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NON-CORROSIVE NON-FLAMMABLE, LOW TOXIC ORGANOPHOSPHORUS DETOXIFYING SOLUTION

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1 Claim

ABSTRACT OF THE DISCLOSURE

A non-toxic, non-corrosive, non-flammable decontaminant for chemical and biological warfare agents, consisting of a solution of 50–70% by volume of monoethanolamine, 30–50% by volume of a glycol, 5–10% by volume of an active antiseptic chlorine compound selected from the group consisting of chloramine-T, dichloramine-T, and calcium hypochlorite solution and 0.5–1.0% by weight of the other ingredients of histidine.

The invention described herein may be manufactured and used by or for the Government of the United States of America for governmental purposes without the payment of any royalties thereon or therefor.

The invention relates generally to a nontoxic, noncorrosive, non-flammable decontaminant for chemical warfare and biological warfare agents.

As is well known, nerve gases are chemical compounds which block nervous activity, and thus either cause temporary loss of human capabilities or cause death in man and many other animals. Among the most lethal of such gases are the organophosphorus chemical warfare compounds of the respective classes known as "G agents" and "V agents."

Various attempts have been made to provide an anti-toxicant which will react with toxic gases to form compounds relatively harmless to military personnel in contaminated areas and of little reaction with stainless steels and other metals. Since World War I strong oxidizing and hydrolyzing agents have been used to decontaminate chemical warfare agents. Such decontaminants, however, have the disadvantages of being toxic to personnel and corrosive to equipment. A search therefore, was undertaken by the Army during World War II for a nontoxic, noncorrosive, non-flammable decontaminant. This effort resulted in the development of a decontaminating solution known in the art as DS2, consisting of 70% diethylenetriamine, 28% methyl Cellosolve and 2% sodium hydroxide. This solution is noncorrosive to most metals in the absence of water, but it is toxic and flammable. Its decontaminating capability has been found to rapidly deteriorate upon exposure to the atmosphere because the diethylenetriamine component reacts with atmospheric moisture and carbon dioxide to form an inactive compound.

It is therefore an object of this invention to provide a new and effective decontaminant for chemical warfare agents which can also be used to kill or inhibit biological warfare agents.

Another object is to provide an organophosphorus decontaminant which is nontoxic, noncorrosive and non-flammable.

Another object is to provide a decontaminating solution which involves noncorrosive, stable, active chlorine in a nucleophilic alkanolamine system.

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A further object is to produce a decontaminant comprising essentially monoethanolamine and active chlorine combined with various selected solvents, which decontaminant, upon application, will not irritate the skin and will not corrode metals and plastics.

Still another object is to provide a decontaminating solution that is effective in neutralizing the infectivity of biological warfare agents and in destroying the cholinesterase-inhibiting properties of organophosphorus compounds.

These and various other objects will be apparent from the following description and appended claims, with the understanding that the various ingredients used may be varied without departing from the invention.

The organophosphorus chemical warfare agents owe their effectiveness to their ability to inhibit and damage the enzyme cholinesterase, an enzyme which controls the hydrolysis of acetylcholine, a substance which is immediately involved in conduction and transmission of nerve impulses. Within seconds after exposure to a lethal dose of an organophosphorus compound, unconsciousness occurs and death ensues within minutes unless therapy is instituted.

A substance was therefore sought which would simulate the chemical agent-enzyme interaction. Such a substance, to be effective, must react with organophosphorus chemical warfare agents so as to block their capacity to react with cholinesterase.

Reactions considered were of two kinds:

(a) Those in which the reagent attacks a negative site, i.e., the reagent is electrophilic (positively charged ions). This is the case when an organophosphate (the reagent) phosphorylates the esteratic site of cholinesterase (the reactant). (b) Those in which the site attacked is positive, i.e., the reagent is nucleophilic (negatively charged ions).

With these two types of reactions in mind, a search was undertaken to discover or develop a nucleophilic agent that would attack the electrophilic site of organophosphorus compounds and in turn be phosphorylated.

Because of its availability, structure and cholinesterase-inhibiting properties, dimethyldichlorovinyl phosphate (DDVP) was chosen as the organophosphorus compound to be used in developing this decontaminating agent. Tetraethylpyrophosphate (TEPP) was also used for some evaluations because it is the most toxic organophosphorus insecticide readily available. Theoretically, any nucleophilic agent that attacks the electrophilic site of DDVP should also attack similar sites on organophosphorus chemical warfare agents.

A series of amines was tested for ability to neutralize activity of DDVP and TEPP against cholinesterase. Monoethanolamine and diethylenetriamine, decontaminants hereinafter referred to as MEA and DETA, respectively, diluted 50% with isopropyl alcohol proved to be the only amines effective in decontaminating DDVP. This alcohol was used as a carrier because it lowers the freezing point, is economical and it has biological warfare agent decontaminating properties. However, its flash point of 72° F. is low. Therefore, hexylene glycol possessing the same properties but with a flash point of 230° F. was substituted. MEA was chosen as the preferred decontaminant over DETA (the active ingredient in DS2) because it is less reactive with carbon dioxide, is less toxic and is equal in its ability to decontaminate DDVP.

The inhibition tests were conducted as follows with Table I showing reactions of various organophosphorus compounds with MEA solutions.

One-tenth milliliter of the mixture of organophosphorus

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compound and decontaminating solution was added to a 10 milliliter volumetric flask containing one milliliter of cholinesterase solution (2 milligrams per milliliter phosphate buffer, pH 6.8, 0.15 mole). The two solutions were mixed and allowed to react for 5 minutes at room temperature (25° C.). At the end of 5 minutes the volumetric flask was brought to the mark with indophenyl acetate solution (0.5 gram indophenyl acetate added to one liter of pH 6.8, 0.15 mole, phosphate buffer, mixed for 20 minutes, filtered and stored in the refrigerator), incubated for 10 minutes in a water bath at 37° C., and the reaction stopped by adding one drop of 1% DDVP in hexylene glycol. This solution was then compared with

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As shown in Table I, MEA solutions were not effective against VX chemical warfare agents (a specific agent in the V class) unless chloramine-T or calcium hypochlorite (HTH) was added to the solutions. Also, another active chlorine compound such as dichloramine-T may be employed in the MEA solution. Because the chlorine produces an electrophilic inductive effect, it has been found to increase the susceptibility of the organophosphorus VX compound to attack by the MEA nucleophilic solution thereby decontaminating the VX agent.

The following Table II, shows the effect of MEA decontaminating solution on animal skin not yet subjected to the presence of chemical or biological warfare agents:

TABLE II.—EFFECT OF MEA DECON SOLUTIONS ON ANIMAL SKIN

Animal	Area of cutaneous exposure	Ml. MEA sol.	MEA sol.	Exposure time, hr.	Results
Rabbit...	Back, clipped area 3 cm. ² .	1	60% MEA, 35% ethylene glycol, 5% HTH sol. (50 g. HTH/100 ml. H ₂ O).	3	The area that had been washed with water after the exposure period showed no signs of irritation. Small areas that were not washed showed signs of irritation and sores developed 5-6 days later.
Do.do.....	do.....	1	70% MEA, 30% ethylene glycol, 10% w./v. chloramine-T.	4	Do.

a known positive (uninhibited cholinesterase-indophenyl acetate solution) by visual color comparison or by optical density readings at 500 microns on a spectrophotom-

Table III shows that MEA solutions are effective in detoxifying DDVP and TEPP when applied to contaminated skin in a reasonable time after exposure.

TABLE III.—DECON EFFECTIVENESS OF MEA SOLUTIONS ON DDVP AND TEPP CONTAMINATED SKIN

TEPP	DDVP	Animals	Area of exposure	MEA applied	Results
	3 drops for 20 sec...	Mouse.....	Back, unclipped.	50% MEA, 50% ethylene glycol. 10 drops applied. Not washed off.	No toxic symptoms could be observed over a 7-day test period.
	3 drops for 30 minutes.	Mouse (control).	do.....	None.....	Mouse died 30 min. after exposure.
	8 drops for 2 hours.	Guinea pig..	Back, clipped 2 cm. ² area.	50% MEA, 50% ethylene glycol. Excess MEA-sol. applied. Severe symptoms had developed before applying decon solution.	Animal recovered in 48 hours.
	8 drops for 5 sec.....	do.....	do.....	50% MEA, 50% ethylene glycol. Excess MEA-sol. applied.	No toxic symptoms could be observed over a 7-day test period.
	2.5 ml. (700 mg./kg.) for 2 hours.	Rabbit.....	Back, clipped 3 cm. ² area.	70% MEA, 30% hexylene glycol, 1% by wt. histidine. 5 ml. applied after severe symptoms had developed.	Animal was found dead 18 hours after the application of MEA solution.
1.....	1.5 ml. (700 mg./kg.) for 3 hours.	Rabbit (control).	do.....	None.....	Animal died 3 hours after exposure.
1 ml. (700 mg./kg.) for 5 sec.		Rabbit.....	do.....	60% MEA, 30% hexylene glycol, 10% HTH sol. (50 g./100 ml. water). 5 ml. applied.	No toxic symptoms could be observed over a 7-day test period.

eter. A positive test is indicated by the change in color of indophenyl acetate solution from amber to brick-red, while no color change indicates a negative test.

Evidence given in the above table indicates that monoethanolamine solutions may possibly penetrate the skin and act as an antidote by competing with the cholin-

TABLE I.—REACTIONS OF GB, VX, DDVP, AND TEPP WITH MEA SOLUTIONS
[Reaction time: 5 minutes. Temperature: 25° C.]

Ml. decon solution	Ml. agent	MEA, percent by vol.	Hexylene glycol, percent by vol.	Ethylene glycol, percent by vol.	Isopropyl alcohol, percent by vol.	Histidine, percent by weight	Chloramine-T, percent by weight	HTH solution, 50 g./100 ml. water, percent by vol.	Destroyed cholinesterase-inhibiting property
1.....	1-DDVP	50			50				Yes.
1.....	1-DDVP	50	50			0.5			Yes.
1.....	1-TEPP	60	30				10		Yes.
5.....	1-TEPP	60	30					10	Yes.
1.....	1-DDVP	60	40			0.5	10		Yes.
5.....	1-DDVP	70	30			1.0			Yes.
5.....	1-DDVP	60	40			0.5	5		Yes.
2.....	1-GB	70	30			1.0			Yes.
5.....	1-VX	70	30			1.0			No.
5.....	1-VX	70	30				5		Yes.
5.....	1-VX	60	30					10	Yes.
5.....	1-VX	70		30			10		Yes.
5.....	1-VX	60		30				10	Yes.
2.....	2-DDVP	50		50					Yes.

esterase for the active (electrophilic) site on the DDVP molecule.

As shown in Table IV, studies conducted with plexiglass and various metals showed MEA and MEA-chlorine solutions to be noncorrosive under total immersion conditions.

There were no visible growths in any of the tubes containing the MEA-chlorine treated *Serratia marcescens* and tryptose broth. There were no *Serratia marcescens* colonies on any of the plates inoculated with decontaminating-culture mixture or dilutions thereof.

In order to be sure that the inoculated plates could sup-

TABLE IV —EFFECT OF MEA SOLUTIONS ON METALS AND PLASTIC

Material	MEA solution	Hours immersed at 25° C	Weight loss from corrosion	Visual observation
Aluminum 6061-T.....	60% MEA, 30% hexylene glycol, 10% HTH (1 g./2 ml H ₂ O).....	72	No weight loss..	No change.
Plexiglas Type II.....	do.....	¹ 168	do.....	Do.
Aluminum 6061-T.....	70% MEA, 30% hexylene glycol, 5% by weight Chloramine-T.....	168	do.....	Do.
Aluminum Type 2042-T3.....	70% MEA, 30% ethylene glycol, 10% Chloramine-T by weight.....	168	do.....	Do.
Do.....	60% MEA, 30% hexylene glycol, 10% HTH sol. (50 g. HTH/100 ml H ₂ O).....	¹ 168	do.....	Do.
Aluminum Type 7075-T6.....	70% MEA, 30% ethylene glycol, 10% Chloramine-T by weight.....	168	do.....	Do.
Stainless steel type 301.....	do. gg.....	168	do.....	Do.
Magnesium in aircraft.....	60% MEA, 40% hexylene glycol, 5% by wt. histidine, 5% Chloramine-T by weight.....	96	do.....	Do.
Plexiglas Type II.....	70% MEA, 30% ethylene glycol, 10% Chloramine-T by weight.....	168	do.....	Do.

¹ Solution gelled after exposure to the atmosphere for 96 hrs.

Tests of the decontaminating solution against bacteria were conducted, the results of which are shown below in Table V, in which 2 milliliters of a 24-hour tryptose broth culture of *Serratia marcescens* bacteria were placed in each of 3 sterile 20 millimeter test tubes. Two milliliters of the MEA-chlorine decontamination solution (60% MEA-40% hexylene glycol-0.5% histidine and 5% chloramine-T by weight) were added to two of these tubes. Two milliliters of sterile tryptose broth were added to the third tube. All tubes were shaken manually to insure thorough mixing of the contents. One and 5 minutes respectively after adding the decontaminant, the free chlorine was neutralized by adding 6 drops of 3% hydrogen peroxide to each tube. Each tube was shaken and 3 milliliters removed. One milliliter was added to a 9-milliliter sterile distilled water dilution blank, and two petri dishes were inoculated with 1 milliliter each. The petri dishes were poured immediately with tryptose agar. Serial dilutions in sterile distilled water were made, and the 10⁻² and 10⁻⁴ dilutions were plated in duplicate on tryptose agar. Ten to 15 milliliters of tryptose broth were added to the residual material in the first two test tubes to see whether *Serratia marcescens* would grow out.

The tube containing only culture and broth was diluted serially and 10⁻⁶, 10⁻⁷ and 10⁻⁸ dilutions were plated in duplicate.

The pH of the mixture, even diluted 1×10⁻⁴, was about 11 which is too high for optimum growth of most bacteria. Therefore, a second set of tubes was prepared and treated as above except that immediately before removing aliquots for dilution and plating, the pH was lowered to 8.5 or 9 by adding 1 to 2 milliliters of concentrated HCl.

All inoculated materials were examined after incubation for 96 hours at room temperature.

port growth of *Serratia marcescens*, all #2 plates, shown in Table V were streaked with a loopful of 48-hour tryptose broth culture of *Serratia marcescens*, and a tryptose agar slant was likewise streaked for comparison. Twenty-four hours later, the slant and all plates, except those containing the undiluted inoculum, showed a luxuriant, coral-colored growth along the line of the streak. There was no growth on the plates with the undiluted inoculum.

Hydrogen peroxide, which was used to neutralize free chlorine in these tests, is itself a decontaminating agent for bacteria. In order to determine whether exposure to hydrogen peroxide under these test conditions was destructive to *Serratia marcescens*, a test was run using 6 drops of 3% H₂O₂ against 2 milliliters of 24-hour tryptose broth culture for 1 minute. Plates of this mixture, undiluted and diluted 10⁻¹ and 10⁻² yielded confluent growth.

The bacteria and molds shown in Table V are contaminants. This high level of contamination is due to leaving the plates open for 15 to 20 minutes after pouring in order to allow evaporation of condensate from inside the lids of the plastic petri dishes.

It was concluded that (a) the MEA-chlorine solution mixed 1:1 with *Serratia marcescens* culture destroyed the organisms in one minute; (b) the H₂O₂ as used in these tests had little effect on the viability of *Serratia marcescens*; and (c) low dilutions of the MEA-chlorine solution were inhibitory to growth of *Serratia marcescens* and air-borne bacteria and molds on tryptose agar plates.

To be effective, a nontoxic, nucleophilic, decontaminating agent should act as an enzyme reactivating agent if applied within a short time after contamination. The extent of reactivation of organophosphate-inhibited serum cholinesterase that can be obtained with nucleophilic dephosphorylating agents depends upon how long the enzyme

TABLE V.—TESTS OF MEA-CHLORINE DECONTAMINATING SOLUTION AGAINST *SERRATIA MARCESCENS*

		Plate counts							
		Undiluted		10 ⁻²		10 ⁻⁴		10 ⁻⁷	
Plates.....		#1	#2	#1	#2	#1	#2	#1	#2
Materials:									
Bacteria broth.....								{67 Sm	47 Sm.
Decon 1 min.....	0.....	0.....		{4 b....	2 b....	4 b....	6 b....	{1 m	8 m.
				{17 m....	6 m....	10 m....	7 m....		
Decon 5 min.....	0.....	0.....		{1 b....	0 b....	0 b....	0 b....		
				{10 m....	3 m....	2 m....	2 m....		
Decon 1 min. HCl.....	0.....	0.....		{0 b....	0 b....	3 b....	4 b....		
				{3m....	1 m....	1 m....	1 m....		
Decon 5 min. HCl.....	Not plated..	Not plated..	0.....	{2 b....	1 b....	6 b....			
				{2 m....	2 m....	0 m....			

NOTE.—Bb=bacteria; m=molds; Sm=*Serratia marcescens*.

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and inhibitor have been left in contact. It has been proposed that the phosphorylation of cholinesterase leads initially to "phosphorylated enzyme I," which gradually changes to Type II. Type I (histidine-phosphorylated) can be readily dephosphorylated by appropriate nucleophilic agents, but not Type II (serine-phosphorylated). The system appears to be a step forward in the development of a universal decontaminating solution. This system appears stable, i.e., it has available chlorine after a two-month storage period in a transparent bottle with numerous short atmospheric exposures.

From the foregoing, it is apparent that a nonflammable, noncorrosive, low toxic MEA-chlorine solution has been developed that is effective in sterilizing *Serratia marcescens* (1.2×10^8 /milliliter), in 1 minute in a 1:1 ratio, and destroying in 5 minutes the cholinesterase-inhibiting properties of DDVP, TEPP, GB, and VX in a 1:5 ratio.

Other uses of the detoxifying solution are as a disinfectant and as a decontaminant of other organophosphorus compounds, such as insecticides.

Obviously, many modifications and variations of the present invention are possible in the light of the above teachings. It is therefore to be understood that within the scope of the appended claims the invention may be practiced otherwise than as specifically claimed.

What is claimed is:

1. A non-corrosive, non-flammable, low toxic nucleo-

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philic solution for use in detoxifying organophosphorus compounds and neutralizing the infectivity of biological warfare agents comprising essentially:

- 50-70% by volume of monoethanolamine;
- 30-50% by volume of a glycol;
- 5-10% by volume of active antiseptic chlorine compound selected from the group consisting of chloramine-T, dichloramine-T and calcium hypochlorite solution; and
- 0.5-1.0% of the weight of the above ingredients of histidine.

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