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MLSR No. 75

A COMPARISON OF TOXIC PROPERTIES OF THE V-AGENTS WITH GB (U)

Francis N. Marzulli

December 1955

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Medical Laboratories Special Report No. 75

A COMPARISON OF TOXIC PROPERTIES OF THE V-AGENTS WITH GB (U)

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Medical Laboratories Special Report No. 75

A Comparison of Toxic Properties of the V-Agents With GB (U)

ABSTRACT (S)

Intravenous and percutaneous toxicity data obtained using rabbits are analyzed to compare V-agents and GB with respect to inherent toxicity and factors related to percutaneous effectiveness, such as the cumulative percutaneous action of a single application and the rate of percutaneous traversal.

The following summarizes the findings of this analysis:

1. Inherent toxicity (LD50 I.V. at 24 hr.) - EA 1517 and GB > EA 1511 and EA 1508.
2. Percutaneous toxicity (LD50, decontamination at 24 hr. and at 2 min.).
 - a. 24 hr. - EA 1517 > EA 1511 > EA 1508 > GB.
 - b. 2 min. - EA 1511 > EA 1517 > EA 1508 > GB.
3. Agent effectiveness (LD50 at 15 min.).
 - a. Inherent - GB and EA 1517 > EA 1511 > EA 1508.
 - b. Percutaneous - EA 1517 > GB > EA 1511 > EA 1508.
4. Cumulative Percutaneous Action $\left(\frac{\text{percut. LD50 at 15 min.}}{\text{percut. LD50 at 24 hr.}} \right)$ - EA 1511 > EA 1508 > EA 1517 > GB.
5. Percutaneous Traversal (skin surface to blood stream) $\left(\frac{\text{I.V. LD50}}{\text{percut. LD50}} \right)$.
 - a. Percent traversing at 2 min. - EA 1511 > EA 1508 > EA 1517 > GB.
 - b. Percent traversing at 24 hr. - EA 1511 > EA 1517 > EA 1508 > GB.
6. Lethal Conversion factor from 24 hr. to 15 min. $\left(\frac{\text{LD50 I.V. at 15 min.}}{\text{LD50 I.V. at 24 hr.}} \right)$.
GB and EA 1517, 1.2; EA 1511, 1.6; EA 1508, 2.6.

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Medical Laboratories Special Report No. 75

A Comparison of Toxic Properties of the V-Agents With GB (U)

I. BACKGROUND.

(C) The recent development of a series of highly-toxic, percutaneous anticholinesterase compound (1, 2, 3), the so-called V-agents, has necessitated the evaluation of their multifaceted CW potential. Unlike GB, these agents are relatively nonvolatile, and when applied to the skin as a single application continue to penetrate over a period of many hours. This physical attribute provides them with a cumulative percutaneous toxic action and is intimately associated with their unique property of being nearly as toxic by the percutaneous as by the intravenous route.

(C) Most of the V-agents provide a lethal potential when applied to the skin in quantities which would be virtually impossible to detect. Their primary limitation when so used is the fact that death is delayed. On the other hand, larger quantities enable some of them to match the speed of the percutaneous action of GB; such quantities are still only a fraction of the amount of GB required.

(C) Up to the present, these laboratories have been concerned with obtaining information on the toxicology of the V-agents, and attention has been directed toward determinations of the median lethal doses in a variety of animals by several routes of administration. The present report is intended to review some of the published and unpublished toxicity data in order to assess more completely such factors as agent effectiveness, cumulative percutaneous action (as described above), and rate of penetration of the V-agents.

II. EXPERIMENTAL.

A. Data.

(C) In Table IA, data are presented giving (a) the median lethal dose for intravenously administered GB, EA 1511, EA 1517 and EA 1508 based upon deaths occurring during a 24-hour period; (b) the median lethal dose for these same agents administered percutaneously and decontaminated with saturated calcium hypochlorite at 24 hr. or 2 min., based upon deaths occurring during a 24-hour period; and (c) the median lethal dose for these agents administered percutaneously or intravenously based upon deaths occurring up to 15 min. after administration of agent. Data for (a) and (b) have been reported recently for EA 1511, EA 1508, and EA 1517 (1, 2, 3) and for GB (4, 5). Data for (c) covering GB have been reported previously (4) whereas those for V-agents are reported here for the first time. All the data in this report were obtained with rabbits (clipped for percutaneous studies), and involve this species limitation.

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B. Analysis.

1. Inherent Toxicity.

(C) In Table IA (item a) it can be seen that for the rabbit, EA 1517 and GB are inherently more toxic than EA 1511 and EA 1508, for the intravenous toxicity of the former compounds is two or more times greater than that of the latter two.

2. Percutaneous Toxicity.

(S) When left (uncovered) on the bare, clipped skin of the rabbit for 24 hr., these materials (item b, Table IA) have approximately the following relative potencies when compared with GB, an arbitrary reference standard assigned the value 1.

<u>Agent</u>	<u>LD50</u> (mm ³ /kg.)	<u>Potency</u>
EA 1517	0.04	900
EA 1511	0.08	450
EA 1508	0.16	225
GB	36.3	1

(C) Prevention of free evaporation would be expected to narrow the difference in percutaneous potency between GB and the V-agents. This effect is seen to some extent where effects of volatility are largely cancelled by decontamination a short period after application of agent.

(S) When the agents are left (uncovered) on the bare, clipped skin of the rabbit for only 2 min. (item c, Table IA), their relative potencies are as follows:

<u>Agent</u>	<u>LD50</u> (mm ³ /kg.)	<u>Potency</u>
EA 1511	0.89	420
EA 1517	6.3	64
EA 1508	11.0	35
GB	385	1

(U) Reversal of the positions of EA 1511 and EA 1517 in this series may be related to the efficiency of the decontaminant in removing the agents at 2 min. rather than to differences in their relative penetrating powers.

3. Agent Effectiveness.

a. Inherent.

(S) CW agent effectiveness is defined as the ability to

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produce incapacitation and death within specified periods of time. Thus, a practical and important measure of CW agent effectiveness is the dose required to produce 50% lethality within a period of 15 min. after administration of agent. The intravenous doses of the 4 agents under consideration (Table IA, item e) which achieve this rate of lethality are as follows:

<u>Agent</u>	<u>LD50</u> (mm ³ /kg.)	<u>Potency</u>
GB	0.018	1.0
EA 1517	0.019	0.9
EA 1511	0.060	0.3
EA 1508	0.130	0.1

The selection of the first 15 min. after injection as an observation period (LD50 at 15 min.) is based upon the following considerations:

(U) (1) Death within 15 min. would be consistent with military interest in a chemical agent which provides rapid incapacitation; i.e., the intravenous dose of GB which kills in 15 min. incapacitates in about 1 min.

(C) (2) Death from percutaneous administration of EA 1511 and EA 1508 cannot be accomplished in less than 15 min. with many times the dose required for practical field use.

(U) The aforementioned comparisons are valid because the general mechanism of action of all the agents under comparison is the same. The use of the intravenous route for obtaining a measure of the inherent agent effectiveness has a firm practical basis in connection with chemical warfare agents since effects by this route most closely approximate those obtained by inhalation.

b. Percutaneous.

(S) A major interest of the Chemical Corps is the practical problem of assessing the percutaneous effectiveness of the V-agents in comparison with that of GB. The absolute amounts of material which must be applied to the skin (Table IA, item d) in order to kill 50% of the rabbits in 15 min. are as follows:

<u>Agent</u>	<u>LD50</u> (mm ³ /kg.)	<u>Potency</u>
EA 1517	(7.0)	10
GB	66.7	1
EA 1511	316	0.2
EA 1508	316	0.2

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4. Cumulative Percutaneous Action.

(U) Since cumulative percutaneous toxic action (as described in paragraph 1, page 3) is characteristic of the V-agents when these materials are applied to the skin, it is important to provide a numerical measurement of this factor. The ratio $\frac{\text{LD50 percut. (15 min.)}}{\text{LD50 percut. (24 hr.)}}$ should

describe the extent of cumulative percutaneous action. Involved herein are (a) volatility, (b) rate of penetration through the skin, (c) rate of uptake by the blood stream, (d) inherent effectiveness, (e) rate and degree of detoxification or elimination, and possibly other factors.

(S) A comparison of the relative positions of the agents under study (Table IB, item d/b) for cumulative percutaneous toxic action is as follows:

<u>Agent</u>	<u>Ratio d/b</u>	<u>Potency</u>
EA 1511	(4000)	2222
EA 1508	(2000)	1111
EA 1517	(180)	100
GB	(1.8)	1

(C) Thus cumulative, percutaneous action in this series of comparisons is greatest with EA 1511 and EA 1508 and least with GB. EA 1517 occupies a somewhat intermediate position.

(U) From what has already been said, it is apparent that decontamination is most effective against those agents whose percutaneous toxicity is based to a great degree upon cumulative percutaneous action.

5. Percutaneous Traversal (Skin Surface to Blood Stream).

(U) A practical measure of the fraction applied (uncovered) to the skin which effectually reaches the blood stream can be made by comparing the intravenous toxicity figure with the percutaneous, as expressed by the ratio $\frac{\text{LD50 I.V.}}{\text{LD50 percut.}}$. In order to obtain a measure of the amount traversing this route at 2 min. and at 24 hr., LD50's based upon decontamination at 2 min. and at 24 hr. were used, deaths being recorded over a 24-hour period in both cases.

(S) The relative amounts of material effectively traversing from the skin to the blood stream in 2 min. are given for these agents as follows (Table IB, item a/c):

<u>Agent</u>	<u>Ratio a/c</u>	<u>Potency</u>
EA 1511	(0.02)	1000
EA 1508	(0.0045)	100
EA 1517	(0.002)	50
GB	(0.00004)	1

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(S) The relative amounts of material effectively traversing this course in 24 hr. are as follows (Table IB, item a/b):

<u>Agent</u>	<u>Ratio a/b</u>	<u>Potency</u>
EA 1511	(0.45)	950
EA 1517	(0.35)	740
EA 1508	(0.31)	660
GB	(0.00043)	1

(C) As has been stated previously, the 2-minute figures are complicated by the unknown effectiveness of the decontaminant. Other differences in these 2 series of potencies involve differences in factors such as volatility, penetration, absorption and elimination for 2 min. vs. 24 hr. It is clear from the 24-hour potency series that a much greater fraction of EA 1511, EA 1517 and EA 1508 is eventually absorbed into the blood stream than of the highly volatile GB, when these materials are applied uncovered to the skin.

6. Relation Between Dose and Lethal Rate.

(C) A comparison of the 15-minute and 24-hour intravenous LD50's provides some index of the amount of material required to hasten death from 24 hr. to 15 min. (Table IB, item e/a). The ratio $\frac{\text{LD50 at 15 min.}}{\text{LD50 at 24 hr.}}$ is 1.2 for GB and EA 1517, 1.6 for EA 1511 and 2.6 for EA 1508. Thus little more GB (or EA 1517) is required to kill in 15 min. than is required to kill in 24 hr. However, over twice as much EA1508 is required to accomplish the same hastening effect. This factor should be taken into consideration in preparing tables of munitions expenditures.

7. Penetration.

(U) In order to obtain a measure of these agents' ability to penetrate the skin, it is necessary to exclude volatilization of the compound. This involves covering the contaminated area of skin. This provides an absolute measure of penetration potentialities of an agent. Although this has been planned, it has not been done at these laboratories for any of these agents, so that this type of comparison cannot be made. Militarily, these determinations have little practical value, for free evaporation cannot be prevented in the field. Hence for chemical warfare, the percutaneous toxicity of materials applied to the skin and left uncovered is the more useful determination.

III. ACKNOWLEDGMENTS.

(U) Acknowledgments are extended to the following members of the Field Toxicology Branch for their cooperation in conducting toxicity studies solely for the purpose of this report: Lillian Conn, Joseph Wiles, Ogle B. Cope, Paul Hott, Bronon Bahosh, Mathew McMinn, Richard G. Horton.

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JP

Table I (S)

A.

Comparison of the median lethal doses of various V-agents and GB, when administered intravenously and percutaneously to rabbits. Data are based upon deaths recorded at 15 min. or 24 hr.

Item	Route	Experimental Conditions		Median Lethal Dose			
		Decontamination	Observation Period	EA 1511 mm ³ /kg.	EA 1508 mm ³ /kg.	EA 1517 mm ³ /kg.	GB mm ³ /kg.
a	I.V.	---	24 hr.	0.036	0.050	0.015	0.0155
b	percut.	24 hr.	24 hr.	0.08	0.16	0.04	36.3
c	percut.	2 min.	24 hr.	0.89	11.0	6.3	385
d	percut.	15 min.	15 min.	>316	>316	7.0	66.7
e	I.V.	---	15 min.	0.060	0.13	0.019	0.018

B.

Ratios of median lethal doses given in A, and the particular characteristics with which these ratios are associated.

Characteristic Assessed	Ratio			
	Items	EA 1511	EA 1508	EA 1517
Cumulative action (percut.)	$\frac{d}{b}$	>4000	>2000	180
Effective penetration at 2 min.	$\frac{a}{c}$	0.04	0.0045	0.002
Effective penetration at 24 hr.	$\frac{a}{b}$	0.45	0.31	0.35
Effect of dose on lethal rate (I.V.)	$\frac{e}{a}$	1.6	2.6	1.2

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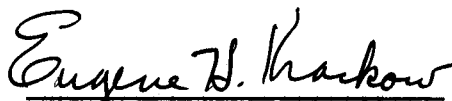
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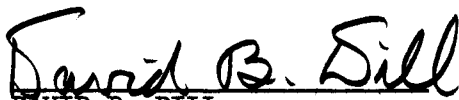
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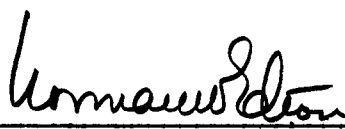
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1. EA 1508, tox., I.V. and percut., rabbits, compare GB
2. EA 1511, tox., I.V. and percut., rabbits, compare GB
3. EA 1517, tox., I.V. and percut., rabbits, compare GB

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1. Callahan, J.F. *The Relation between Skin Thickness and the Penetration Rate of VX through Skin*. In *Research Program of the Field Toxicology Branch*; CRDL TM 20-27; Callahan, JF, et al. Eds.; Directorate of Medical Research, U.S. Army Chemical Research and Development Laboratories, U.S. Army Chemical Center: Edgewood Arsenal, MD, 1962; UNCLASSIFIED Report. **CBRNIAC-CB-118810 Dist. E.**

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