DEPARTMENT OF THE ARMY
UNITED STATES ARMY INTELLIGENCE AND SECURITY COMMAND
FREEDOM OF INFORMATION/PRIVACY OFFICE
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December 1, 1999

Freedom of Information/
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Mr. John Greenewald, Jr.

Dear Mr. Greenewald:

Reference is made to your letter of June 28, 1999, to the US Army Foreign Science and Technology Center (FSTC), requesting a copy of the document titled The Modern Possibilities of Prevention and Therapy in Animal Poisioning with Different kinds of Nerve War Gases, number 862059, dated December 6, 1969, under the Freedom of Information Act. This document was provided to us by the National Ground Intelligence Center (NGIC), which has replaced the FSTC and received on November 23, 1999.

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If you have any questions regarding this action, feel free to contact Mr. Smith at (301) 677-3847. Please refer to case #263F-00.

Sincerely,

for Russell A. Nichols
Chief, Freedom of Information/
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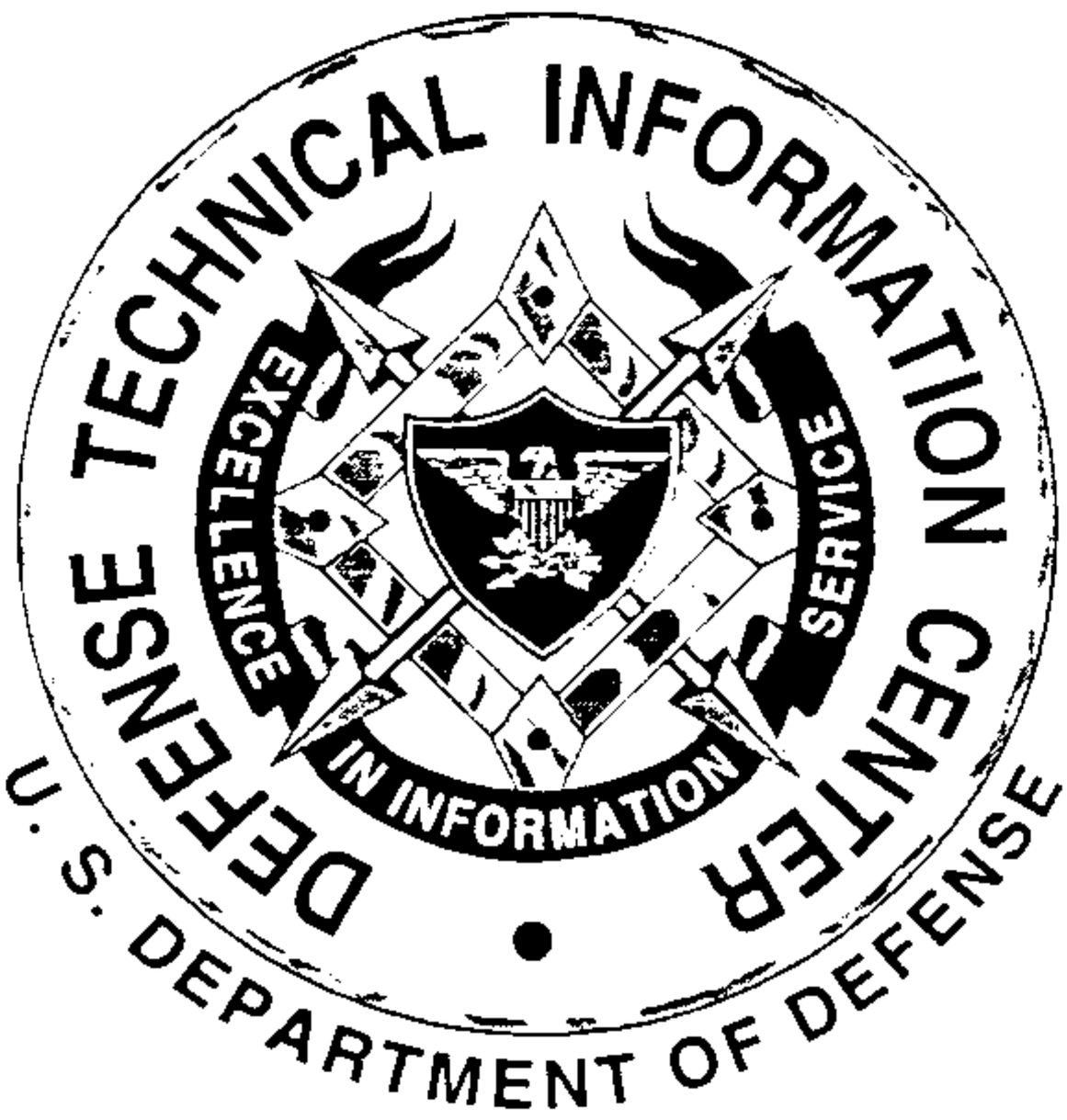

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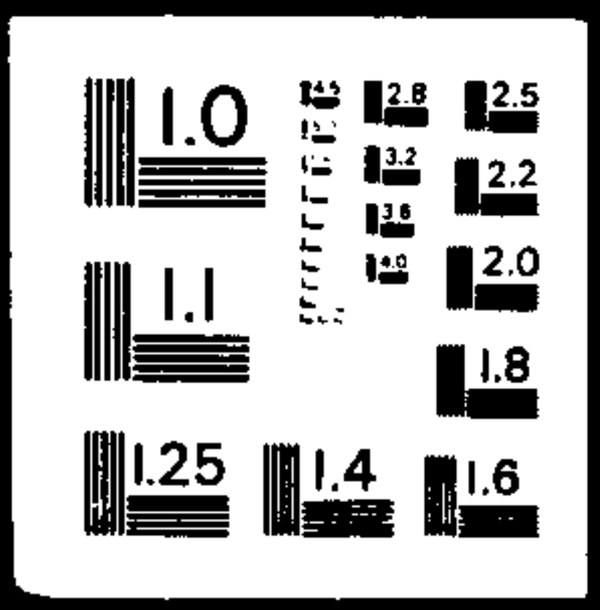

FSTC-HT-23-096-70

U.S. ARMY FOREIGN SCIENCE AND TECHNOLOGY CENTER

THE MODERN POSSIBILITIES OF PREVENTION AND THERAPY IN ANIMAL POISONING WITH DIFFERENT KINDS OF NERVE WAR GASES

COUNTRY: YUGOSLAVIA

TECHNICAL TRANSLATION

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FSTC-HT-23-096-70

THE MODERN POSSIBILITIES OF PREVENTION AND THERAPY IN ANIMAL POISONING WITH DIFFERENT KINDS OF NERVE WAR GASES

by

Bogdan Bosković and Radovan Jović

Source: VOJNOSANITETSKI PREGLED
No. 4, 1969, pp. 179-182.
Serbo-Croatian

Translated for FSTC by ACSI

According to the specific features of the chemical structure, the mechanisms of influence on living organism, and the tactical-technical characteristics, we can at present distinguish three groups of potential military nerve poisons: trilons, "Vx" poisons and "F" poisons.

Trilons are already well-known conventional military nerve poisons (NBot), synthesized over the 1936-1943 period in Germany. Tabun, sarin, and soman were extensively investigated in the post-war period and the ineffectiveness of modern therapy in soman poisoning gives this poison a particular significance in the theoretical and practical sense.

In the scientific literature there are no data available on the chemical structure of "Vx" poisons. Semenov (reference 17) describes "Vx" poisons as poorly vaporizable fluids without odor or taste which are allegedly one hundred to a thousand times more toxic than all Bot. known up to now. They penetrate the skin very easily if used in the form of liquid aerosols or vapors. Drops invisible to the naked eye falling on human skin suffice to cause death. These gases are particularly dangerous when they are used in the aerosol form and when they get into the organism through the respiratory organs. The symptoms of poisoning by Vx poisons through the skin are manifested in fasciculation (trembling) of the muscles at the site of contamination, difficult breathing, increased salivation, cramps, and paralysis of respiratory and other transverse-streaked musculature. It should be noted that all of these symptoms occur late, sometimes even several hours after the time of contamination, in contrast to the other NBot, in which the symptoms appear after several minutes or at the very latest one hour after the time of contamination, depending upon the doses of the poison used. It should be noted that the danger of contamination by Vxagents lies in the fact that personnel can be poisoned even if there is a protective mask but they are not covered by protective clothing.

According to Z. Sinenfeld (3) the Vx should be assigned to the group of phosphorylthiocholines, which were already synthesized by Ghosh and Newman in 1955 (11). These are organic phosphorous compounds which contain thiocholine in the molecule. The toxicity of these compounds and their anti-

cholinesterase activity are ten times greater than those of sarin (1, 6, 20).

Fluorophosphorycholines (F poisons) were synthesized by Swedish scientists (19). They are choline derivatives, but contain sarin residue in the molecule. In their structure these compounds are similar to the natural substrate of cholinesterase, acetylcholine (ACh), and, according to the data of Tammelin, are more toxic than sarin. These are powerful inhibitors of cholinesterase (ChE) and the enzyme which is inhibited by the units in vitro PAM-2Cl cannot reactivate (9). These poisons easily penetrate the skin. According to Bowers et al. (4), a compound of this type after percutaneous administration in humans causes a psychic change in the form of anxiety, psychomotor depression, mental disturbance, and insomnia with hallucinations. In intensity all of these symptoms were in correlation with the degree of ChE inhibition in the blood. No data are available in the scientific literature concerning the possibilities of protection from poisoning with these compounds.

According to the data of Semenov (17) the toxicity of presently known chemical warfare agents for humans exceeds the the toxicity of hyperite by several tens of thousands of times (Table 1).

Table 1

Percutaneous toxicity of chemical warfare agents in man(1)

Poison	Lethal dosage in mg	Toxicity index
Hyperite	4000 to 5000	1.0
Trilons	100 to 200	30.0
Model "F" poisons	2 to 10	734.0
Model "Vx" poisons	0.1 to 0.2	30,000.0

(1) The table gives the minimum lethal dosages for a man weighing 70 kg, as calculated on the basis of the data of Semenov (17).

Since in scientific literature there is very little information on the possibilities of protection and therapy in poisoning by phosphorthiocholines and fluorophosphorylcholines (model "Vx" poisons) and since therapy and prevention in poisoning with soman (a representative of the conventional NBot) are ineffective, it was of interest to investigate these possibilities. This paper shows the effectiveness of modern antidotes (oximes and cholinolytics) in prophylaxis of poisoning of animals by various chemical warfare agents.

Materials and methods

1. Substances. As a representative of trilon we used pinacholiloxy-methyl-phosphoryl-fluoride (soman), and as a representative of the model "F" poisons we used methylfluorophosphorylcholine (MeFfH); as representatives of the model "Vx" poisons we used diethyl-S-(2-diethylamino-ethyl) thiophosphate-oxalate (amiton), diethyl-S-(2-diethylamino-ethyl) thiophosphate-methylsulphomethylate (amiton-4), O-ethyl-S-(2-diethylamino-ethyl methylthiophosphonate (edemo) and etoxy-2-ethyl-thioethyl-thiomethyl-phosphin oxide-methylsulphomethylate (GD-42). For protection we used: atropine sulphate and bis (4-hydroxyminomethyl-piridine (1) methyl)-ether dichloride (toxogonin ® [2] Lü16, Bi16).

2. Acute toxicity and protective effect of atropine and oxime. For these experiments we used mice of both sexes weighing from 15 to 30 grams. The toxicity of the investigated organophosphorous compounds and the protective effect of atropine, toxogonin, and their combinations were determined according to the method of Behrens and Körber (2). After calculating LD_{50} we took the result of survival of the animals 24 hours after injection of the poison.

For these experiments we used fresh solutions of these substances in a 0.9% solution of NaCl. The poisons were injected subcutaneously and the antidotes intraperitoneally in a dosage of 10 ml/kg animal weight.

3. Determination of activity of ChE in the blood of rats in vivo. Cutting the tail of rats we first of all took 0.04 ml of blood to determine the normal (control) activity of ChE. Then the rat was administered the poison subcutaneously in the dosage of 2/3 of the previously determined LD_{50}. Exactly 30 minutes after the rats were injected with the poison the tail was once again cut and blood was taken to analyze the degree of ChE inhibition. Immediately after taking this blood the rats were injected with toxogonin (25 mg/kg) intraperitoneally. Five minutes after the toxogonin injection the rats were killed and blood was taken for measuring the ChE activity. The percentage of ChE reactivation was calculated according to Childs (8).

The activity of ChE was determined by the electrometric method according to Michel, as modified by Stevanovich and Jovich (18). Each result in the table represents the mean value of ChE activity obtained in five rats. The mean values and the standard error were calculated according to Eramens(10).

Results

The acute toxicity of the organophosphorous compounds (OPC) investigated is shown in table 2.

2) Toxogonin ® trademark of the firm Merck AG, Darrstadt (Germany).

Table 2

Acute toxicity of nerve poisons (mouse, s. c.)

Poison Model	OPC	$LD_{50} \pm S_x$ mg/kg
"TRILONS"	SOMAN	0.160 ± 0.02
"P" Poisons	MeFPH	0.150 ± 0.04
"Vx" poisons	AMITON	0.220 ± 0.04
"	AMITON-4	0.057 ± 0.006
"	EDEMO	0.022 ± 0.004
"	GD-42	0.032 ± 0.005

The acute toxicity in mice, as determined by the average lethal dosages (LD_{50}), shows that the values for soman and MeFPH are approximately the same and that the toxicity of Vx poisons is 3 to 7 times greater than that of the first two.

In the group of model Vx poisons there is a considerable difference in toxicity among the individual compounds. Thus, for example, edemo is exactly 10 times more toxic than amitone whose toxicity by quaternization (amitone-4) increases severalfold. Regardless of these differences, according to the classification by Loomis (15) all of these compounds are among the extremely toxic substances.

The protective effect of atropine, toxogonin, and their combinations (table 3) shows that these antidotes are not equally effective in protecting animals poisoned with various OPC.

Atropine sulphate protects 50% of the animals poisoned with a 1.0 (GD-42) to 3.6 LD_{50} dosage of poison (amiton); this represents a relatively weak protection. On the other hand, the protective strength of toxogonin varies to a considerably greater degree depending on the poison. It provides a relatively weak protection, just as does atropine, in mice poisoning with amiton and amiton-4 (protective index is 52 as against 196).

The combined utilization of atropine and toxogonin affords practically no protection in poisoning of mice with soman and MeFPH, but provides good protection in poisoning with edemo and GD-42, and exceptional protection in poisoning with amiton (protective index of 691) and amiton-4 (protective index of 388). The current treatment of poisoning with organic phosphates

virtually excludes simultaneous utilization of symptomatic (atropin) and causal (toxogonin) antidotes. Our results show the great effectiveness of this combination in poisoning with some OPC.

Table 3

Protective effects of atropine, toxogonin, and their combinations in s. c. poisoning of mice with nerve BO_t [1].

OPC	LD_{50} / mg / kg / with protection			Protective index [2]		
	ATROPINE /A/	TOXOGONIN /T/	ATROPINE + TOXOGONIN	A	T	A+T
SOMAN	0.208	0.208	0.230	1.3	1.3	1.4
MeFPH	0.180	0.195	0.200	1.2	1.3	1.3
AMITON	0.790	11.400	152.00	3.6	52	691
AMITON-4	0.170	11.200	22.20	3.0	196	388
EDEMO	0.055	0.076	0.58	2.5	3.5	26
GD-42	0.030	0.320	0.91	1.0	10	28

1) Atropine (10 mg/kg, i.p.), toxogonin (25 mg/kg, i.p.), and mixtures of atropine and toxogonin were injected 10 minutes before the poison.

2) Protective index = $\frac{LD_{50} \text{ with protection}}{LD_{50} \text{ without protection}}$

The reactivating effects of toxogonin in regard to the activity of cholinesterase in the blood of rats inhibited by the compounds investigated in vivo are not the same (Table 4).

The results from Table 4 show that toxogonin does not reactivate cholinesterase inhibited with soman and MeFPH, i.e., in those poisons in which toxogonin and combinations of toxogonin and atropine provide hardly any protection. On the other hand, toxogonin showed an extraordinary and rapid reactivating effect in comparison to cholinesterase inhibited by the group of "Vx" poisons, the reactivating effect ranging from 87.9% (edemo) to 100% (GD-42).

Table 4

Reactivating effects of toxogonin relative to ChE in the blood of rats inhibited by nerve RO_t in vivo

POISON /mg/kg, s. c./	ChE activity (in percentage of control values)		Percentage of reactivated ChE
	30 min. after inhibitor	5 min. after toxogonin	
SOMAN (0.06)	11.4 ± 2.7	9.4 ± 3.0	0
MePfH (0.10)	24.1 ± 1.0	27.6 ± 1.9	4.0
AMITON (0.10)	34.2 ± 4.9	95.0 ± 5.0	92.4
AMITON-4 (0.03)	39.7 ± 5.4	94.8 ± 3.0	91.6
EDEMO-3 (0.01)	42.1 ± 3.2	93.4 ± 3.0	87.9
GD-42 (0.02)	30.4 ± 4.6	100	100.0

Discussion

The high toxicity and exceptionally powerful anticholinesterase activity of "Vx" and "F" poisons is due to the fact that these compounds are similar in their structure to acetylcholine, the natural substrate of cholinesterase (20).

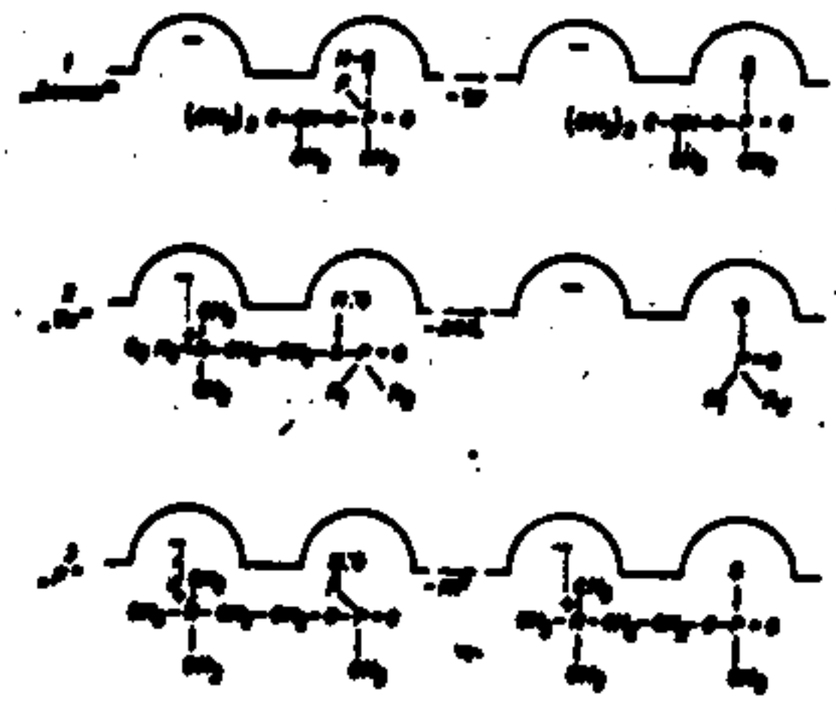
Particularly toxic are quarternary compounds (amiton-4, GD-42). The orientational and inductive effects are attributed to the cation head of these quarternary compounds. The orientational effect is related to the creation of ion bonds between the cation head of the molecule of the OPC and the anion side of the cholinesterase (see diagram), whereby the molecule of the enzyme, and the inductive effect is related to the increase in the phosphorizing activity of the inhibitor (22). However, regardless of the difference in toxicity between the individual groups of OPC, the mechanism of their toxic effect is basically the same. All of them irreversibly inhibit AChE; this is of essential significance for conveying the nerve impulses. As a result of the inhibition of AChE there occurs an accumulation of acethylcholine in the nervous system, tissues, and organism, so that poisoning with these compounds actually represents poisoning with endogenic acetylcholine.

The protective effects of the combination of atropine and toxogonin in poisoning of animals with soman and MeFfH are insignificant. This agrees with the findings of Loomis and coworkers (14) and Heilbronn and coworkers (12). In the scientific literature there are no data on the possibilities of protection in the poisoning of animals with MeFfH.

In poisoning with OPC of the phosphorylthiocholine type (model substances for "Vx" poisons) the protective effects of the combination of atropine and toxogonin are very good. Coleman and coworkers (7) and Lehman and coworkers (13) also obtained excellent protection in mice with amiton poisoning, or phospholine poisoning, which also belong to the group of model "Vx" poisons. The good protective power of atropine and oximes in poisoning with phosphorylthiocholines can be explained by the easy reactivation of the cholinesterase by the oximes, as can also be seen from our results (Table 4).

The reactivating effect of toxogonin with regard to cholinesterase inhibited by the OPC investigated can be explained by the interaction between these poisons and ChE, as is shown in the diagram below.

Anion side Ester side After interaction



The reaction between soman and AChE (diagram, number 1) leads to the renoval of the fluorine (F), and the cumbersome pinacholyloxy group prevents the connection of the toxogonin on the anion side AChE (5), thereby also the reactivating of the enzyme of the inhibited soman. MeFfH reacts with AChE as a "bifunctional" unit, i.e. after renoval of the fluorine. It also blocks both the anion and ester side of the enzyme (20). This prevents connection of the toxogonin on the anion side and reactivation of the inhibited enzyme (diagram - number 3).

According to Tammelin (20, 21) the reaction between phosphorylthiocholine and AChE develops from the release of thiocholine and the simultaneous phosphorizing of the ester side of the enzyme (diagram - number 2).

Since the anion side of the AChE inhibited by the phosphorylthiocholines remains free, the toxogonin can be bound to this center of the enzyme. This permits effective orientation of the oximes in the direction of the phosphorous group of the inhibitor, as well as successful reactivation.

Data from the scientific literature, as well as our results, show that there is basically a good correlation between the protective and reactivating effects of oximes, although the protection in poisoning with some OPC can also occur without reactivation of cholinesterase by the oximes (16).

Conclusion

The combined utilization of atropine and reactivator cholinesterase effectively protects animals poisoned with phosphorylthiocholines (model "Vx" poisons).

The prophylaxis of poisoning with soman (trilons) and methyl-fluorophosphorylcholine ("F" poisons) is ineffective and presents a problem which requires new efforts for the purpose of finding suitable antidotes.

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13. ABSTRACT

An investigation has been made of the acute toxicity of soman, methylfluorophosphoryl choline (MePfH), amiton, amiton-4, edemo, and Gd-42, and of the protective effect of atropine, toxogonin, and combinations thereof in subcutaneous poisoning of mice. The reactivating effect of toxogonin in relation to ChE in the blood has been investigated in rats, after administration of the poisons in question. Edemo and Gd-42 have been found to be the most toxic, and amiton the least toxic. The best protective effect of atropine and toxogonin in combination has been achieved in poisoning with amiton and amiton-4, a significantly lesser effect in poisoning with edemo and Gd-42, and the least protective effect in poisoning with soman and MePfH.

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14. KEY WORDS	LINK A		LINK B		LINK C	
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Nerve poisons						
Soman						
Methylfluorophosphoryl choline						
Amiton						
Amiton-4						
Edemo						
Gd-42						
Atropine						
Toxogonin						

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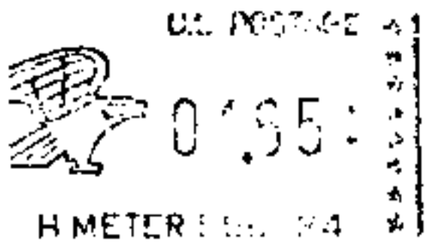

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SEP 7 1999

Mr. John Greenewald, Jr.

Dear Mr. Greenewald:

This is in reply to your letter dated June 28, 1999, which was received in this office August 31, 1999, requesting information under the Freedom of Information Act (FOIA). Under Department of Defense rules implementing the FOIA, published at 32 CFR 286, your request was categorized as **"other."**

Release of document AD 862059, *The Modern Possibilities of Prevention and Therapy in Animal Poisoning with Different Kinds of Nerve War Gases*, dated November 6, 1969, may only be performed by the appropriate Army releasing activity. Therefore, your request has been forwarded to the Army office below for processing and direct response back to you.

Commander
U.S. Army Freedom of Information
and Privacy Acts Office
7798 Cissna Road, Suite 205
Springfield, VA 22150-3197

Please direct all future correspondence related to document AD 862059 to the above organization.

Please understand that other members of the public may submit a FOIA request for copies of FOIA requests received by this office, or the names of those who have submitted requests. Should such occur, your name, and, if asked for, a copy of your request will be released; however, your home address and home telephone number will not be released. You may be contacted by other private citizens who have obtained your name by using such a request. However, correspondence from the Defense Department about your request will be on official letterhead.

To date, no assessable fees have been incurred for services from the Defense Technical Information Center related to the processsing of this request. If you have any questions, please call me at (703) 767-9194.

Sincerely,

Kelly D. Akers

KELLY D. AKERS
FOIA Program Manager

DEPARTMENT OF THE ARMY
FREEDOM OF INFORMATION AND PRIVACY ACTS OFFICE
7798 CISSNA ROAD
SPRINGFIELD, VIRGINIA 22150-3197

REPLY TO
ATTENTION OF

October 7, 1999

FOIA #99-1611

Mr. John Greenewald, Jr.

[redacted]

Dear Mr. Greenewald:

This letter responds to your Freedom of Information Act request, dated June 28, 1999, addressed to the Defense Technical Information Center (DTIC). That agency forwarded your request to this office on September 7, 1999, for appropriate referral within the Department of the Army. Your request is for a copy of Army Document (AD) Number 862059, entitled "The Modern Possibilities of Prevention and Therapy in Animal Poisoning With Different Kinds of Nerve War Gases," dated December 6, 1969.

As a matter under its purview, we have referred your request, along with a copy of that document obtained by this office, to the following agency for review, release determination, and direct reply to you:

National Ground Intelligence Center
220 7th Street N.E.
Charlottesville, VA 22902-5396
Telephone: (804 980-7603

If this office can be of further assistance to you, please contact Phyllis Walls, of my staff, or myself, at the above address. The telephone number is (703) 806-7137.

Sincerely,

Rose Marie Christensen

Rose Marie Christensen
Chief, Freedom of Information
and Privacy Acts Office