaction mixture is agitated vigorously in the presence of air. In this instance the nitrogen tetroxide is generated in situ according to the reaction

$$2NaNO_2+2HCl\rightarrow 2NaCl+NO+NO_2+H_2O$$

Nitric oxide (NO) immediately is converted to nitrogen dioxide (NO_2) by the oxygen of the air

$$NO+\tfrac{1}{2}O_2\rightarrow NO\ [or\ \tfrac{1}{2}N_2O_4]$$

NO_2 is, of course, equivalent in function to N_2O_4. Accordingly, one mole of nitrite salt results in the formation of one-half mole of nitrogen tetroxide (N_2O_4), or one mole of NO_2.

One advantage of this method of preparing the oxydisulfides is that they can be readily manufactured in the field, and even at the site at which they are to be applied, for example, to vegetation as an aqueous spray. Thus, the more stable monosulfide can be stored indefinitely and shipped under conditions which might cause some decomposition of the oxydisulfide.

The thiuram monosulfide may be oxidized to the oxydisulfide by cold concentrated nitric acid but the reaction is difficult to control. Peroxygen compounds such as peracetic acid, hydrogen peroxide, and t-butyl hydroperoxide may be used to prepare oxydisulfide, but in general, the yields are low.

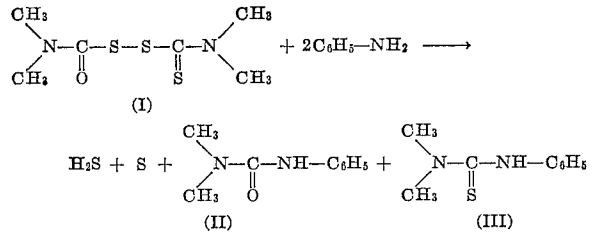
The process of the present invention is illustrated by the following examples.

Example 1

Tetramethylthiuram monosulfide (416 g., 2.0 moles) was slurried in 1500 ml. water and 500 g. crushed ice. The mixture was stirred vigorously while nitrogen tetroxide (55 g., 0.6 mole) admixed with air was bubbled in through a fritted glass dispersing tube, and air alone was bubbled into the mixture through an adjoining fritted glass dispersing tube. The temperature was maintained at 0–10° C. The yellow slurry of tetramethylthiuram monosulfide turned white after all the nitrogen tetroxide had been added (50 minutes). The white solid was filtered at the pump and dried in a vacuum oven at 50° C. The yield was 415 g., or 93% of theoretical (based on tetramethylthiuram monosulfide). Melting point was 103–106° C. Calculated for $C_6H_{12}N_2OS_3$ or dimethylcarbamoyl dimethylthiocarbamoyl disulfide: C=32.2%, H=5.36%, N=12.5%, O=7.15%, S=42.8%. Found: C=32.3%, H=5.52%, N=12.37%, O=7.01%, S=42.8%. The infrared spectrum of this material shows a strong absorption in the region ascribed to carbonyl groups.

Infrared evidence and elemental analysis suggest the structure shown in (I) below. To verify this structure, the material was treated with two moles of aniline in toluene. Hydrogen sulfide was given off, and free sulfur was detected in the solids precipitated from toluene by petroleum ether. The crude solid was separated into two main products, A and B, by treatment with boiling water. Portion A, which dissolved in boiling water, precipitated out in the cold. It melted at 127–132° C., and its melting point was not depressed by mixing with a pure sample of 1,1-dimethyl-3-phenylurea (II). The infrared spectrum of A was identical with that of a pure sample of (II).

Portion B, which was insoluble in boiling water, was recrystallized from ethanol and was identified as 1,1-dimethyl-3-phenylthiourea (III), by melting point and by its infrared spectrum.



(I)

(II) (III)

Example 2

Tetramethylthiuram monosulfide (20 g., 0.1 mole) was slurried in 150 ml. toluene at room temperature (20° C.) with vigorous stirring. Nitrogen tetroxide (4.6 g., 0.05 mole) admixed with air was bubbled into the mixture. The solids slowly dissolved and when all of the nitrogen tetroxide had been added (15 minutes), the yellow foam on top of the reaction mixture had turned white. The toluene solution was decolorized with charcoal and filtered. The oxidized material was precipitated as snow white crystals by addition of petroleum ether. The solid was filtered at the pump and dried in a vacuum oven at 50° C. Yield of dimethylcarbamoyl dimethylthiocarbamoyl disulfide was 21 g., or 94% theory.

Example 3

N,N-dimethyl-N',N'-dibutylthiuram monosulfide (29.2 g., 0.01 mole) was dissolved in 50 ml. of chloroform at room temperature (20° C.) and stirred vigorously while bubbling in nitrogen tetroxide (4.6 g., 0.05 mole) admixed with air. After the nitrogen tetroxide had all been added, the chloroform was evaporated under reduced pressure, leaving a light yellow viscous oil. The yield of isomeric mixture of dimethylcarbamoyl dibutylthiocarbamoyl disulfide and dibutylcarbamoyl dimethylthiocarbamoyl disulfide was 30 g., or 97% of theory.

Examples 4 to 11

Eight additional oxydisulfides were prepared according to the method of Example 3, using 0.05 or 0.1 mole of thiuram monosulfide and half a mole of N_2O_4 (admixed with air) per mole of monosulfide, as shown in the table below. All the products were light yellow, viscous oils.

Example	Thiuram Monosulfide Used					Oxydisulfide Yield, Percent
	Groups on N		Groups on N'		Moles	
4	CH_3	CH_3	C_2H_5	C_2H_5	0.1	95
5	C_2H_5	C_2H_5	C_2H_5	C_2H_5	0.05	92
6	C_2H_5	C_2H_5	C_4H_9	C_4H_9	0.12	96
7	C_4H_9	C_4H_9	C_4H_9	C_4H_9	0.05	96
8	CH_3	CH_3	C_6H_{13}	C_6H_{13}	0.05	95
9	CH_3	CH_3	$CH_2CH_2OCH_2CH_2$		0.1	94
10	CH_3	CH_3	$(CH_2)_5$		0.1	83
11	CH_3	CH_3	$C_6H_5CH_2$	$C_6H_5CH_2$	0.1	83

Examples 12 to 15

Tetramethylthiuram monosulfide (208 g., 1 mole) was slurried in 1200 ml. toluene and air bubbled first through weighed portions of nitrogen tetroxide was led through the slurry as in Example 1. Reactions were carried out with and without external ice cooling. There was some evolution of sulfur dioxide at the higher reaction temperatures. Reactions were judged complete when the yellow foam on top of the reaction mixture had turned white. Unused N_2O_4 was weighed and the amount used calculated. Solutions were filtered hot, concentrated to 800 ml. and the oxydisulfide precipitated by adding petroleum ether. These results are summarized as follows:

Example	N_2O_4 used		Reaction Temp., ° C.	SO_2 Odor	Yield Oxydisulfide	
	Gms.	Moles			Gms.	Percent
12	23	0.25	to 60	Strong	141	63.5
13	10	0.11	30–35	Some	180	81
14	8	0.09	20–25	Slight	186	84
15	10	0.11	15	Faint	200	90

Example 16

In order to determine whether a solvent of any kind is necessary in the oxidation of a liquid thiuram monosulfide, a catalytic amount of nitrogen tetroxide (0.5 g. 0.006 mole) was carried by a stream of air into tetraethylthiuram monosulfide (8 g., 0.03 mole), which is a liquid, in a test tube cooled externally by ice. Air was bubbled through for 15 minutes. The dark color of the thiuram monosulfide became much lighter as it was converted to the oxydisulfide, namely diethylcarbamoyl diethylthiocarbamoyl disulfide. Yield of light amber oil was almost quantitative.

Example 17

A wettable powder (WP) formulation of tetramethylthiuram monosulfide having the following composition was prepared:

	Percent by weight
Tetramethylthiuram monosulfide	92
McNamee clay (a soft clay)	5
Dispersing agent [1]	2
Wetting agent [2]	1
Total	100

[1] The sodium salt of a mixed alkyl aryl sulfonate.
[2] A high molecular weight alkyl benzene sodium sulfonate or in some instances where indicated isooctyl phenyl polyethoxy ethanol, the latter being preferred because of a lower tendency to produce foaming. Other wetting agents could be used, however.

The preferred particle seize of the wettable powder is in the order of 3–5 microns. The average particle size of commercially available tetramethylthiuram monosulfide is in the neighborhood of 10–20 microns. Accordingly, it is desirable to grind the material to particle size of about 3 to 5 microns. It has been found that the oxidation of the monosulfide to the oxydisulfide proceeds more rapidly and is more complete when the particle size of the material is as indicated. It is not necessary however to grind the monosulfide together with the clay, dispersing agent and wetting agent although it is convenient to do so.

The percentage of the monosulfide in the wettable powder may be varied within wide limits. The particular formulation set forth above would permit the use of 2 pounds of formulated chemical per 100 gallons of water spray which is convenient and a proportion widely used in agricultural sprays.

There is no resetriction on the type and quality of clay or other carrier that might be used. Any inexpensive approximately neutral clay would be satisfactory. Dispersing agents other than the one mentioned could also be used.

The wettable powder formulation comprising the monosulfide was blended with various proportions of sodium nitrite and then dispersed in 200 gallons of water in a 600 gallon capacity spray tank provided with a motor driven agitator. The sodium nitrite used was the commercial 99.5% pure grade. The oxidation was carried out by adding to the monosulfide-nitrite suspension one equivalent of acid per mole of nitrite present in order to liberate nitrous acid. Any acid, inorganic or organic, which can liberate nitric oxide from sodium nitrite can be used. Two forms of sodium bisulfate were used as sources of sulfuric acid; the monohydrate which is equivalent to 35% sulfuric acid, and the "globular" which is equivalent to 38% sulfuric acid. Phosphoric acid (85%) was also used as another type of acid. The mixture was agitated vigorously by the paddles, and by recycling the liquid by means of the pump, the liquid being exposed to the air at all times. In carrying out the oxidation there is sufficient liquid in the tank to almost cover the paddles to insure maximum agitation and exposure to air. With the type of equipment used (Herosite-lined Bean Royal 55 spray tank) the tank was approximately one-third filled with liquid.

Runs were made in the spray tank using the formulations tabulated below, each prepared in 200 gallons of water. The ratio of nitrite to the 92% oxydisulfide wettable powder (WP) formulation above varied from 0.2:10 to 1.5:10 parts by weight. In each case, except for runs 10 and 15 in which triple amounts were used, 4 pounds of the wettable monosulfide formulation were blended with the stated quantity of sodium nitrite and then dispersed in 200 gallons of water in the spray tank with vigorous agitation. Then the quantity of acid material calculated to liberate all the nitric oxide from the nitrite was added and the time required for the yellow color of the monosulfide to be replaced by the white color of the oxydisulfide was noted. The pH of the slurry when conversion was complete was determined by means of commercially available narrow range pH paper. Unground monosulfide wettable powder was used in runs 12 and 13 to determine the effect of particle size upon conversion. No wetting agent was used in run 13 although the dispersing agent was present in 2% concentration as it was in all the wettable powder formulations. In run 14 the alkyl benzene sodium sulfonate was replaced with the isooctyl phenyl polyethoxy ethanol as the wetting agent. The results of the tests are tabulated below:

Formulation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Wettable Powder, lbs./200 gal.	4	4	4	4	4	4	4	4	4	12	4	*4	*4	4	------
Wetting agent:															
Na salt of mixed alkyl aryl sulfonate	V	V	V	V	V	V	V	V	V	V	V	V	------	------	V
Isobutyl phenyl polyethoxy ethanol	------	------	------	------	------	------	------	------	------	------	------	------	------	V	------
Defoamer, 10% silicone emulsion, oz./200 gal.											1.5				
Sodium nitrite, lb./200 gal.	0.3	0.3	0.3	0.4	0.5	0.6	0.4	0.5	0.6	1.2	0.4	0.4	0.4	0.4	1.2
Ratio, parts nitrite per 10 parts W.P	0.75	0.75	0.75	1	1.25	1.5	1	1.25	1.5	1	1	1	1	1	1
Acid source, lb./200 gal.:															
$NaHSO_4$(35% H_2SO_4)	0.5	------	0.59	------	------	------	------	------	------	------	------	------	------	------	------
(38% H_2SO_4)	------	------	------	------	------	------	0.73	0.91	1.09	------	------	0.73	0.73	0.73	2.19
H_3PO_4	------	0.5	------	0.66	0.82	0.99	------	------	------	1.98	0.66	------	------	------	------
Conversion time, minutes	20	25	20	15	12	9	15	10	8	13	20	[1] 17	(2)	16	[3] 7–12
pH after conversion	3.5–4	3.5–4	3.5–4	3.4–5	3.5–4	3.0	3.5–4	3.5–4	3–3.5	3.5–4	3.5–4	3.5–4	3.5–4	3.5–4	3–3.5

[1] Unground formulation dispersed poorly; contained small lumps of unchanged monosulfide.
[2] All of this unground formulation containing no wetting agent floated to the surface and failed to react.
[3] Conversion was essentially complete in the liquid phase in 7 minutes but a total of 12 minutes was required to convert monosulfide caught in the foam.
*Unground.

When 1.5 parts of sodium nitrite per 10 parts of 92% active wettable monosulfide formulation were used at the rate of 4 pounds per 200 gallons, the reaction was complete in 9 minutes. The preferred ratio of nitrite to monosulfide is believed to be about 1.25:10 at which level the reaction required 12 minutes to go to completion. This amount is believed to provide for the loss of small quantities of N_2O_4 or NO_2 which may take place during the reaction. However, when agitation is not so vigorous, and/or the supply of air is limited, larger quantities of nitrite, for example 0.8 mole of NO_2 per mole of monosulfide or 2.4 parts nitrite per 10 parts monosulfide 92% active wettable powder, may be desirable thereby to complete the reaction in 6–7 minutes.

Example 18

The procedure described in the foregoing example was repeated using the same equipment, using a different wetting agent and testing the effect of added Dow Corning Antifoam B. The basic wettable powder formulation was the same except that the wetting agent (also present in the amount of 0.5 part by weight) was a liquid nonionic surfactant, nonyl phenyl polyethylene glycol ether. The amount of sodium nitrite used based on the amount of monosulfide wettable powder was kept constant at 1.25

parts per 10 parts of wettable powder. The acid used to liberate the nitric oxide was sodium bisulfate, globular (38% H_2SO_4). The results of the four tests are presented in the following table:

Run No.	Antifoam, parts	Monosulfide, WP lbs.	Conversion time, min.	Dispersion	Foam
1	0	4	12 all but foam / 15 total	Excellent	Small.[1]
2	0.2	4	16	Not as good as Run No. 1.	None 1st 10 min.[2]
3	0	12	14	Excellent	Small (1" deep).
4	0.2	12	16	do	A little lower than Run No. 3.

[1] Traces of yellow in foam at 12 min., were gone in 15 min. Moderate agitation was used.
[2] Some monosulfide floated on surface the 1st 10 min., then a small quantity of foam appeared. No trace of unreacted monosulfide in foam after 16 min.

The following five examples illustrate the facteriostatic and fungistatic activity of representative oxydisulfide compounds of the invention and the use thereof in soap to impart bacteriostatic activity thereto.

Example 19

The bacteriostatic activity of representative oxydisulfides of the invention was measured by the use of the zone of inhibition method. To demonstrate fungistatic activity the agar incorporation method was used for *Aspergillus niger*.

Oxydisulfide Compounds	Lowest Concentration Inhibiting Growth, p.p.m.			
	Aspergillus niger	*Bacillus subtilis*	*Staphylococcus aureus*	*Salmonella typhosa*
Tetramethyl	300	10	50	20
Dimethyldiethyl	1,000	300	300	300
Tetraethyl	+1,000	50	50	50
Dimethyldibutyl	+1,000	300	300	300
Diethyldibutyl	+1,000	10,000	10,000	10,000
Dimethyldihexyl	+1,000	300	300	500
N,N-dimethyl-N'-diethylene-oxy	500	300	300	100
N,N-dimethyl-N'-pentamethylene	500-1,000	100	50	50
N,N-dibenzyl-N'-N', dimethyl	+1,000	1,000	10,000	500

In the above tabulation the plus sign means greater than.

Example 20

The bacteriostatic activity of representative oxydisulfides was determined in soap. In one test, one percent of each oxydisulfide was blended into a separate portion of Ivory soap (a neutral white high grade toilet soap consisting of a mixture of 80% sodium soap and 20% potassium soap produced from a 70% tallow and 30% coconut oil glyceride blend), the soap compressed into plugs, and the plugs placed on separate agar plates inoculated with *Bacillus subtilis, Staphylococcus aureus* and *Salmonella typhosa*. The plates were prepared by adding 18-hour nutrient broth cultures of each of the test bacteria (20 ml./l.) to melted and cooled sterile nutrient agar. The agar was carefully blended, then placed in sterile Petri dishes (20 ml./dish) and allowed to harden. The plates were incubated for 24 hours at 37° C., then the diameter of the zone of no bacterial growth around each plug was measured in millimeters. In the second test, substantivity to skin or hide (as a measure of its retention on the skin) was determined by soaking untanned calfskin buttons in an 8% solution of the test soap (prepared above) in water (0.008% concentration of each oxydisulfide in water), rinsing the buttons three times in an equal volume of distilled water, then placing them on nutrient agar plates inoculated and subsequently treated as those described in the first test, above. Parallel tests were also made using compounds other than the oxydisulfides, which compounds are enclosed in brackets in the following table of results.

Oxydisulfide Compounds	Average Diameter of Zone of Inhibition in mm.					
	Soap Plug			Hide Substantivity		
	Bacillus subtilis	*Staphylococcus aureus*	*Salmonella typhosa*	*Bacillus subtilis*	*Staphylococcus aureus*	*Salmonella typhosa*
Tetramethyl or $(CH_3)_2N—CO—S—S—CS—N(CH_3)_2$	32	28	23	13	14	14
$[(CH_3)_2N—CS—S—CS—N(CH_3)_2]$	3	0	0	0	0	0
$[(CH_3)_2N—CS—S—S—CS—N(CH_3)_2]$	14	16	16	14	9	10
$[(CH_3)_2N—CO—S—CS—N(CH_3)_2]$	13	10	10	0	0	0
Dimethyldiethyl	18	19	20	8	7	4
Tetraethyl or $(C_2H_5)N—CO—S—S—CS—N(C_2H_5)_2$	6	19	18	3	8	5
$[(C_2H_5)_2N—CS—S—CS—N(C_2H_5)_2]$	8	7	5	0	0	0
$[(C_2H_5)_2N—CS—S—S—CS—N(C_2H_5)_2]$	6	2	4	0	0	3
Dimethyldibutyl	14	17	14	4	6	2
Diethyldibutyl	18	24	16	6	11	4
Tetrabutyl	8	5	6	0	0	0
Dimethyldihexyl	21	17	18	6	3	7
N,N-dimethyl-N'-diethyleneoxy	18	16	18	6	5	6
N,N-dimethyl-N'-pentamethylene	27	18	19	8	5	8
N,N-dibenzyl-N',N'-dimethyl	13	22	12	2	7	4

These comparative tests show that the tetramethyl oxydisulfide, i.e., dimethylcarbamoyl dimethylthiocarbamoyl disulfide, is markedly superior in the baceteriostatic activity and skin substantivity to the bracketed compounds, namely bis-(dimethylthiocarbamoyl) - monosulfide or tetramethylthiuram monosulfide, bis-(dimethylthiocarbamoyl)-disulfide or tetramethylthiuram monosulfide and dimethylcarbamoyl dimethylthiocarbamoyl monosulfide. The same is true of the tetraethyloxydisulfide, i.e., diethylcarbamoyl diethylthiocarbamoyl disulfide.

Example 21

Further tests of the bacteriostatic activity of the tetramethyloxydisulfide, i.e., dimethylcarbamoyl dimethylthiocarbamoyl disulfide, at a concentration of 0.1% in soap were performed, and the results are compared below with those obtained using the same concentration of tetramethylthiuram disulfide. Other tests were made on the oxydisulfide at 0.25% and 0.5%.

Test Compound	Average Diameter of Zone of Inhibition in mm.					
	Soap Plug			Hide Substantivity		
	Bacillus subtilis	*Staphylococcus aureus*	*Salmonella typhosa*	*Bacillus subtilis*	*Staphylococcus aureus*	*Salmonella typhosa*
Tetramethyl oxydisulfide (.05)	20	22	15	1	0	0
Tetramethyl oxydisulfide (0.1%)	21	22	19	3	3	3
Tetramethyl oxydisulfide (.25%)	27	25	22	3	6	6
Tetramethyl-thiuram disulfide (0.1%)	10	3	0	0	0	0

These results show that tetramethyl oxydisulfide is an active bacteriostat in soap at a far lower concentration than is tetramethylthiuram disulfide.

Example 22

Since discoloration of bacteriostatic soap when exposed to light or when held in storage is a very important factor in consumer acceptance, tablets of Ivory soap containing varying amounts, from 0.25% to 1.0%, of tetramethyl oxydisulfide were made up and subjected to sunlight aging tests and to accelerated shelf aging (heat aging tests).

Sunlight color stability was tested by exposing the tablets of treated soap to direct sunlight for two weeks, rotating the tablets one-quarter turn daily. Color after exposure to sunlight was recorded. Heat aging stability, which is an accelerated aging test comparable to many months' storage on the shelf at room temperature, was tested by placing the bars of treated soap in a 125° F. oven for two weeks and noting their color at the end of the exposure period. Controls for both these tests were stored in the dark at room temperature for two weeks and their color noted at the end of that time.

Hide substantivity tests were performed on the aged soaps, using the various test soaps at 8% concentration in water in order to determine whether storage or light had an adverse effect on substantivity to skin.

Tetramethyl Oxydisulfide, Percent in Soap	Color	Average Diameter of Zone of Inhibition, mm.		
		Bacillus subtilis	*Staphylococcus aureus*	*Salmonella typhosa*
Aged 14 days in sunlight:				
1.0	White	11	10	10
0.75	do	5	8	5
0.50	do	5	4	3
0.25	do	1	0	1
Aged 14 days in 125° F. oven:				
1.0	Sl. yellow	12	8	8
0.75	do	11	8	7
0.50	White	10	10	6
0.25	do	2	0	1
Unaged:				
1.0	do	13	13	14
0.75	do	10	8	11
0.50	do	10	10	6
0.25	do	8	6	3

These results show that a concentration of at least 0.5% of tetramethyl oxydisulfide is needed to withstand the effect of sunlight aging in order to retain a satisfactory hide substantivity. Also a concentration of 0.5% is desirable to maintain hide substantivity through the severe heat aging test.

Example 23

Hand washing tests were performed with Ivory soap containing 1% of tetramethyl oxydisulfide, using a modification described by Quinn et al., in Applied Microbiology, vol. 2, 202–4 (1954) of the split-use hand washing test. In the split-use test, one hand of a subject is covered with a neoprene glove while he washes his hands in a specified manner with the medicated soap, then he uncovers that hand and covers the other with a neoprene glove while he washes his hands in fresh wash water with nonmedicated soap. The hand washed with nonmedicated soap thus serves as a control for the hand washed with medicated soap.

In the modified test, the hands, one covered with a neoprene glove, are soaped for 20 seconds by briskly rubbing the moistened soap bar with a small amount of water held in the cupped hands, then the lather is worked up as far as the wrists for 75 seconds, the hands rinsed for 20 seconds while immersed in the wash water, and finally drained for 20 seconds. The left hand is covered while the right hand is washed with medicated soap, and the right hand is covered while the left hand is washed with nonmedicated soap.

Each subject washes his hands three times a day, at the same times, for five days. Sterile wash water (1500 ml.) and sterile gloves are used for the second washing on the third and fifth days of the test, and nonmedicated soap is used for both hands at these times so that none of the test compound will be present in the wash water to influence the results of bacterial counts which are made then. Aliquots of the wash water in which each hand is washed were planted into nutrient agar pour plates within one minute after completion of washing. The plates were incumbated for 48 hours at 37° C., the number of bacterial colonies were counted, and the number of bac-

teria was calculated for the entire wash water. The results of these tests are summarized below:

Sub. No.	Third Day			Fifth Day		
	Count in Millions		Reduction Percent	Count in Millions		Reduction Percent
	Left	Right		Left	Right	
1	5.0	1.8	64	7.2	0.9045	87.4
2	15.9	2.25	86	39.9	0.3195	99.1
3	15.0	1.5	90	19.995	0.465	97.6
4	20.0	2.8	86	30.495	1.725	94.3
Average			81.5			94.6

These results show that tetramethyl oxydisulfide at 1% in Ivory soap is very effective in reducing the bacterial population on the human skin. In general, the effective range of the oxydisulfides when used as a bacteriostat in soap is about 0.1 to 5% by weight. They are especially useful in alkali metal soaps of the common fatty acids although they may also be used in solid synthetic bars.

Example 24

One (1) inch circles of doubled diaper fabric were washed in a beaker containing 499 ml. water and 1 gram of a built alkyl aryl sulfonate detergent at 125° F. The detergent contained 1% by weight of the tetramethyl oxydisulfide. The fabric circles were agitated in the aqueous solution for 5 minutes. They were then removed and dried. The dried samples were placed in separate 4 ounce jars and sterilized by autoclaving with steam. A control sample was washed with the detergent only. After sterilization, the samples were inoculated by pipetting 1 ml. of a 1:100 dilution of 18-hour nutrient broth culture of *Staphylococcus aureus* in special nutrient broth onto each set of samples in the jars. The inoculum was immediately enumerated to determine cell count per milliliter. The jars were incubated for 24 hours at 37° C. After incubation, 99 ml. of sterile distilled water were added to each jar and a serial plate dilution was performed, considering the contents of the original jar to be 1:100 dilution. Tryptone glucose extract agar was used as plating medium. The plates were incubated for 48 hours at 37° C. The count of untreated cotton was compared to the count of cotton washed with detergent containing the oxydisulfide. The percent reduction was calculated by comparing the original count of bacteria added to each group of fabric samples with the number found on each group of fabric samples after incubation.

No. of Bacteria (Thousands)		Percent Reduction of Original Inoculum
Inoculum	200.00	
Detergent alone	120,000.00	
Detergent plus Oxydisulfide	3.8	98.10

Example 25

A xylene solution of tetramethyl oxydisulfide was prepared for treatment of cotton duck (10 ounces per square yard). The duck was placed in a bath of each oxydisulfide concentration and run through squeeze rolls to achieve 100% pickup of treating solution. By this means, the various concentrations based on fabric weight were achieved. After air drying, the samples were subjected to agar plate and soil burial tests. Pieces of treated fabric 1 inch square were used for agar plate tests. Samples 6 inches long containing five of the marked-off 1 inch wide samples were used for soil burial.

In the agar plate tests, triplicate samples 1 inch square for each concentration were placed on mycophil agar and inoculated by pipetting 1 ml. of spore suspension of *Aspergillus niger* onto the surface of the sample and agar.

Readings were taken after 7 and 14 days of incubation at 30° C.

For the soil burial tests, the samples of each test concentration were exposed as outlined in Federal Government Specification CCC–T–191b Soil Burial and Tensile Strength Determination.

Results:

Oxydisulfide Concentration	Evidence of Growth				
	Agar Plate		Original, lbs.	Soil Burial	
	7th day	14th day		After 14 days, lbs.	Percent Retained
Untreated cotton	+++	+++	93.3	0.0	0.0
1%	0	0		89.3	95.71
½%	0	0		45.4	48.66
¼%	0	0		0.0	0.0
⅛%	++	+++		0.0	0.0

Key:
0=no growth.
+=slight growth.
++=moderate growth.
+++=heavy growth.

Example 26

To determine efficacy against paper mill slime, seven samples of slime recovered from paper mills were collected and each cultured for 7 consecutive days. Each slime culture was used as the inoculum in a separate Zone of Inhibition test. No attempt was made to purify these organisms. Consequently, they represent the mixed flora that existed in the paper mill. The tetramethyl oxydisulfide in concentrations in the range of 0.1 to 0.3% by weight was found to inhibit the growth of the organisms.

Example 27

To test for mold-proofing of paper, typical brown kraft paper carefully weighed was used. Measured amounts of test chemicals were added to samples of the paper and subjected to the Lever Brothers Mold-Proofing test. Basically, this test is inoculation by use of a "spore cloud" (of *Aspergillus sydowi, Aspergillus versicolor, Penicillium chrysogenum*) of each of the treated samples and then placing the samples on agar medium. The plates were incubated and rated after 7, 14 and 21 days. The rating system used was:

0=no growth
1=slight growth
2=moderate growth
3=heavy growth
4=paper completely overgrown

The ratings are customarily reported as 011, 123, etc., the three digits of each number denoting the rating at 7, 14 and 21 days. A rating of 000 to 011 is considered excellent, 112 to 123 as good, and 233 to 344 as poor. The paper was treated at two levels of oxydisulfide concentration. The table below compares the rating of two levels of oxydisulfide with those obtained with equal cost concentrations of a commercial slimicide and with an untreated control.

	$5.00 per ton	$10.00 per ton
Untreated	444	444
Tetramethyl Oxydisulfide	011	011
Commercial Dithiocarbamate type	023	012

Example 28

To test the tetramethyl oxydisulfide as a dry cleaning bacteriostat, solutions of the chemical were prepared in trichlorotrifluoroethane and trichloroethylene. Swatches of 1 inch diameter of wool and corduroy (cotton) were prepared. The swatches were wetted thoroughly with the various solvent solutions. One-half of the samples were

pressed with a steam iron. The swatches had an approximate pickup of 100% which means that the concentration of oxydisulfide in the solution was the same as on the swatch. The swatches were air dried and then evaluated by the Zone of Inhibition test using *Staphylococcus aureus* and *Salmonella typhosa* as test organisms. The results are presented in the following table.

Solvent	Oxydisulfide (p.p.m.)	Diameter of Zone of Inhibition, mm.			
		Wool		Cotton	
		Staph. aureus	*Salm typhosa*	*Staph. aureus*	*Salm. typhosa*
Trichlorotrifluoroethane	0	0	0	0	0
Do	500	14	7	23	17
Trichloroethylene	0	0	0	0	0
Do	500	14	12	19	14
Do	1,000	15	10	21	15

Further testing showed that the presence of wetting agents and steam pressing had no effect on biological efficacy.

Example 29

The several oxydisulfides were tested for activity against organisms secured from jet fuel. The bacteria were evaluated by the Zone of Inhibition test and the fungi by agar incorporation method. The results given in the following table are the lowest concentration in parts per million of the oxydisulfide which inhibited growth of the seven microorganisms tested.

Oxydisulfide	Lowest Concentration Inhibiting Growth p.p.m.						
	Bacteria					Fungi	
	A	B	C	D	E	F	G
Tetraethyl	10M	300	------	------	300	1M	------
Dimethyl N-piperidyl	10M	500	------	------	500	1M	1M
Dimethyl morpholyl	10M	1M	------	------	10	500	500
Dihexyl dimethyl	------	10M	------	------	1M	300	300
Tetramethyl	1M	500	10M	10M	10	300	300
Diethyl dimethyl	10M	500	------	10M	500	1M	------
Dibutyl dimethyl	10M	10M	------	------	10M	------	------

Legend: M=thousand.

Example 30

Tetramethyl oxydisulfide was tested as a fungicide for a vinyl resin composition by incorporating it into the following formulation:

	Parts by Weight	
	Control	Test
Vinyl chloride-vinyl acetate copolymer resin VYNW	100	100
Dioctyl phthalate, DIOP	25	25
Ricinoleate type plasticizer, P-4	25	25
Stearic acid	0.3	0.3
Barium-cadmium type stabilizer, Vanstay HT	1.5	1.5
Chelating type stabilizer, Vanstay S	1.5	1.5
Oxydisulfide	0	1

and fluxing on a 325° F. mill for five minutes. The resin was sheeted off the mill and cut into 2 x 4-inch samples which were placed on mineral salts agar and each inoculatsd with a mixed spore suspension of *Aspergillus niger, Aspergillus flavus, Penicillium luteum,* and Trichoderma, T–1. The plates were incubated at 75° F. for 7 to 14 days and observed for evidence of growth on the samples. The results for oxydisulfide-treated vinyl resin as compared with those for a similar control resin containing no oxydisulfide are presented below:

Vinyl resin formulation	Evidence of growth on samples [1]	
	7 days	14 days
Oxydisulfide-treated	0	tr.
Control	++	+++.

[1] Key:
0=no growth.
tr=trace of growth (probably mycelium fragments).
+=slight growth.
++=moderate growth.
+++=heavy growth.

Examples 25 to 30 inclusive demonstrate the utility and efficacy of the oxydisulfides in various media, when applied in suitable amounts and in suitable manner, to material subject to microbiological degradation. Example 25, for instance, demostrates how the oxydisulfides may be used to impart to textiles resistance to fungus and soil-borne organisms. Example 26 demonstrates their efficacy in combatting paper mill slime when introduced into the aqueous fluids of the paper mill. Example 27 shows how paper may be mold-proofed effectively by incorporating the oxydisulfide directly into or onto the paper. In Example 28 the oxydisulfide is effectively added to the dry cleaning solvent thereby to render the garment cleaned resistant to bacteria. The problem of combatting organisms which grow in jet fuel is effectively met by using the oxydisulfides, as illustrated in Example 29. Example 30 illustrates how the oxydisulfides may be used in combatting the attack of fungus on vinyl resin formulations.

In the following examples, 31 to 42, the oxydisulfides were tested as agricultural fungicides.

Example 31

A 65% wettable powder of the oxydisulfide was prepared and applied to potato plants in a regular schedule to combat late blight and other foliar diseases. The spray was applied at 2 lbs. per 100 gallons concentration using approximately 200 gallons per acre at each application. The percent defoliation of plants and final yield of tubers was determined. At harvest there was about 72% defoliation of the treated plants compared with 92% for the controls. Yield of the treated plants was 247 lbs. compared with 207 lbs. for the controls.

Example 32

Tests were carried out in northern Florida during the early annual growing season on the effect of the tetramethyl oxydisulfide on the control of downy mildew (*Pseudoperonospora cubensis*) on cucumbers (Marketer cucumber). The seeds were sown on February 21 and thinned on March 15. The oxydisulfide was applied 13 times at substantially uniform three to four day intervals beginning on March 24 and ending on May 7. The rate of application was 1.6 lbs. per 100 gallons of spray. The fungicide was applied with a single row experimental sprayer which could be equipped with 3, 4, or 6 nozzles. Four applications of the fungicide were made using 3 nozzles which delivered 140 gal. per acre when pressure was maintained at about 200 lbs.; the following four applications were made using 4 nozzles delivering 175 gal./acre; and 5 applications were made using 6 nozzles delivering 230 gal./acre. Downy mildew was first seen on a few plants on April 23. Development thereafter was rapid and the vines in untreated check rows were becoming yellow by May 4. Production in the untreated rows was reduced quite markedly starting with the 4th picking of fruit. Quality also was reduced by the disease. The observations made on the plants treated with test chemical and on the control during the growing season are presented in the following table. Yield data are also presented.

Date observed	Percent Damage	
	Oxydisulfide-treated	Untreated
5/3	2.3	35.0
5/7	20.0	68.3
5/12	45.0	83.0
5/15	73.3	93.0
Total weight of fruit, in lbs.	359	272

Example 33

Field tests were made in northern Florida during the early growing season on the effectiveness of the tetramethyl oxydisulfide in the control of Helminthosporium leaf blight of corn (Gold Cup Sweet corn) (Harris). The seeds were sown on February 26 and thinned on March 25. The oxydisulfide was applied 10 times during the growing season at substantially uniform intervals beginning March 24 and ending May 12. The sprays were applied with the equipment described in Example 32. Three applications were made using 3 nozzles (delivering 140 gal./acre when pressure is maintained at 200 lbs.), three with 4 nozzles (175 gal./acre) and four with 6 nozzles (230 gal./acre). Rate of application was 2 lbs. of oxydisulfide per 100 gal. of spray. After the corn was 4 feet tall, the spray boom was offset to permit travel between the rows. Leaf blight appeared early in April but developed very slowly because of the generally dry weather and relatively cool nights. The disease had only begun to take effect when the corn was ready for picking, so the effect of oxydisulfide on yield was very slight. In order to get a better evaluation of the fungicide, the plots were scored again about a week after picking the ears.

Test	Oxydisulfide	Check
Blight at picking, percent	16.7	26.7
Blight the following week, percent	33.3	41.7
Total weight of ears, pounds	169.0	161.0

Example 34

Tests were also made in northern Florida on the tetramethyl oxydisulfide for the control of Alternaria leaf spot (early blight) of tomatoes. The seeds were sown on January 10, transplanted to pea pots on February 1 and were transplanted on February 26. There were 13 dates of application at uniform intervals beginning March 15 and ending May 19. The chemical was sprayed at the rate of about 1.6 lbs. per 100 gallons of spray using the equipment described in Example 32 and making 3 applications using 3 nozzles, 2 using 4 nozzles and 8 using 6. Early blight developed during May but did not become serious until the last week of the month. By that time it had begun to defoliate the center parts of the plants. The extremely dry season and hot weather in May prevented the development of late blight (Phytophthora infection). Observations on defoliation and on yield are presented in the following table.

Test	Oxydisulfide	Check
Defoliation on 5/24, percent	3.7	33.3
Defoliation on 5/28, percent	15.0	35.0
Defoliation on 6/2, percent	20.0	43.3
Defoliation on 6/7, percent	40.0	61.7
Total fruit, six pickings, pounds	260.0	224.0

Example 35

Field tests were made on the efficacy of the tetramethyl oxydisulfide in the treatment of sugar beet seed. The test area which was in Colorado consisted of single row plots 20 feet long. The seed used was American No. 2 "Monogerm," No. 1 size. A total of 100 seeds was planted per plot with special plot planters. The pathogen was soil fungi complex. The oxydisulfide was applied as a wettable powder containing 75% active ingredient. The rate of application was 1.75 ounces (active oxydisulfide) per 100 lbs. seed. Duplicate tests were run. In the first the average seedling count per 100 seeds was 59% compared with 48% for the control. In the second test, the average seedling count per 100 seeds was 46% as compared with 39% for the control.

Example 36

The tetramethyl oxydisulfide was field tested for seed treatment of two types of hybrid corn designated type "A" and type "B." The application rate was ¾ ounce per bushel. For type "A," cold test germination was 72% as compared with 14% for the untreated control. For type "B," the germination was 82% as compared with 38% for the untreated control. The average of the two types was 77% germination for the treated seed corn as compared with 26% for the untreated control.

Example 37

Field tests were made in New York on the tetramethyl oxydisulfide for efficacy in control of damping off and seed decay complex on Golden Cross bantam sweet corn, Kinghorn wax beans, and "Monogerm" sugar beets all grown on upland mineral soils. Lots of 100 seeds each were treated with fungicide in slurry or in dust form at several rates and for different time intervals. In the first series of tests the oxydisulfide was applied as a slurry prepared from 75% active wettable powder. The rate of application varied from 0.75 to 3.0 ounces of active ingredient per 100 lbs. of seed.

In the case of sweet corn, germination of the untreated control was 66% whereas germination of the treated seed ranged from 77 to 79%. In another test (with the same control), a 50% active wettable powder was applied as a dry mix at the rate of 0.75 ounce of active material per 100 lbs. seeds. Germination of the treated seed ranged from 76 to 80%.

In the case of the wax beans, the oxydisulfide again was applied as a slurry prepared from 75% wettable powder, the rates of application ranging from 0.75 to 3.0 ounces of active material per 100 lbs. of seed. Germination of the untreated control was 80% as compared with 89 to 92% for the treated seed. When 50% wettable powder was applied as a dry mix at the rate of 0.75 ounce of active material per 100 lbs., germination ranged from 89 to 92%.

In similar tests made on the sugar beet seeds, the percent germination for the seeds treated with the slurry ranged from 40 to 57%, and the seeds treated with the dry mix ranged from 46 to 48%, both compared with the control which germinated at the rate of 35%.

Example 38

Field tests were made on the efficacy of tetramethyl oxydisulfide in combatting apple scab of the foliage and fruit of trees (MacIntosh) grown in New York State. The material was applied 12 times at intervals starting April 30 and ending August 17. Applications were timed in such a way as to permit some disease development. The oxydisulfide was applied as a wettable powder containing 75% oxydisulfides and 25% inert ingredients. Rate of application was 1 lb. of active ingredient per 100 gal. of spray. Insecticides were applied throughout the season to keep insect damage at a low level. Foliar data were taken by two observers counting visible lesions for 3 minutes each (6 minutes total time) per tree. Data on primary and secondary scab lesions on the fruit were taken by picking and examining a given number of fruit per tree.

In a parallel test, Kolospray, a commercial fungicide, was applied at 6.0 lbs. through the first cover, 4.0 lbs. through the third cover, and 2.0 lbs. the remainder of the

season. Kolospray contains 81.25% sulfur (a fused bentonite sulfur) and 18.75% inert ingredients. There were 12 applications of the fungicide to the trees during the season. The results of the observations on the foliage appear in the following table.

Material	Rate, lbs. active/100 gal.	Foliage Apple Scab Lesion Counts, 6 min./tree, ave./tree on dates			
		6/11	6/26	7/18	8/2
Oxydisulfide 75% WP	1.00	40.0	60.4	139.0	115.6
Kolospray	Variable [1]	185.2	365.2	891.0	812.4
Untreated		213.4	614.8	+1,000.0	+1,000.0

[1] Kolospray 6.0 lbs. through 1st cover, 4.0 lbs. through 3rd cover and 2.0 lbs. the remainder of the season.

Results of the observation on the fruit are presented in the following table.

Material	Rate, lbs. active/100 gal.	Fruit Apple Scab Lesion Count on 8/24, percent		
		Primary	Secondary	Both
Oxydisulfide 75% WP	1.00	2.0	0.5	0.0
Kolospray	Variable [1]	21.0	6.0	4.5
Untreated		48.5	60.0	37.5

[1] See footnote in preceding table.

Example 39

In order to determine effectiveness of the tetramethyl oxydisulfide as an eradicant of apple scab (*Venturia inequalis*) an orchard test was carried out in New York on MacIntosh apple trees. In separate tests, 1.0 and 2.0 lbs. per 100 gallons of the oxydisulfide (in a formulation) were applied 28 to 30 hours past the initiation of a heavy primary and secondary apple scab infection period. The application was made on June 6. On June 22, lesions were counted on the foliage which had been open for infection at the time of application, namely, from the 8th true terminal leaf to the end of the terminal. Two observers counted the visible lesions for 3 minutes each per tree. For the untreated control, 601 lesions were counted per tree in 6 minutes. For trees treated with 75% active wettable powder formulation of oxydisulfide at the rate of 1.0 lbs. of active material per 100 gallons of spray, 51 lesions per tree were counted, while for trees treated with 2.0 lbs. active per 100 gallons, 26 lesions per tree were counted.

Example 40

Orchard tests were made on the efficacy of the tetramethyl oxydisulfide in combatting cherry leaf spot and brown rot of Napoleon sweet cherries. The orchard was in New York State and applications of the fungicide were made on June 6, 19 and July 2, the latter date being the date when normal harvest began. The fungicide was used in the form of a 75% active wettable powder and was applied at the rate of 1.0 and 2.0 lbs. of active material per 100 gallons of spray. Data were taken by two observers counting infected fruit on the trees for 2 minutes each on June 20, 29, July 9, 13 and 19. The results of the observation of the incidence of brown rot are presented in the table below:

Material	Rate, lbs. active/100 gal.	Average Number of Infected Fruit per Tree, 4 minute Count Time per Tree				
		6/20	6/29	7/9	7/13	7/19
Oxydisulfide 75% W.P.	1.00	3.8	3.5	10.8	13.0	36.2
Do	2.00	3.2	3.0	6.5	8.0	13.0
Untreated		8.5	13.8	87.8	139.2	93.5

Cherry leaf spot developed rapidly on the trees in the middle of August. Ratings were made by two observers on August 27, September 4, 7 and 18 and average ratings were recorded.

The results of the observations made on the incidence of cherry leaf spot are presented in the following table.

Material	Rate, lbs. active/100 gal.	Visual Rating[1] of Leaf Spot Infection on Dates			
		8/27	9/4	9/7	9/18
Oxydisulfide 75% WP	1.00	0.2	2.6	3.2	3.8
Do	2.00	1.5	2.8	3.1	4.0
Untreated		4.0	4.1	4.2	4.8

[1] Rating Key:
0=Practically no infection.
1=Up to 25% of foliage infected.
2=25 to 50 % of foliage infected.
3=50 to 75% of foliage infected.
4=75 to 90% of foliage infected.
5=100% of foliage infected.

Example 41

The material tested in Example 40 was used to determine its effectiveness in combatting cherry leaf spot (*Coccomyces hiemalias*) of Montgomery sour cherries. Tests were also carried out in New York State. Six applications were made during June 7 and August 8. Data were taken by two men rating the infection on each tree on August 23 and 30, September 6 and 18. Cherry leaf spot development was very slow through most of the season until about August 10. By August 23 the untreated trees were nearly 100% infected and by September 18 nearly completely defoliated. The observations are presented in the table below.

Material	Rate, lbs. active/100 gal.	Visual Rating[1] of Leaf Spot Infection on Dates			
		8/23	8/30	9/6	9/18
Oxydisulfide 75% WP	0.50	0.4	0.8	1.0	1.5
Do	1.00	0.2	0.0	0.2	0.7
Untreated		4.1	4.8	4.0	5.0

[1] Rating Key: Same as in Example 40.

Example 42

In order to test the efficacy of the material used in Example 41 against the development of brown rot (*Monilinia fructicola*) on Hale Haven peaches, trees in a New York State orchard were sprayed 7 times during the season beginning May 10 and ending August 15. The rates of application were 1.0 lb. and 2.0 lbs. active tetramethyl oxydisulfide per 100 gallons. Several commercial fungicides were also tested concurrently. Since brown rot failed to develop on the peach fruit while on the trees, the peaches were picked and placed in baskets for disease development. After 6 days in the basket, 24% of the peaches treated with 1.0 lb. active oxydisulfide were found to be infected, and 29% of those treated with 2.0 lbs. active oxydisulfide were infected. Seventy-four percent of the untreated control peaches were infected. From 41% to 73% of the fruit which had been treated with commercial fungicides were infected. After 8 days in the basket, 52% of the peaches treated with 1.0 lb. active oxydisulfide were infected, 56% of those treated with 2.0 lbs. active oxydisulfide were infected, and 85% of the control peaches were infected. From 70% to 87% of the peaches which had been treated with commercial fungicides were infected.

Example 43

To test tetramethyl oxydisulfide as an anti-fouling agent, a solution thereof was prepared in an organic solvent and absorbed onto a silica block and submerged in the ocean at Miami, Florida, for two months. The types of fouling organisms attached to the block after two months

submersion were observed and reported. The results are reported in a numerical system with the following meaning:

0=no repellency of organism to 10=complete repellency of organisms.

Fouling Organism	Rating of Repellency	
	Untreated	Oxydisulfide
Algae	0	10
Amphipods	0	10
Annelids	0	10
Barnacles	0	10
Bryozoans	0	10
Hydroids	0	10
Mollusks	0	10
Tunicates	0	10
Microfouling	0	10

The foregoing examples demonstate the effectiveness and potency of the oxydisulfides in combatting various microorganisms. In Examples 31 to 42 inclusive there is presented a demonstration of how the oxydisulfides may be used effectively in combatting fungus on potato plants, downy mildew on cucumbers, leaf blight of corn, leaf spot (early blight) of tomatoes, soil fungi complex on sugar beet seed, and on hybrid corn seed, damping off and seed decay complex on sweet corn, wax beans and sugar beet seeds, scab on apple trees and fruit, cherry leaf spot and brown rot of sweet cherries, cherry leaf spot of sour cherries and brown rot on peaches as well as the various microorganisms and macroorganisms which cause fouling of surfaces due to marine growth (Example 43. In each instance the oxydisulfide was added to or incorporated in the object to be protected in accordance with known techniques. Tetramethyl oxydisulfide was observed to be effective as a contact pesticide against pea aphids and spider mites when used at a concentration of 0.35%.

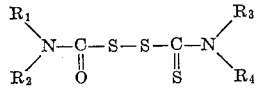
In some instances, the oxydisulfides are applied to the area or the object to be treated by using a carrier, such as a liquid spray or powder. In other cases, a carrier may not be needed or may be undesirable. However, the use or non-use of such carrier, the type of carrier used, the manner and frequency of application, and the dosage levels will be readily apparent to or determinable by those skilled in the art.

The above examples demonstrate that the invention provides a novel process for producing the chemical compounds in very high yield, which compounds are highly useful as bacteriostats, fungistats, agricultural fungicides, and antifouling agents.

It will be appreciated that various modifications and changes may be made in the invention without departing from the spirit thereof and accordingly the invention is to be limited only within the scope of the appended claims.

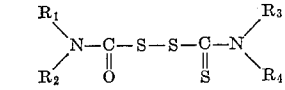
We claim:

1. A method for the preservation of material normally subject to fungus and bacterial attack which comprises applying to such material at least one carbamoyl thiocarbamoyl disulfide having the structural formula



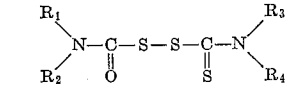
where R_1, R_2, R_3 and R_4 are alkyl from 1 to 10 carbons, cycloalkyl from 5 to 7 carbons or aralkyl from 7 to 10 carbons; where R_1 plus R_2 or R_3 plus R_4 are said alkyl taken together with the adjoining nitrogen atom to form a heterocyclic ring of 4 to 10 carbons; or where R_1 plus R_2 or R_3 plus R_4 are oxyethyl and ethyl taken together with the adjoining nitrogen atom to form a morpholyl ring.

2. A method for the preservation of material normally subject to fungus attack which comprises applying to such material at least one carbamoyl thiocarbamoyl disulfide having the structural formula



where R_1, R_2, R_3 and R_4 are alkyl from 1 to 10 carbons, cycloalkyl from 5 to 7 carbons or aralkyl from 7 to 10 carbons; where R_1 plus R_2 or R_3 plus R_4 are said alkyl taken together with the adjoining nitrogen atom to form a heterocyclic ring of 4 to 10 carbons; or where R_1 plus R_2 or R_3 plus R_4 are oxyethyl and ethyl taken together with the adjoining nitrogen atom to form a morpholyl ring.

3. A method for the preservation of material normally subject to bacterial attack which comprises applying to such material at least one carbamoyl thiocarbamoyl disulfide having the structural formula



where R_1, R_2, R_3 and R_4 are alkyl from 1 to 10 carbons, cycloalkyl from 5 to 7 carbons or aralkyl from 7 to 10 carbons; where R_1 plus R_2 or R_3 plus R_4 are said alkyl taken together with the adjoining nitrogen atom to form a heterocyclic ring of 4 to 10 carbons; or where R_1 plus R_2 or R_3 plus R_4 are oxyethyl and ethyl taken together with the adjoining nitrogen atom to form a morpholyl ring.

4. The method as set forth in claim 1 in which the carbamoyl thiocarbamoyl disulfide is one in which the groups R_1, R_2, R_3 and R_4 are alkyl radicals having from 1 to 10 carbon atoms.

5. The method as set forth in claim 1 in which the carbamoyl thiocarbamoyl disulfide is dimethylcarbamoyl dimethylthiocarbamoyl disulfide.

6. The method set forth in claim 1 in which said disulfide is diethylcarbamoyl diethylthiocarbamoyl disulfide.

7. The method described in claim 1 wherein said material is cellulosic fibers.

8. The method described in claim 1 in which said material is paper.

9. The method described in claim 2 wherein said material is a living agricultural crop.

10. The method described in claim 2 wherein said disulfide is dimethylcarbamoyl dimethylthiocarbamoyl disurfide.

11. The method of combatting fungus on potato plants which comprises applying to such plants at least a fungistatic amount of dimethylcarbamoyl dimethylthiocarbamoyl disulfide.

12. The method of combatting the growth of fungus on cucumbers which comprises applying to the growing plants at least a fungistatic amount of dimethylcarbamoyl dimethylthiocarbamoyl disulfide.

13. The method of combatting fungus attack on growing corn which comprises applying to the plants a fungistatic amount of dimethylcarbamoyl dimethylthiocarbamoyl disulfide.

14. The method of combatting fungus attack of tomato plants which comprises applying to the growing plant at least a fungistatic amount of dimethylcarbamoyl dimethylthiocarbamoyl disulfide.

15. The method of combatting the attack of bacteria and fungus on seeds which comprises applying to said seeds a biostatic amount of dimethylcarbamoyl dimethylthiocarbamoyl disulfide.

16. In the control of scab on apple trees the step of spraying the trees with dimethylcarbamoyl dimethylthiocarbamoyl disulfide in an amount sufficient to inhibit the development of scab.

17. In the control on apple trees of fungus diseases of the fruit and foliage the step of applying to the trees dimethylcarbamoyl dimethylthiocarbamoyl disulfide.

18. In the control on stone fruit trees of fungus diseases of the fruit and foliage the step of applying to the trees dimethylcarbamoyl dimethylthiocarbamoyl disulfide.

19. The process of claim 18 wherein said stone fruit trees are cherry trees.

20. The process of claim 18 wherein said stone fruit trees are peach trees.

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ALBERT T. MEYERS, *Primary Examiner.*

S. J. FRIEDMAN, F. D. HIGEL, *Assistant Examiners.*