AEP-52
(Edition 1)

ASSESSMENT OF EFFECT LEVELS OF CLASSICAL CHEMICAL WARFARE AGENTS APPLIED TO THE SKIN TO BE USED IN THE DESIGN OF PROTECTIVE EQUIPMENT

AEP-52
(Edition 1)

AEP-52
(Edition 1)

NORTH ATLANTIC TREATY ORGANISATION

NATO STANDARDIZATION AGENCY (NSA)

NATO LETTER OF PROMULGATION

September 2003

1. AEP-52 (Edition 1) - ASSESSMENT OF EFFECT LEVELS OF CLASSICAL CHEMICAL WARFARE AGENTS APPLIED TO THE SKIN TO BE USED IN THE DESIGN OF PROTECTIVE EQUIPMENT is a NATO/PFP UNCLASSIFIED publication.

2. AEP-52 (Edition 1) is effective upon receipt.

3. AEP-52 (Edition 1) contains only factual information. Changes to this AEP are not subject to the ratification procedures and will be promulgated on receipt from the nations concerned after endorsement by the AC/225-Land Group 7 in plenary session.

J H ERIKSEN
Rear Admiral, NONA
Director, NSA

National Reservations

Nation	Specific Reservations

Amendments

No	Reference/Date of amendment	Date entered	Signature

Table of contents

AEP-52
(Edition 1)

PREFACE

1. This document, which reports on the effects of classical chemical warfare (CW) agents on military personnel, is the output of a Team of Experts (TOE) on Percutaneous Toxicity which transitioned into Working Group 1 (WG1) under the auspices of AC225/LG-7.

2. Initially, the group, through its Terms of Reference, was tasked with providing toxicity data with respect to chemical warfare agents in order to provide the basis for design criteria for CW protective clothing as enshrined in NATO document AEP-38, but it was quickly realised that this information was critical for other aspects of CW defence such as collective protection and decontamination. Thus, the output from the group supports the tasking from the DCI specifically in the areas of SF4 (PPE) and SF5 (COLPRO). The output from this report will directly influence the design and requirements for clothing, respirators, collective protection (colpro), decontamination and detectors.

3. Though originally tasked with consideration of toxicological effects with relevance to all CW agents used in a wide range of military scenarios, the group recommended, for reasons explained within, that the group focus on the effects of vesicants (HD and L) and the nerve agents (GA, GB, GD, GF and VX) in war-fighting scenarios only. This recommendation was endorsed by LG7 and for that reason, issues related to toxic industrial chemicals and materials (TICs and TIMs) in operations other than war (OOTW) are not addressed in this document.

4. Bounding the problem in this way has enabled the group to arrive at consensus levels for the scenarios and materials of concern in a way that would not have been possible if the wider matrix of scenarios and materials had also been addressed with concomitant paucity of data and hence, much greater scope for contention within the group.

5. Wherever possible, the group has used existing human toxicity data for its estimates, being acutely aware of the errors that will certainly ensue in extrapolating data derived from animal models, to humans. However, in some instances, because of a lack of human data, some extrapolation from animal data was necessary. The latter are clearly annotated.

6. The group carried out its task by reviewing data published in the literature. Wherever possible original source data was used. The group debated the robustness of the data from the various sources using its expertise and experience and arrived at consensus figures, based on critical toxicological analysis of the data. In order to give greater confidence to future users of this AEP in the

7. reported values, the report contains annexes which detail the assessment criteria and thought processes which were applied by the group to the original source data

8. (also supplied in an accompanying CD-ROM as electronic documents) in order to provide some degree of provenance to the conclusions.

9. In conclusion, this work addresses the military issues associated with percutaneous absorption (i.e. absorption through exposed skin) of classical CW agents relevant to war-fighting scenarios for the purposes of aiding decisions in equipment design and aiding the commander in the field of assessing risk. A strong caveat must be included at this point: WG1 feels strongly that the data within this report was derived for the purposes stated in the preceding sentence and is adequate for that purpose. **It is NOT suitable in its current form for casualty estimation and operational analysis assessments.**

On behalf of Working Group 1

(signed) P. R. Norman
Chair AC225/LG7/WG1

CHAPTER I

INTRODUCTION

1.1. The purpose of this work is to produce a series of values pertaining to the percutaneous toxicity of defined chemical warfare (CW) agents which are agreed within the NATO community and can be used to inform NATO doctrine and specification development with respect to chemical and biological (CB) warfare defence activities. Originally, a Team of Experts (TOE) was established in 1999 in order to agree pass/fail criteria with respect to CW agent penetration characteristics on NBC personal protective equipment (PPE). The intent was that this data would replace the interim pass/fail values that had been incorporated into D101 (The Protective Clothing Triptych, which transitioned into AEP-38) with authoritative data, agreed by the NATO nations. Sensible and validated values are essential in this respect, since these will determine the overall performance specification of an NBC protective ensemble.

1.2. The performance of protective equipment is often defined in terms of a protection factor (PF). Given a knowledge of the anticipated chemical challenge (defined in the output of the Challenge Sub-Group to LG-7 (CSG), and a knowledge of the level of contamination that might be permitted on the skin (note: not a no effect level, but a level of defined effect which might not have significant military implications), a protection factor (the ratio of the challenge dose to the permitted penetration dose) can be determined which is relevant to the design specification.

1.3. Although originally intended to address the issues associated with clothing, it became apparent during the first meeting of the TOE that the data reviewed by the group had relevance to other NBC protection issues and the remit of the discussion broadened accordingly. The WG met for the first time between 5 and 7 February 2001 at the TNO Prins Maurits Laboratory in The Netherlands. The meeting was attended by delegates from Canada, France, Germany, The Netherlands, United Kingdom and United States of America. Apologies were received from Belgium, Hungary and The Czech Republic. The WG was tasked to continue the work of the TOE, established in January 1999 to determine percutaneous toxicity levels for chemical warfare agents in order to inform and guide equipment manufacturers and to aid decision making in the field. The terms of reference (TORs) adopted by the WG were those of the TOE, namely:

TOR 1 – Mission: “The TOE is to review toxicity data for classical CW agents in order to inform LG7 of the short term, percutaneous dose effects. This will facilitate operational risk assessment and assist in establishing design criteria for chemical defensive equipment”
TOR 2 – Objective: “The TOE is to support the tasking from the DCI specifically in the areas of SF4 (PPE) and SF5 (COLPRO). The output from this study will directly influence the design and requirements for following clothing, respirators, colpro, decon and detectors.

TOR 3 – Method: The WG will comprise an appointed chairman and representatives from member nations and invited experts. It will meet twice a year.

TOR 4 – Tasks: The WG will exchange information on percutaneous toxicity of classical agents, will develop agreed human toxicity data and provide a progress report to each LG-7

1.4. The TOE agreed, and this agreement has been subsumed by the WG and agreed by LG-7, that it would focus only on operational effects (i.e. short-term, acute effects), and only on effects of classical CW agents, namely mustard(HD), lewisite(L), G-agents (GA, GB, GD and GF) and VX. The reasons for this decision being:

1.4.1. Nearly all available human toxicity data that has been historically measured has been in respect of acute exposures (i.e. comparatively short duration and high concentration) as were then expected on a chemical battlefield. There is a huge lack of data with respect to longer term, chronic exposures to CW agents. It is this type of data which is likely to be relevant to many Operation Other Than War (OOTW) scenarios and in this respect the data would or should be analogous to accepted health and safety exposure limits (such as occupational exposure limit, OEL). Such data does not exist for many CW agents.

1.4.2. Such chronic exposure data that is available for many Toxic Industrial Chemicals and Materials (TICs and TIMs) is already enshrined in internationally agreed health and safety documentation.

1.4.3. The large number of scenarios that might be envisaged for OOTW meant that the group would have spent much of its valuable time discussing scenarios rather than engaging in the topic of its expertise, i.e. assessing the value of the toxicological data that was available.

1.4.4. Other classical agents such as hydrogen cyanide (AC), cyanogen chloride (CK) and phosgene (CG) were not considered to offer a serious percutaneous hazard because of their high volatility and were not addressed in this study, though as dual use industrial chemicals their toxicities are addressed in standard toxicological databases.

1.5. The group has thus specifically excluded issues of long term, low level exposure such as might be encountered in OOTW. The group believes that though this issue is important, it is a much more difficult problem to assess and to include it within its current remit would result in slow progress on operational issues. The group feels that sufficient data exists to enable considered assessment and determination of acute percutaneous toxicity values to be made with respect to the war-fighting scenario and classical CW agents.

1.6. The group utilised existing human data wherever possible. This was to minimise the requirement to extrapolate from data derived from animal experiments and to attempt to minimise the danger of misquoted and badly assessed data being used in drawing conclusions. Extrapolation from animal data is difficult and can, it is believed, lead to erroneous conclusions. In all cases, priority and weight have been given to reported human experimental data. Reports from the USA, UK and Canada were evaluated and consideration was given to exposure conditions i.e. numbers of subjects exposed, agent concentrations, exposure times, temperature, humidity, location and area of exposed skin, and whether or not there was clothing covering body parts. The signs and symptoms of poisoning, the dosages of agents used and relevant signs of poisoning were evaluated. Account was taken of human data from routes of exposure other than percutaneous if the information provided relevant information to the interpretation of the percutaneous data e.g. information on dose response-relationships, signs and symptoms of poisoning. Where limited or no human data was available relevant animal studies were assessed. Due regard was made to the known differences in skin permeability and response characteristics between species. The group has also decided to utilise primary source data wherever possible, not reviewed data. In order to assist in this process and to make the reasons for conclusions obvious to subsequent readers, the report documents, in as convenient a form as possible, the thought processes adopted in assessing the data so that future readers of the report will understand how various figures were derived. To facilitate this even further, the group intends to publish copies of all the unclassified literature it used in its deliberations in order to aid and inform users of the report. The group worked to the following criteria (paragraphs 1.6.1. to 1.6.4.).

1.6.1. In all cases, priority and weight have been given to reported human experimental data. Reports from USA, UK and Canada were evaluated and consideration was given to exposure conditions i.e. numbers of subjects exposed, agent concentrations, exposure times, temperature, humidity, location and area of exposed skin, and whether or not there was clothing covering body parts. The signs and symptoms of poisoning, the dosages of agents used and relevant signs of poisoning were evaluated.

1.6.2. Account was taken of human data from routes of exposure other than percutaneous if the information provided relevant information to the interpretation of the percutaneous data e.g. information on dose response relationships, signs and symptoms of poisoning.

1.6.3. Where limited or no human data was available relevant animal studies were assessed. Due regard was made to the known differences in skin permeability and response characteristics between species.

1.6.4. The three levels of effect are defined in chapter 2 in terms of military requirements of operational performance. They are not defined in terms of LCt_{50} and ECt_{50} since these definitions require: a) a level of statistical analysis and certainty that cannot

be provided by the human experimental data and b) cannot be derived with the necessary degree of certainty from animal experiments.

1.7. In order to make good use of the infrequent meetings of the group, the WG functioned by producing “strawmen” prior to each meeting, making use of data provided by all participating nations. The "strawmen” were circulated prior to the meeting for consideration. During the meeting, the group then redrafted each “strawman” appropriately to produce a final version and a set of agreed toxicity values.

1.8. The report addresses the military issues associated with percutaneous absorption of classical CW agents relevant to war-fighting scenarios for the purposes of aiding decisions in equipment design and aiding the commander in the field of assessing risk. **A strong caveat must be included at this point: WG1 strongly considers that data contained within this report was derived for the purposes stated in the preceding sentence and is adequate for that purpose. It is NOT suitable in its current form for casualty estimation and operational analysis assessments.**

1.9. Detailed technical considerations leading to selection of recommended values for G-agents, VX, HD and L are given at annexes A to D, respectively. References consulted are cited at annex E whilst annex F to the document is a CD-ROM containing scanned copies of all unclassified source data in Adobe Acrobat® .PDF format.

CHAPTER 2

DEFINITIONS AND ASSUMPTIONS

2.1. In order to promote clarity for both the user and the WG when formulating the report, a series of definitions and assumptions were agreed before detailed discussions commenced. This avoided ambiguity and misunderstanding, saving considerable time in the assessment process. The definitions and assumptions adopted by the group are set out in this chapter. The values recommended in this document are derived specifically for use in the design of NBC protective equipment. Under NBC clothing, conditions of high relative humidity and temperature exist and are, in effect, the worst case conditions which maximise the effect of HD and exacerbate nerve agent effects.

2.2 Three levels of militarily siginificant effect were defined with respect to assessment of nerve agent (i.e. GA, GB, GD, GF and VX) toxicology. The three levels of effects are given below with full definitions in paragraphs 2.3.1. to 2.3.3.

2.2.1. Danger of death
2.2.2. Significant decrement in military performance
2.2.3. Negligible military impact

2.3. These levels are defined below in terms of the military requirements of operational performance. The WG felt that these definitions are critically important, since they allow the members to assess data against a constant parameter set. They are not defined in terms of LCt_{50} and Ect_{50} since these definitions require: a) a level of statistical analysis and certainty that cannot be provided by the human experimental data and b) cannot be derived with the necessary degree of certainty from animal experiments. These three levels of effect were initially defined in relation to the effects of nerve agents, though very similar definitions have been derived for mustard and lewisite. These have been related to relevant levels of military significance to be used in designing/developing personal protective equipment, collective protective equipment, decontamination equipment, and chemical detection equipment.

2.3.1. Danger of death

A dose of agent that will cause personnel to become critically ill and be at risk of death. Doses higher than that proposed for the threshold may increase the risk of death very significantly and a large proportion of personnel will die without intensive medical intervention.

2.3.2. Significant decrement in military performance

A dose of agent that will result in a number of personnel becoming physically unable to undertake their military duties e.g. vomiting, serious physical weakness and tremor. The ability of the military unit to complete its mission would be seriously compromised.

2.3.3. Negligible military impact

No signs of poisoning but with a significant cholinesterase (ChE) inhibition. Signs may be evident, but these will not impact military operations. A dose of agent that produces no incapacitating signs of poisoning with initial exposure but may, with subsequent attack, render personnel highly susceptible to the toxic effects of nerve agent; this may be due to a cumulative effect of ChE inhibition by nerve agent (>30-40% inhibition).

2.4. A related set of definitions was agreed in respect of HD and L. It should be noted that effects due to L occur quickly whilst onset of HD effects are delayed. For HD, the higher the concentration, the shorter the time to onset of effects. Should military operations require, personnel may be able to complete their immediate mission.**These values must not be adopted for other situations such as casualty estimation and peace time operations.**

2.4.1. Danger of death

Most personnel will be sufficiently seriously burned that they will be unable to carry out their military duties for several months, will require intensive medical support, and some may die from their injuries.

2.4.2. Significant decrement in military performance

Exposed personnel may suffer at least moderate skin burns in the sensitive areas and may vesicate or desquamate in these areas. Some personnel may be sufficiently burned, either generally or in sensitive areas that they may be unable to carry out their duties for a period of 1day to 3 weeks. Some medical support may be required after this level of exposure.

2.4.3. Negligible military impact

Personnel may experience some minor irritation and/or erythema of the sensitive areas (genitalia, axillae, neck and the folds of the elbow and knee).

2.5. Both vapour and liquid percutaneous hazards were addressed. Vapour challenges are defined in terms of their Ct, i.e. the integration of the concentration against time curve for the challenge and are expressed in the normal units of $mg.min.m^{-3}$. Liquid challenges are usually expressed in terms of mass of agent per kilogram bodyweight. Where expressed as an amount per person, the assumption that the "standard" person weighs 70 kg is made.

CHAPTER 3

AGREED AND RECOMMENDED TOXICITY VALUES FOR CW AGENT PERCUTANEOUS CHALLENGE

CAVEAT

WG1 considers that data contained within this report was derived for the purposes stated in the preceding sections and is adequate for those purposes. It is NOT suitable in its current form for casualty estimation and operational analysis assessments.

Definitions of effect can be found in section 1.5

3.1. G-Agent Vapour Challenge

Discussion of data leading to these recommendations with respect to GA, GB, GD and GF can be found at annex A.

*Table 3.1.1. Assessed and recommended Ct levels for hazard of **GA vapour** on the skin of the human torso and limbs (excluding head and neck region and estimated from exposure times of 4-9 minutes)*

Effect	Range/ $mg.min.m^{-3}$	Recommended Value/ $mg.min.m^{-3}$
Danger of death	10000-15000	12500
Significant decrement in military performance	5000-10000	7500
Negligible military	2000-4000	3000

*Table 3.1.2. Assessed and recommended Ct levels for hazard of **GB vapour** on the skin of the human torso and limbs (excluding head and neck region and estimated from exposure times of 4-20 minutes).*

Effect	Range/ $mg.min.m^{-3}$	Recommended Value/ $^{-3}$
Danger of death	7000-9000	8000
Significant decrement in military performance	2500-7000	3000
Negligible military	1000-2500	2000

*Table 3.1.3. Assessed and recommended Ct levels for hazard of **GD vapour** on the skin of the human torso and limbs (excluding head and neck region and based on potency ratio to GB).*

Effect	Range/ mg.min.m^{-3}	Recommended Value/ mg.min.m^{-3}
Danger of death	Lack of data	2000
Significant decrement in military performance	Lack of data	750
Negligible military	300-400	350

*Table 3.1.4. Assessed and recommended Ct levels for the percutaneous hazard of **GF vapour** to humans, based on potency ratio to GB*

Effect	Range/ mg.min.m^{-3}	Recommended Value/ mg.min.m^{-3}
Danger of death	Lack of data	2000
Significant decrement in military performance	Lack of data	750
Negligible military	300-400	350

3.2. G- Agent Liquid Challenge

*Table 3.2.1.Assessed and recommended percutaneous hazard levels for **Liquid GB** on humans*			
Effect	Agreed Dose (mg per man)	Range (mg per man)	% Blood AChE Inhibition
Danger of death	500	400-600	90
Significant decrement in military performance	400	300-500	80
Negligible military impact	300	250-350	50
Note: LD_{50} for GB quoted as 1500 mg/man			

*Table 3.2.1.Assessed and recommended percutaneous hazard levels for **Liquid GF** on humans*			
Effect	Agreed Dose (mg per man)	Range (mg per man)	% Blood AChE Inhibition
Danger of death	No data	75-100	90
Significant decrement in military performance	30	No data	70
Negligible military impact	15	No data	50

3.2.1. There is insufficient available data to draw valid conclusions for GA liquid. However, the following observation can be made. Whole body exposure shows no symptoms at 2000 mg.min. m^{-3} at 0-10% RBC ChE Inhibition. There is no reliable data for GD liquid effects.

3.3. VX Vapour Challenge

See annex B for derivations and chapter two for definitions.

*Table 3.3.1 Assessed and recommended Ct levels for the percutaneous hazard of **VX vapour** to humans.*

Effect	Range/mg.min.m^{-3}	Recommended Value/ mg.min.m^{-3}
Danger of death	90 - 470	200
Significant decrement in military performance	35 - 100	60
Negligible military	20 - 40	30
Estimates based on exposures of 1.5 to 75 minutes Due to inherent variability between individuals and different areas of skin it is not possible to refine the data to account for inherent differences in skin penetration		

3.4. VX liquid percutaneous challenge.

See annex B for derivations and chapter two for definitions.

*Table 3.4.1. Assessed and recommended levels of percutaneous hazard or **VX liquid** to humans*

Effect	Agreed Dose for the cheek (mg per man)	Agreed Dose for the body (mg per man)	% Blood AChE Inhibition
Danger of death	0.35	2.80	90
Significant decrement in military performance	0.14	2.45	70
Negligible military impact	0.035 – 0.070	1.75	50

The liquid penetration data was determined at 32ºC (RH 20%). For each ~ 10ºC rise in temp these numbers should be reduced by 30-50%.The group knows of no data to relate the effect of RH on the percutaneous toxicity of VX. It is expected that at high RH the penetration of VX will increase, hence the onset of signs of poisoning will be more rapid. Due to inherent variability between individuals and different areas of skin it is not possible to refine the data to account for inherent differences in skin penetration

3.5. Sulphur Mustard (HD) percutaneous vapour challenge:

See annex C for derivations and chapter two for definitions

*Table 3.5.1. Assessed and recommended levels for hazard of **HD vapour** to skin of the human torso and limbs estimated from exposure times of 10 – 60 minutes.*

Effect	Range/ mg.min.m^{-3}	Recommended value/ mg.min.m^{-3}
Danger of death	750 - 1500	1000
Significant decrement in military performance	100 - 400	200
Minor symptoms	10 - 100	50

3.6. Sulphur Mustard percutaneous liquid effects

See annex C for derivations and chapter two for definitions.

	Range (g/man)	Recommended value (g/man
Danger of Death	Available data does not permit a reliable range to be estimated	>1.0
Significant decrement of military performance	0.8-1.0	0.8
Negligible military impact	0.1-0.3	0.2

3.7. Lewisite (L) percutaneous effects

3.7.1. Available data on L toxicity is documented at annex D. However, there is a paucity of original source data so the recommendatiopns of the WG in this respect must be used with extreme caution. The WG suggests that a **safety factor of at least 3 be applied** to the values derived from referenced literature sources (see annex D), to allow for documented uncertainties in human toxicity effects of L , as well as recent animal experiments which suggest that L may be considerably more toxic than HD. Applying a safety factor of 3 to the data in table 3.7.1., the group derived the recommended values in table 3.7.2.

Table 3.7.1.: Values taken from the review by Gates (original data sources not reviewed). Exposure expression times not noted.

	Vapor ECt_{50} ($mg.min.m^{-3}$)	Liquid Dose (mg)*
Death (by body exposure)	100000	2800
Vesication of skin	1200 – 1500	0.014
Serious corneal damage	1500	0.1

*Not noted whether these values represent minimum, or median effective doses.

Table 3.7.2.: Recommended maximum L exposure levels for humans

	Recommended Vapor ECt_{50} ($mg.min.m^{-3}$)	Recommended Liquid Dose (mg)
Death (by body exposure)	33,000	930
Vesication of skin	400 - 500	0.005
Serious corneal damage	500	0.030

ANNEX A

COMMENTS AND NOTES ON G AGENT TOXICITY DATA

A1. G Agent Percutaneous Toxicity

1.1. The contents of this annex represent notes based on the discussion of the group and are not precisely structured documents.

1.2. For assumptions made, see chapter 2

A2. GB Vapour

2.1 Evidence Considered

See annex E

2.2 Assessment of Hazard of GB vapour through Human Skin

2.2.1 Levels of cholinesterase inhibition and signs and symptoms of poisoning.

A number of studies have attempted to correlate the lethality (as estimated by LD_{50}) of GB with cholinesterase inhibition. A comprehensive summary by Cullumbine et al (1954) quoted estimates of percutaneous liquid GB LD_{50} (11.8 mg/kg slope 6.7) and ChE_{50} (2.5 mg/kg) for close clipped rabbits. The same authors quote a number of LD_{50} /ChE_{50} ratios for different species (rat 3.2, guinea pig 3.3, monkeys 6.3 and pigeons 4.0) which they use as justification for using a ratio of 4.25 to estimate the LD_{50} for percutaneous liquid GB in man from the estimate of the ChE_{50}.

From these data Cullumbine estimated an LD_{50} in man of 1500-1700 mg/man using LD_{50} /ChE_{50} ratio of 4.25 and an estimated ChE_{50} of 400 mg/man. Inspection and review by the Skin Toxicity TOE revealed that the line of “best fit” to the ChE inhibition versus dose graph, used by Cullumbine et al, was too shallow. The TOE recommended that a line with a steeper slope was more justified and the ChE_{50} should be nearer to 300 $mg.man^{-1}$ giving an LD_{50} of 1275 mg/man or about 18 $mg.kg^{-1}$.

Cullumbine et al (1954) reported studies in man which correlate the symptoms of GB poisoning with cholinesterase inhibition. A total of 396 men were exposed to liquid GB percutaneously, either on bare skin or through layers of cloth. Exposures were carried out in a chamber at 21.1°C (70%RH) with each man wearing respiratory protection being exposed to droplet of liquid GB either directly on to the skin or through one or two layers of clothing. Seven of the 396 men showed signs of poisoning, two of whose symptoms were considered as life threatening. No man with a blood cholinesterase inhibition less than 80% showed any symptoms other than a local cutaneous response (“e.g. sweating, blanching, itching and a feeling of cold”). Of the 14 men whose cholinesterase was inhibited by more than 80 %, 7 were symptom free, 5 had nausea, dizziness and vomiting and two experienced inco-ordination, convulsions and respiratory arrest and of these last

two, one died. These two individuals had cholinesterase inhibitions of 94 and 96% respectively. Given that the individual who survived required medical intervention and may have died without it, the available human data supports a threshold for death of 90% inhibition.

It should be noted that the variation in the range of responses was very large and commented upon by the authors.

2.2.2 Human studies

There are only three recorded studies of the percutaneous toxicity of GB in man. The study reported by Cullumbine et al (1954) described above, one reported by McGrath et al (1951) and Freeman's work.

Freeman et al (1953) [102] reported experiments where eleven human subjects were exposed to liquid GB (99% pure by isoproxy radical P & F) on the forearm. The subject's arms were examined with a hand lens prior to exposure and questionable spots protected, but were neither washed nor otherwise prepared. Each subject was masked and their hand placed in a hood where GB was applied as discrete 4-5mg drops from a "drod" micro-syringe up to 25-550mg (equivalent to 6-229), for the largest doses the upper arm was also used as a dosing site. The area of spread was visualised by adding 0.14% luxol-fast blue but the authors present no evidence of any effect this may have had on the rate of penetration of the agent. The disappearance of agent from the surface of the skin was measured by sampling a small part of the drop when it was "almost dry" using a cotton applicator soaked in o-tolidine (3% in acetone) applied firmly to representative drops. The applicator was then dipped into 2% aqueous sodium perborate, an orange colour indicating more than 1µg. Sampling and testing in this way was continued until negative. Subjects were tested for psychometric performance, plasma (ChE) and red blood cell cholinesterase (RBC ChE) - Analysed in 3 different laboratories, electrocardiogram (ECG), muscular grip strength and fatigability, temperature, and sweating response of the skin. Control tests were carried out over 2 hours on the day of application or the previous day. From 30 minutes to at least 6 hours series of blood samples taken. Ambient room temperature 24°C (26-50%). Air movement controlled and adjusted using fume hood opening to give about 30 $m.min^{-1}$ at the arm.

The GB spread immediately to a very thin film with apparent complete evaporation within less than two minutes in some instances - hair prevented both spreading and evaporation. Three of the subjects who received 25-50mg showed no inhibition of RBC-ChE. Whereas the RBC-ChE of the six subjects given 300-500mg were inhibited by 14-18%. There was no consistent effect on plasma ChE. RBC-ChE activity remained constant over 6 hour period in ten control subjects. Systemic effects were absent or minimal. Two subjects reported transient Diarrhea and one a mild state of elation. Other effects were local sweating and cooling. No fasciculation was observed or effects on ECG, muscular strength or fatigability. There were "suggestive alterations" in psychometric function which required further evaluation. Local sweating was present in all within a few minutes and persisted for hours to weeks with the longer durations being produced by the larger

amounts. The sweating was reported to cease abruptly, change from continuous to intermittent and could be elicited by mild stimulation (i.e. exercise not producing generalised sweating).

The WG assessed the data derived below:

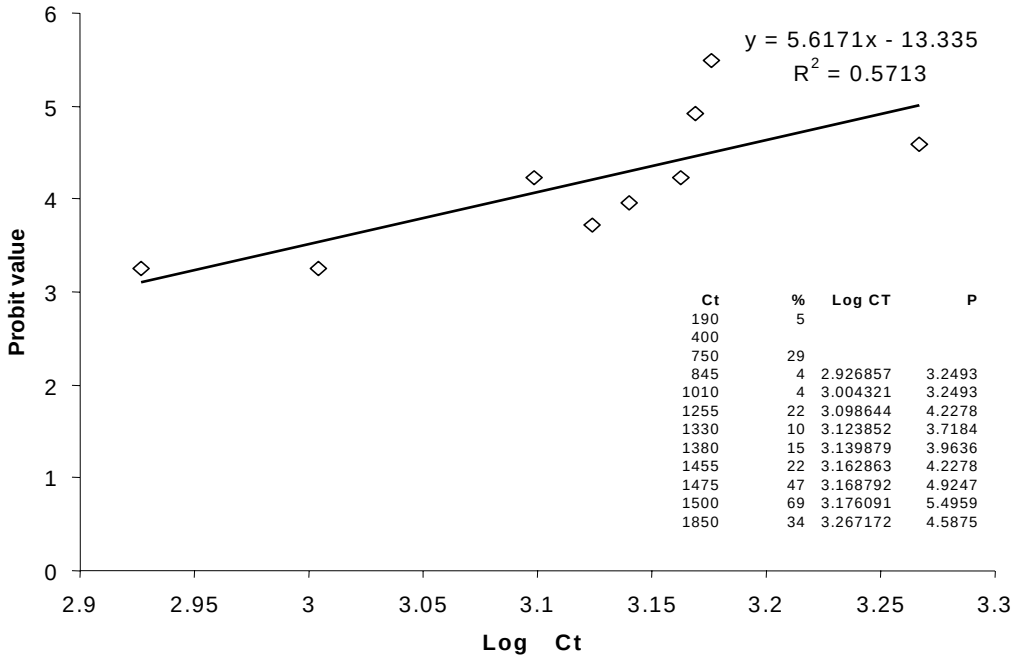
McGrath et al (1951) exposed human subjects to GB under controlled conditions. In preliminary experiments volunteer's forearm. The arms of six subjects were exposed in a 386L chamber to Ct's of 1160, 2330 and 4170 mg.min.m^{-3} (C = 115 mg.m^{-3}) and 3930, 4380 and 8030 mg.min.m^{-3} (C = 200 mg.m^{-3}). Four out of six showed local sweating but no other effects. This was strongest in the warm environments and limited to the exposed area. The two subjects who received the largest and smallest dose were the two who did not sweat.

On the basis of this 13 volunteers were exposed whole body in a 4000L chamber wearing an M-9 gas mask with leak proof headpiece, E2R4 and a new M-11 canister (masks were tested for effectiveness, in a CS filled chamber, with subjects wearing overalls). Subjects were exposed sitting quietly on a stool. Fourteen subjects were exposed (one man twice) in the range 190 (C=21 mg.m^{-3}) to 1850 mg.min.m^{-3} (C = 93 mg.m^{-3}). One subject was exposed twice. Blood samples taken 24 hours, 15 minutes before and 1 hour and 24 hour after exposure.

Red cell cholinesterase inhibitions ranged between -8% to 69% and the only symptom observed was local sweating. The majority of individuals were exposed to Ct's around 1500 which gave inhibitions around 50%. The subject who was exposed to 750 mg.min.m^{-3} (C = 36 mg.m^{-3}) arm only and had 29% inhibition, was re-exposed 129 days later to 1330 mg.min.m^{-3} (C = 81 mg.m^{-3}) showed 10% inhibition but the arm previously exposed remained moist for 30 days. The RBC ChE inhibitions in groups of Ct = 190-1010 mg.min.m^{-3} and 1255-1850 mg.min.m^{-3} were significantly different and inhibition was first observed in the range 700-1000 mg.min.m^{-3}. The authors estimated of RBC ChE_{50} was 2000 mg.min.m^{-3} based upon a line through the data. The RBC ChE_{50} human:monkey ratio was used to calculate the Ct expected to produce symptoms in man. These were 8600 mg.min.m^{-3} to cause and 12,800 mg.min.m^{-3} to cause apprehension.

The human cholinesterase data from the whole body exposures carried out by McGrath (24 hours post exposure) has been subject to probit analysis (figure X) in order to confirm the relationship between Ct for ChE inhibition. From the equation of the regression line shown in Figure X the Ct at a probit value of 5 is 1867.5 mg.min.m^{-3} and is not substantially different from the authors original estimate of 2000 mg.min.m^{-3}. The Ct resulting in an 80% inhibition, the inhibition above which Cullumbine (1954) report symtpoms in human subjects, approximates to 3000 mg.min.m^{-3}. It should be noted that Cullumbine (1954) exposed subjects to liqudi whereas McGrath's subjects were exposed to vapour which questions the validity of comparing the two studies. However, in the absence of more definitive data the WG considers this is the best approximation and should be regarded as interim estimates until further data becomes available.

To establish the bottom of the range for the negligible effects the methods used for biological monitoring of workers at Porton were consulted. Individuals are not normally considered to be showing signs of inhibition until their red blood cell cholinesterase deviates from ten prior measurements by more than 2.5 standard deviations. This represents between 6% and 15% inhibiton (determined by intra and inter-individual variation (E Gosden, personal communication). An approximation of the ECt_{10} taken from figure 2.1 (McGrath, 1951) is therefore taken as the bottom of the range for Negligible Effects.



Ct	%	Log CT	P
190	5		
400			
750	29		
845	4	2.926857	3.2493
1010	4	3.004321	3.2493
1255	22	3.098644	4.2278
1330	10	3.123852	3.7184
1380	15	3.139879	3.9636
1455	22	3.162863	4.2278
1475	47	3.168792	4.9247
1500	69	3.176091	5.4959
1850	34	3.267172	4.5875

Figure 2.1

Probit analysis of the red blood cell cholinesterase inhibition after whole body exposure of human volunteers to GB. Raw data is shown in the superimposed table and the individual with a negative inhibition was excluded. The calculations of the Ct's for cholinesterase inhibitions of 10%, 50% and 80% from this data are:

Ct_{10} = 10^((3.7184+13.335)/5.6171) = 1086.4 ~ 1000 mg.min.m^{-3}
Ct_{50} = 10^((5+13.335)/5.6171) = 1837.1 ~ 2000 mg.min.m^{-3}
Ct_{80} = 10^((5.8416+13.335)/5.6171) = 2594.0 ~ 3000 mg.min.m^{-3}

Figures are rounded because of the paucity in the data giving low confidence in the accuracy of the estimates.

Studies in animals

2.2.3. Liquid GB:

Studies in animals usually used death as the end point measuring classical LD_{50}. These are summarised in the table below:

Table 2.1 LD_{50}s of liquid GB

SPECIES	LD50 (mg/kg)	Slope	Comments	Ref
Goat	0.913	3.0	Depilated; 5 groups of 2	Muir et al (1948)
Rabbit	1.26		Depilated; n=40	Muir et al (1950)
Rabbit	11.6	3.3	Clipped; n=25; 5 groups	Muir & Callaway (1950)
Rabbit	2.36	4.2	Depilated; n=20; 4 groups	Muir & Callaway (1950)
Rabbit	5.1	6.6	Depilated, six groups of 7-23	Ainsworth & Truckle (1950)
Rabbit	3.5	6.3	Depilated, clothed, six groups of 7-14	Ainsworth & Truckle (1950)
Rabbit	11.8	6.7	Clipped; n=70	Cullumbine et al (1954)
Rabbit	12.4	5.7	Clipped clothed	Cullumbine et al (1954)
Rabbit	2.7	4.1	Depilated; n=70	Cullumbine et al (1954)
Rabbit	2.2	3.4	Depilated, clothed; n=71	Cullumbine et al (1954)
Rat	11	1.8 ± 0.9	Depilated; n=139	Muir et al (1948b)
Rabbit	26	3.3	Clipped; crude material adjusted for purity. 2 groups of 14	Marzulli et al (1952)
Pig	112	4.6	Clipped; crude material adjusted for purity 3 groups of 7	Marzulli et al (1952)

Data derived from animals which had been depilated must be excluded from estimates of the LD_{50} in that species. Muir and Callaway (1950) showed convincingly that depilation decreased the LD_{50} of liquid GB in rabbits as did Cullumbine et al (1954). This places the LD_{50} of liquid GB in rabbits at 11-12 $mg.kg^{-1}$. This figure is of the same order of magnitude as the LD_{50} in man estimated by Cullumbine et al of 18 $mg.kg^{-1}$.

The work reported by Marzulli et al (1952) in pigs is compromised because the GB used was impure containing 20% “propoxy” and trace amounts of tri-butylamine. The authors applied a simple arithmetic correction for the mass of applied substance which was not GB but this does not take into account any enhancement or retardation of penetration which may have taken place.

2.2.4 GB Vapour

There are three reports of studies which measured the toxicity of GB vapour in animals. Ainsworth (1950) exposed partially depilated rabbits to concentrations of between 14 and 1100 $mg.m^{-3}$ (t = 7-420 min) until death, recorded the time to death and calculated the LCt. This study suffers from two disadvantages 1) depilation increasing the sensitivity of the

animal and 2) a lethal dose being absorbed before death occurs. A clear result however was that partially depilated rabbits could survive in vapour concentration of 17 $mg.m^{-3}$ for 420 min (Ct = 7200 $mg.min.m^{-3}$) only exhibiting “slight symptoms”. Ainsworth observed that the lethal dosage was fairly constant over the concentration range 40-200 $mg.m^{-3}$, but outside of this range the value increased.

In a separate study Oberst et al (1952) reported LCt_{50}s in clipped rhesus monkeys in which care was taken to avoid inhalation absorption of agent. These animals were exposed until dead with the following results.

Temperature (°F)	Relative Humidity (%)	LCt_{50} ($mg.min.m^{-3}$)
70-80	55-85	8800
80-90	90-97	9700
90-100	90-97	3600

The LCt_{50} may be dependent upon temperature and humidity and it may be assumed that other signs of toxicity will also be. Given that these animals were exposed until death, it is almost certain that the calculated LCt_{50}s underestimate the toxicity of the agent as a consequence of the delayed absorption of agent from the skin. By how much is not known.

Animal studies: McGrath et al (1951) [103] exposed rhesus monkey's body only in a 4000 L constant-flow chamber sampled by bubblers and analysed using peroxide-o-dianisidine method. Ten animals were close clipped (hair 1/16") 3 days prior to the exposure and immediately before any abrasions sealed with collodion. Animals were restrained at wrists and ankles and head passed through rubber seal held in place by wood stock so that only the body below the neckline was exposed. Monkeys were not washed after exposure and blood samples taken 24 hours & 15 minutes before and at frequent intervals after the start of gassing. Red blood cell cholinesterase was measured by the Michel method. Exposures were to constant concentrations of GB, ChE and signs noted at various times to give Ct's.

Minor sysmptoms, muscle twitches, generalised body tremors developed gradually and sometimes sporadically allowing no definite end point to be defined. The first definite, objective symptom was sudden marked apprehensiveness observed at 4.3 times dose required for rbc 50%. This was characterised by baring of teeth, trying to bite the operator and crying out loudly when approached. The second definitive symptom was a sudden dilatation of the pupils from four to eight millimetres. At this time the animals were unconscious and were removed from the chamber immediately after dilatation occurred. The blood of some animals was very dark in colour toward end of gassing. Some showed marked constriction of the pupils after being withdrawn from the chamber and placed in a cage. It can be concluded that concentrations of less than 70 $mg.m^{-3}$ do not kill monkeys up to 14,180 $mg.min.m^{-3}$ but greater than 80 $mg.m^{-3}$ can (though this is based on a group size of 2); apprehension was observed at Ct's of 3290-12,450 $mg.min.m^{-3}$. For individual monkeys there was a fairly close correlation between the Ct producing apprehension and

ChE_{50} - 4.3 ± 0.9 (3.0-5.5). Similarly, there was a correlation between the Ct causing dilatation and ChE_{50} - 6.4 ± 1.4 (4.6-8.8).

2.2.5 Estimates of toxicity for percutaneously absorbed GB vapour

Of the three criteria "Burden of inhibition" is the only one where the dose may approximate to the ChE_{50}, the thresholds for death and Significant decrement in military performance require a knowledge of the relationship between dose and effect (i.e. the slope of a probit analysis).

From the human data available (McGrath et al (1951), Cullumbine et al(1954)) a conservative estimate of the ChE_{50} would be 1255-1850 $mg.min.m^{-3}$. On this basis an "Negligible military impact" level of 1200-2000 $mg.min.m^{-3}$ is proposed. Based on the TOE re-appraisal of the papers by McGrath et al (1951) Oberst et al (1952) the LCt_{50} for man probably lies between 10,000 and 15,000 a "threshold for death" of 8000 (range 7000-9000) is proposed. Assuming that this is the value which corresponds to an approximate 90% inhibition of ChE, a figure of 6000 (range 5000-7000) is proposed as that which would correspond to a figure of 80% inhibition and would therefore be the "onset of nausea and vomiting" indicated by the studies reported by Cullumbine et al (1954).

Table 2.2 Recommended levels for hazard of GB vapour on the skin of the human torso and limbs (excluding head and neck region).

Effect (section 1.5)	Range/$mg.min.m^{-3}$	Agrred Value/
Danger of death	7000-9000	8000
Significant decrement in military performance	2500-7000	3000
Negligible military	1000-2500	2000

A3. GA Vapour

3.1 Evidence considered

There are few documented experimental studies of the exposure of humans to GA and the existing estimates of toxicity are based on very limited information. Previous assessments, by all routes of entry, have been based upon early human exposures and extrapolations from animal experiments. In some cases the assessment of toxicity has been derived from a comparison of the relative potency of GA to that of other G agents. The estimates of hazard proposed by the TOE remain of limited confidence.

In many early nerve agent studies using animals, the skin was chemically depilated. This is well known to increase the penetration of chemicals through skin. Evidence derived from such studies has not been used to derive estimates for this assessment.

In this assessment the following studies/reports have been evaluated:

See annex E

3.2 Assessment of Hazard of GA vapour through Human Skin

3.2.1 Levels of cholinesterase inhibition and signs and symptoms of poisoning.

Unlike VX and GB, no relationships between the level of cholinesterase inhibition and the signs and symptoms of poisoning, have been established for GA. Consequently it has not been possible to directly relate symptoms and signs of poisoning to ChE inhibition levels, although limited measurements in humans and rhesus monkeys give some guidance on effects (Krackow and Fuhr, 1949).

3.2.2 Human studies

Apart from some information derived from German sources during World War II, there is only one documented study in which men were exposed to GA vapour (Krackow and Fuhr, 1949). This US study was undertaken in two parts: a preliminary investigation, using forearm exposure, of 4 men to Cts of 200-400 mg min m^{-3} to assess hazard levels, followed by whole body exposure, of another group of 16 men, to Cts of between 520-2000 mg min m^{-3}. In only one instance was an important decrease in RBC AChE determined (Ct 1090 mg min m^{-3}; RBC AChE depression at 15 min of 45%). In all other cases, up to a Ct of 2000 mg min m^{-3}, no significant decrease in RBC AChE was measured up to 24 hour after exposure. In no instance did any subject have signs or symptoms of exposure.

It was concluded that humans, who were masked, could be safely exposed to GA vapour up to a Ct of 2000 mg min m-3 without any signs or symptoms of exposure. A small but statistically significant decrease in plasma and RBC ChE (<10%) occurred between 1000 - 1300 mg min m-3.

3.2.3 Studies in animals

Krackow and Fuhr (1949) also undertook studies in rhesus monkeys which had been clipped 4 days before exposure. The heads of the animals were outside the exposure chamber and care was taken to prevent inhalation of desorbed vapour at the end of the exposure. The detailed results and conclusions were largely based upon one satisfactory exposure. Cholinesterase determinations were carried out during the exposure and were shown to be very sensitive to small increments in Ct. At 5 min (Ct 300 mg min m^{-3}) ChE was 10% inhibited, at 10 min (Ct 600) it was 55% inhibited and it continued to decrease to 99% inhibition at 30 min (Ct 1850 mg min m^{-3}). The monkey was sign free during this period. Shortly after this time (32 min) the monkey exhibited symptoms of poisoning (muscle twitching, respiratory distress and restlessness). At 45 min the monkey became very apprehensive and was removed from the chamber at 50 min (Ct 3100 mg min m^{-3})

with a ChE of 0%. It remained prostrate, with shallow breathing but by 1 day had recovered and was able to move around its cage.

The studies, in the other six monkeys, showed that monkeys exposed to GA vapour (body only) can be killed by a Ct of 5000-9000 mg min m^{-3} (t = 34-161 min). The authors noted that progression of symptoms is sufficiently slow that removal from the toxic environment on the onset of signs should enable survival.

Marquand and Kethley (1946) undertook a limited study of weapons grade GA (purity not stated) in dogs and rabbits. Both clipped and unclipped animals were used and it was claimed that there was no difference in toxicity due to clipping. Gassing was continued until the animals showed marked signs or died, hence the value for LCt50 is likely to be a significant overestimate. In the initial studies the agent was shown to contain significant quantities of cyanide but this was resolved in the last 2 rabbit exposures (2/4 deaths at 26,000 mg min m^{-3} (t = 120min); ¾ deaths at 35,000 mg min m^{-3}) (t = 283min). All dogs exposed to a Ct in excess of 80,000 died, one died at 78,000, those at 45,000, 60,000 and 80,000 mg min m^{-3} lived.

Information, derived from interrogation of German CW personnel, indicate that body only exposure of dogs to 18,000 – 21,0000 mg min m^{-3} was a $LCt_{90\text{-}100}$. However the experimental conditions and data were not reported (Evans,1946)

3.2.4 Estimates of toxicity for man for percutaneously absorbed GA vapour

Existing estimates of toxicity of GA by a variety of routes indicates that its intrinsic toxicity is between ½ to ¼ of that of GB, depending upon the route of administration and the species investigated (Muir, Callaway and Burgess, 1950). Many earlier estimates of its toxicity to man have included a comparison of this relative toxicity to GB because of the paucity of information upon which to make a confident assessment.

Current national estimates of toxicity to humans by the percutaneous route for absorbed vapour are LCt_{50} of between 10,000 and 20,000 mg min m^{-3} and ICt_{50} of between 5000 and 12,000 mg min m^{-3}. These estimates are based largely upon the work of Krackow and Fuhr (1949) in humans.

Review of the animal data indicate that the percutaneous toxicity of GA is, to some extent, a function of species size with mice and guinea pig being the most sensitive, monkeys are intermediate and rabbits and dogs least so. It is unclear where humans are placed on this axis, however the estimated LCt_{50} by this route for GB is between 10,000-15,000 mg min m^{-3}. Bearing in mind the lower volatility of GA and the slower skin penetration of this agent over GB (occluded) similar estimates of toxicity would be prudent.

The studies of Krackow and Fuhr (1949) clearly indicate that a Ct of 2000 mg min m^{-3} is safe for humans. Remarkably, exposures of rhesus monkeys to Cts of up to 1200 mg min m^{-3} produced a graded decrement in RBC ChE of more than 90% in some cases, signs however were not critical, though possibly incapacitating, shortly after these levels were

achieved. Dermal absorption appears to be slow but progressive even after the source of the vapour is removed, which suggests that a depot of sorbed agent may remain in skin for some time. Dogs and rabbits appear to be more resistant species.

The duration of exposure, on the basis of the limited amount of data available, does not appear to have a major influence on the Ct required to produce effects between the times of 10 min to 360 min.

On the basis of the following considerations:

a. That intrinsic toxicity of GA is between ¼ and ½ of that of GB,

b. That a Ct of 2000 mg min m^{-3} is a proven no-effect level in humans with evidence of ChE inhibition in only one human subject,

c. That the lethal dose to rabbits and dogs is between 18,000 – 26,000 mg min m^{-3},

d. That the lethal dose to monkeys is between 5000 – 9000 mg min m^{-3} on continuous exposure and that there is evidence that the skin of rhesus monkeys is between 2-4 times more permeable than human skin,

the WG proposes the following hazard levels:

Table 3.1 Recommended levels for hazard of GA vapour on the skin of the human torso and limbs (excluding head and neck region){mg min m^{-3}}

Effect (section 1.5)	Range/mg.min.m^{-3}	Agrred mg.min.m^{-3}
Threshold for death	10000-15000	12500
Significant decrement in military performance	5000-10000	7500
Negligible military impact	2000-4000	3000

A4. GD Vapour

4.1 Evidence considered

There are few experimental studies of exposure of humans to GD. One significant human exposure has been documented together with the signs and symptoms of poisoning and the treatments administered. Another has investigated the effects of skin application of low doses in animals and humans. All existing estimates are based, in the main, on limited studies in animals. Hazard levels have, to some extent, been related to that of GB with some additional considerations based on knowledge of its relative toxicity and physico-chemical properties.

No studies in which skin has been depilated have been used to derive estimates in this assessment.

In this assessment the following studies/reports have been evaluated:

See annex E

4.2 Assessment of Hazard of GD vapour through Human Skin

4.2.1 Human studies

Unlike GB, the relationships between cholinesterase and signs and symptoms of poisoning for GD are not well defined. In a human exposure to a Ct of 5.5 mg min m^{-3} over a period of 1min 40 sec, three men who had no respiratory protection suffered significant signs and symptoms of poisoning (Ladell,1961). The signs and symptoms including nausea and vomiting, tight chest include bronchial spasm, muscle weakness and pain, miosis and headaches. These signs and symptoms persisted for up to two days and responded well to atropine treatment. The whole blood cholinesterase depression differed between the men but did not achieve maximum depression until after the first day (day 5 was lower than day 1). At the end of the first day the depressions were 62%, 41% and 74%. Recovery was not complete until beyond 57 days. The mean WB ChE depression was determined to be 59% which was equivalent to marching men exposed to GB at a Ct of 15 mg min m^{-3}, and which represents a respiratory dose of 6 µg kg^{-1}. The authors believed that this would have been equivalent to the absorption of a total dose of 400-500µg if GD had the same AChE activity as GB. The paper includes a calculation upon the absorbed dose using the ratio of percutaneous/systemic (i.v.) ChE_{50} for VX (30:1). This leads to the assumption that the total percutaneous dose was 4.5 mg. If distributed evenly over the face, neck and hands (1100cm^2) this is equivalent to a dose of 4µg cm^{-2}. The comments by Ainsworth in this paper disputed the assumptions based upon VX used by Ladell. Based upon knowledge of skin uptake in vitro and physicochemical properties Ainsworth suggested that 2mg of GD were absorbed through skin in this demonstration.

In the same study, three men, with respiratory protection were also exposed at the same time to the same dose of agent. They experienced no signs or symptoms of poisoning

other than a rapid onset of miosis on leaving the chamber and removing their respirators. Regrettably the WB ChE depression was not determined in the masked men. The authors concluded that the important route of exposure to GD vapour was via the lungs and eyes (including those who suffered miosis after mask removal). However, the delay in depression of ChE indicates some skin absorption, since it is not clear from the report whether the men removed and changed their clothing after exposure. It was also concluded that, if the interpretation was correct, then desorption of GD is very slow and agent which is absorbed into the keratinous layers of skin remains to penetrate.

Cullumbine et al (1954) undertook limited studies with liquid GD (together with GB and GF) in humans and rabbits. Thirty eight masked men were contaminated with liquid GD on the volar forearm at doses of from 10-40 mg. The mean red cell ChE was determined and the data is as follows:

Table 4.2.1.1 Effect of GD on RBC ChE

Administered Dose/mg	No of Subjects	Mean 24 hour RBC-ChE(± S.D.)
10 (single drop)	3	34.3 (6.0)
10 (0.5 mg drops)	3	12.7 (6.4)
15 (single drop)	3	26.0 (11.0)
20 (single drop)	3	18.3 (3.9)
30 (single drop)	8	22.3 (9.3)
40 (single drop)	18	27.1 (9.0)

Too few subjects were contaminated with GD to allow a relationship between contamination dosage and ChE inhibition to be determined. Provisionally it appeared that the ChE_{50} for bare skin for GD is much greater than 40 mg/man and probably around 60-70 mg/man (~1mg kg^{-1}). Some of these men experienced local effects of sweating and “twitching” at the sites of application. Most of these effects were apparent at 30 min after dosing.

In this study the slopes of the human and rabbit data for GB are similar and suggest that the mechanisms of toxicity for GB are the same in the two species. From the rabbit data the ratio of LD_{50}/ChE_{50} (4.25) is relatively constant for different skin conditions (clothed and unclothed). It leads to an estimate of the percutaneous toxicity of GB for man of 1500 mg to 1700 mg of GB per man. The data for GD in both rabbits and humans are much fewer and less reliable for GB. The rabbit data suggest a slope similar to that of GF (1.63) and a LD50/ChE50 ratio of 5. Using a similar approach for GD, an LD_{50} estimate of 350 mg of GD per man was proposed.

Silver (1964) refers to a study in 23 volunteers in which GD vapour was applied to the volar surface of the forearm. The agent was applied in a dynamic cup through which air flowed at a range of velocities. Whole blood ChE was measured at intervals. No subject showed any gross signs of poisoning although sweating occurred over the application area. The ChE depressions were, in general, dose related with insignificant depression

below 1µg kg^{-1}. Doses were increased to up to 37.5 µg kg^{-1} and graded inhibitions up to 22% were obtained at 30 µg kg^{-1}. One individual at this dose had an inhibition of 65% and in this individual the air flow through the application cup was virtually zero. The total applied dose of 1.4 mg contrasted markedly with the Cullumbine (1954) finding of 70 mg.

4.2.2 Animals studies

As GD was discovered in 1944 only limited investigations were carried out in Germany before the end of the war. Evans (1946) reported that the inhalation LCt_{50}s by inhalation of GA, GB and GD in rhesus monkeys, dogs and cat were as follows:

Table 4.2.2.1: Inhalation LCt_{50} (mg kg^{-1})

Agent	Rhesus Monkey	Dog	Cat
GA	150	250-300	150-200
GB	100-150	80-100	80-90
GD	50-75	15-20	50-60

In the same report the following $LCt_{90\text{-}100}$ was cited for dogs, (body only):
GA: 18,000-21,000; GB: 12,000-18,000; GD 6,000-9,000 mg min m^{-3}. No experimental details were provided.

A key assessment of the relative toxicities of percutaneously applied liquid G-agents was undertaken by Cullumbine et al (1954). The cited LD_{50}s for GB, and GD on clipped bare rabbit skin, within this study, are: GB 11.8 mg kg^{-1}; GD 0.7 mg kg^{-1}. The estimates of GD toxicity obtained were however unreliable and derived by visual interpolation. The ChE_{50} are: GB 2.5 mg kg^{-1} and GD 0.13 mg kg^{-1}. The LD_{50}/ChE_{50} ratios in this species are thus: GB 4.7 and GD 5.0.

Using excised pig skin Tregear and Dirnhuber (1961) showed that there was little decomposition of G agent on evaporation. G-agents penetrated rabbit skin faster than VX and the steady penetration rates under closed cells for GB, GD and GF were similar (1.3; 0.9 and 1.3 µg cm^{-2} min^{-1}). When free evaporation was permitted, at 32C, the amount of agent, that penetrated rabbit skin, was in approximate inverse proportion to the agent volatility (fraction penetrated: GB 0.0013; GD 0.0078 and GF 0.064). Penetration continued after all liquid had evaporated from the skin surface; this is consistent with the above studies in man (Ladell, 1961).

4.2.3 Estimates of toxicity for man for percutaneously absorbed GD vapour

The existing estimates of the human toxicity of GD are based upon very limited data. The current national estimates have evolved over the past 50 years but are nonetheless based upon very little human data and limited animal data (Silver, 1954; Howd et al. 1986). Some credibility has been attached to the early German studies in dogs despite the fact that no experimental details (exposure concentrations, times, clipped/unclipped; statistical analysis) were provided. Current UK estimates are similar to that of the US and are based

on UK and US studies of the period and are within the range 1400 to 6000 mg min m^{-3} (LCt_{50}) and 700 to 1000 mg min m^{-3} (ICt_{50}).

A recent US assessment has proposed the following hazard levels for GD (NRC Report, 1997) :

LCt_{50} 2,500 mg min m^{-3}

ECt_{50} (Threshold effects) 300 mg min m^{-3}

The LCt_{50} proposed in the NRC report is based upon toxicity information obtained for GB and assumes that GD is 4 times more potent than GB. This is supported by empirical evidence, from an internal US report (Van de Waal and Zeffert 1970), that the percutaneous potency of GD vapour is at least 2-5 times that of GB. The confidence in these estimates was rated as low by the TOE due to lack of data.

The exposure of unmasked humans to a Ct of 5.5 mg min m^{-3} of GD (Ladell 1961) shows that a depression of WB ChE by 62% and 74% was associated with vomiting and severe muscle weakness/collapse, whereas a depression of 41% induced nausea, but no vomiting. (It should be noted however that there is evidence to suggest that the rate of inhibition of ChE may have a role to play in the triggering of vomiting – see GA assessment). Furthermore the rate of ChE recovery in these individuals was prolonged and at the end of 1 month was still almost 50% inhibited in those who had vomited. This rate of recovery is in line with the turnover of red cells which occurs at approximately 1% per day. Masked personnel showed no adverse effects that could be attributed to dermal absorption of GD (although ChE was not measured).

Studies by Cullumbine et al (1954) compared the toxicities of liquid GB, GD and GF in humans and estimated percutaneous LD_{50} of each agent to be: GB 1.5-1.7 g/man, GD: 0.35 g/man and GF: 0.54 g/man. Those authors deduced a ChE_{50}, for GD, of 0.06-0.07 g/man.

Silver (1964) also assessed GD vapour exposure and in the one individual who attained a 65% depression of ChE there was no vomiting or other signs of poisoning. It is worthy of note that the air flow over the applied GD in this individual was virtually zero so that this dose may be regarded as at least partially occluded.

Animal studies confirm the relatively higher toxicity of GD over that of GB. The original German work (Evans et al 1946) implies a ratio of 2 in rhesus, 5 in dog, 1.5 in cats for inhalation exposure. For liquid agent, applied to clipped rabbit skin, Cullumbine et al (1954) determined that the ratios of LD_{50}/ChE_{50} for GB and GD were of 18 and 19 respectively. Rabbit skin is, however, a poor model for human skin and in addition Tregear and Dirnhuber showed that at 32C the fraction of liquid GD that penetrates rabbit skin compared with that of GB is 0.0078:0.0013. The authors concluded that the difference in the fraction which enters skin (6-fold) implies that the intrinsic ratio of toxicities of GD/GB is three–fold, which is in line with other estimates of 3 to 5.

With these considerations, the proposed toxicity levels for GD, are as follows. They are based upon these assumptions: 1) GD is approximately 3-4 times as toxic as GB, 2) the amount of GD absorbed from dermal application is higher than that for GB (though not as great as that recorded for rabbit), 3) the slope of the dose-mortality and dose/ChE inhibition lines are similar.

Table 4.1 Recommended levels for hazard of GD vapour on the skin of the human torso and limbs (excluding head and neck region) mg min m^{-3}.

Effect (section 1.5)	Range/mg.min.m^{-3}	Recommended Value/mg.min.m^{-3}
Threshold for death	Lack of data	2000
Significant decrement in military performance	Lack of data	1500
Negligible military	300-400	350

A5. GF Vapour

5.1 Evidence considered

There are no documented studies of the effects of vapour-only exposure of humans to GF vapour, and there is only one study which has assessed the effects of liquid GF in humans (Cullumbine et al 1954). The existing human estimates of the toxicity of GF vapour appear to be based solely upon a single study in monkeys coupled to judgements upon the relative toxicities of liquid GF to other liquid G-agents derived from studies in animal species (Cullumbine et al 1954).

As part of these assessments some cognisance has been taken of the physico-chemical properties of GF and its closer similarity to GA and GD (rather than GB) as evidenced by vapour pressure (Hunt G.A. CBD TP 624, Jan 1994).

AGENT	VAPOUR PRESSURE mmHg x 10^4 at 25 ^{0}C
GA	350
GB	21,000
GD	3,400
GF	440

In this assessment the following reports/studies have been evaluated:

See annex E

5.2 Assessment of the hazard of GF vapour through human skin.

5.2.1 Human studies

The only study with GF in humans that assessed the degree of inhibition of RBC ChE was carried out with liquid application to the inner side of the forearm (Cullumbine et al , 1954). 21 men, wearing respirators, were exposed to either single or multiple drops of liquid GF. 6 men received a single 5 or 10 mg drop and 15 men received between 5 and 30 mg doses as 0.5mg drops. Within the same experimental design, men were also exposed to liquid GD and GB so that comparative responses could be made.
Single drop exposures of six men to 5 or 10 mg of GF on bare skin produced mean 24 hour maximum ChE inhibitions of 31.3 and 35% respectively. The same doses given as 0.5 mg drops produced mean inhibitions of 3.0% and 0%. 20 and 30mg given as 0.5mg drops produced 24 hour inhibitions of 16.7 and 57.3 %. None of the men experienced any signs or symptoms of effects.

For comparison, GD as single drops of 10mg and 15mg produced mean inhibitions of 34.3 and 26.0% and as multiple 0.5mg drops inhibitions of 12.7% (10mg), 18.3% (20mg), 22.3% (30mg) and 27.1% (40mg). Some of these men experienced local effects of sweating and “twitching” at the sites of application. Most of these effects were apparent at 30 min after dosing, but in one case not until 8 hours after dosing. On the basis of these studies it was estimated that the ChE_{50} for bare skin contamination of human skin with liquid agent was around 30 mg/man for GF and around 60-70 mg /man for GD suggesting a potency ratio of around 2. The LD_{50} for GF was estimated to be 540 mg/man and that for GD as 300-350 mg/man. These differences in the toxicity may reflect not only differences in the intrinsic systemic toxicity of these agents, but also the differences in volatility of the two agents (see table).and their skin penetration rates.

5.2.2 Animal studies

The toxicity of percutaneously applied liquid GF to rabbits was also determined by Cullumbine et al (1954) (LD_{50} 0.57 mg kg^{-1}) however, the skin was chemically depilated which invalidates the results for human estimates of toxicity.

Two studies by McGrath et al (1953) have reported the toxicities of percutaneously absorbed GF in rhesus monkeys . The first study was done in clipped monkeys, following initial assessment in mice, and the second in clipped monkeys wearing various items of clothing. Care was taken to avoid inhalation absorption of agent however the exposures were conducted until the animals died. The experimental design (exposure until death) has seriously compromised the interpretation of the results. It is almost certain that the calculated LCt_{50}s significantly underestimate the toxicity of the agent as a consequence of the delayed absorption of agent from the skin. The estimated LCt_{50} using this methodology for rhesus monkeys is 10,000 mg min m^{-3} with 95% confidence limits of 6900 -14500 and a slope of 1.85.

5.3 Estimates of toxicity for man for percutaneously absorbed GF vapour

The existing estimates of human toxicity are based upon extremely limited data. The current US and UK estimates are based upon scant information and predominantly upon a single study in monkeys. The methods which involved continuous exposure to death (McGrath et al,1953) result in low confidence in the accuracy of these results.

The historical estimates by the USA have been related to the studies of McGrath et al (1953) and in general assess the toxicity of vapour percutaneously to be 15,000 (LCt_{50}) and 8,000 mg min m^{-3} (ICt_{50}). The rationale for these estimates is unclear.

More recent US estimates (NRC report, 1997) recommend an estimate of 2,500 mg min m^{-3} for the LCt_{50} for percutaneously absorbed liquid. The rationale for this lowering appears to reside entirely in the caution generated by the low degree of confidence that can be placed upon the limited experimental data.

In proposing estimates of the toxicity of this agent, at the three levels of hazard defined by the TOE, it has not been possible to develop the previous arguments further. Clearly the estimates derived from the monkey studies of McGrath et al (1953) are only of value in setting an upper value on the toxicity in this species. It is highly probable that the true estimate of toxicity in this species is lower than that derived in the study as the animals were continuously exposed until they died.

It is proposed that the studies of Cullumbine et al (1954), in humans, provide the best evidence for the relative percutaneous toxicities of GB, GD and GF. The volatility of GB on skin is such that any direct comparison of percutaneous toxicity with the intermediary volatility agents, GD and GF should be treated with caution since GB is more volatile and will evaporate more rapidly from skin. This would be expected to occur to a much lesser extent for GD and even less so for GF. However the studies of Tregear and Dirnhuber (1961) show that the steady penetration rates of liquid GB, GD and GF through rabbit skin, under closed cells, were 1.3, 0.9 and 1.3 µg cm^{-2} min^{-1} respectively. Based upon his findings, Cullumbine et al (1954) proposed that the relative percutaneous toxicities of GB, GD and GF are 1.0, 5 and 3, suggesting that the intrinsic toxicities of these agents are the most important determinants of their percutaneous toxicities, certainly from liquid application.

Taking these considerations into account the proposed Ct values for the three levels of hazard proposed by the TOE are as follows:

Table 5.1 Recommended levels for the percutaneous hazard of GF vapour to humans.

Effect (section 1.5)	Range/mg.min.m^{-3}	Recommended Value/ mg.min.m^{-3}
Threshold for death	Lack of data	2000
Significant decrement in military performance	Lack of data	1500
Negligible military	300-400	350

A6. GB Liquid

Levels	% Blood AChE Inhibition	Agreed Levels (mg/man)	Range mg/man	Effect
1	90	500	400-600	Threshold for death (3)
2	80	400	300-500	Decrement in military performance
3	50	300	250-350	No signs but burden of inhibition(1)
	LD_{50}		1500	(2)

Notes:
(1) Author concluded ID_{50} to be 350 mg/man. Inspection of data indicated quoted range is better estimate.
(2) Cullumbine estimate of LD_{50} percent liquid based on ration of ChE_{50}: LD_{50} of 4.25.
(3) Cullumbine extrapolation from Figure 1.
(4) This data was derived from forearm data.

A7. GF LIQUID

Levels	% Blood AchE Inhibition	Agreed Dose (mg/man)	Range mg/man	Effect
1	90		75-100 (1)	Threshold for death
2	70	30		Decrement in military performance
3	50	15		No signs but burden of inhibition

Notes

1. Extrapolated from Graphs in TP 399

AEP-52
(Edition 1)

ANNEX B

COMMENTS AND NOTES ON VX TOXICITY DATA

B1. VX Agent Percutaneous Toxicity

1.1. The contents of this annex represent notes based on the discussion of the group and are not precisely structured documents.

1.2. For assumptions made, see chapter 2 and annex E for references considered

B2. VX Vapour Data

Levels	% Blood AChE Inhibition	Agreed Ct Levels ($mg.min.m^{-3}$)	Reported Ct Ranges (whole body exposure)	Effect
1	90	200	89-467	Threshold for death
2	70	60	36-102	Decrement in military performance
3	50	30	20-37	No signs but burden of inhibition

Notes:

1. This data determined at 32ºC (RH 20%). For each ~ 10ºC rise in temp these numbers should be reduced by 30-50%.

2. The WG knows of no data to relate the effect of RH on the percutaneous toxicity of VX. It is expected that at high RH the penetration of VX will increase, hence the onset of signs of poisoning will be more rapid.

3. Due to inherent variability between individuals and different areas of skin it is not possible to refine the data to account for inherent differences in skin penetration.

4. No miosis results from system absorption of VX. Miosis might occur from off gassing of VX vapour from facial skin.

B3. VX LIQUID

See references in annex E

Applied to Cheek

Levels	% Blood AChE Inhibition	Agreed Dose (µg kg^{-1})		Effect
1	90	5		Threshold for death
2	70	2		Decrement in military performance
3	50	0.5-1.0		No signs but burden of inhibition

Applied to torso

Levels	% Blood AChE Inhibition	Agreed Dose (µg kg^{-1})		Effect
1	90	40		Threshold for death
2	70	35		Decrement in military performance
3	50	25		No signs but burden of inhibition

Notes

1. The levels for liquid have been separated between the forearm (body) and the cheek which may be indicative of the sensitive areas of the body.

ANNEX C

COMMENTS AND NOTES ON HD TOXICITY DATA

C1. HD Percutaneous Toxicity

1.1. The contents of this annex represent notes based on the discussion of the group and are not precisely structured documents.

1.2. For assumptions made, see chapter 2 and annex E for references considered.

C2. General Approach

Since sulphur mustard (HD) is one of the few CW Agents used in combat during WWI and trials were carried out on human volunteers between WWI and WWII, and during WWII, there are many reports of its effects in man. Given the unique nature of human skin, compared to animal skin, priority and weight has been given here to human data.

This report assesses the hazards of cutaneous exposure to HD vapour. Consideration has been given to the area of the body exposed, the conditions of exposure (temperature and humidity) and how these compare to the conditions under CB protective clothing.

The three levels of effects are defined in this document in terms of the military requirements of operational performance. They are not defined in terms of LCt50, ECt50 or threshold doses since these definitions require: a) a level of statistical analysis and certainty that cannot be provided by the human experimental data and b) cannot be derived with the necessary degree of certainty from animal experiments.

For the purposes of setting pass/fail criteria for protective clothing, in an operational military context, knowledge of the effects of chemical agents on the skin at high temperatures (35-40°C) and high humidity (90+%) is required. The figures recommended in tables 7.1 are consistent with chamber trials where subjects were exposed under tropical conditions of temperature and humidity and were actively sweating during exposure.

The trials reported by Heinen et al (1945) suggested that an increase in temperature (from 21°C to 37°C) enhanced HD injury, both in terms of area involved and severity. Whether this was entirely due to the sweating induced or has some component due to temperature increase alone is unclear.

Increasing the humidity will clearly increase the evaporation time of the sweat which enhances HD injury, but, since the studies from which the figures below are drawn were carried out on profusely sweating volunteers, no correction need be made for increased humidity.

All the data used to derive the recommendations below were from trials carried out on subjects who had not been exposed to HD before. HD is well known to sensitize subjects on repeat exposures but the effects of sensitization to HD have not been considered in this report.
carry out their military duties for several months, will require intensive medical support, and some may die from their injuries.

C3. HD Vapour

3.1 Evidence Considered.

Of all the CW agents the “King of the War Gases”, sulphur mustard (HD), is the most studied in man. A large number of volunteer trials were carried out by the UK and US between 1919 and 1945 and were reviewed by several authors at the end of WWII. Some of these reviews are quite extensive and permit conclusions to be drawn direct from human data where the original reports are no longer available. In recommending toxicity estimates for HD vapour for effects other than death the authors have been able to use exclusively human data. Data derived from animal studies were only used in assessing the lethal dose of HD to humans.

In this assessment reports indicated in annex E were considered.

C4. Assessment of human studies

Human volunteers have been exposed either whole body or on selected regions of the body – normally the arm. In addition, whole body exposures were undertaken in a controlled chamber environment and in the field, under a variety of climatic conditions. Each of these situations is considered separately.

4.1 Exposure of the forearm

Several groups of workers have attempted to establish the threshold Ct for erythema in man. The first systematic studies were probably those reported by Marshal et al (1918). These authors investigated differences in susceptibility of individuals to mustard gas contamination of the skin by measuring the minimum amount of time to cause erythema on forearm skin exposed to saturated vapour at 20°C. Vapour was generated using a cotton plug soaked in liquid HD, in a small test tube inside a larger tube containing water (presumably to buffer any temperature changes). Prepared tubes were left for a time to allow any other vapours to evaporate before addition of HD. Exposures were carried out by holding the mouth of the small tube firmly on the forearm for fixed time.

There was a marked variation in the amount of time it took to produce a burn with saturated vapour (n=54) and coloured skin seemed to be less susceptible than white skin Reactions times of between 1 and 600 seconds were recorded and a frequency distribution of this data shows that the distribution of minimum times to produce effects approximates to log-normal (figure 1a), very similar to the distribution of penetration rates analyzed by Chilcott (2000). Assuming a volatility of 610 $mg.m^{-3}$ at 20°C, it is possible to

convert the minimum burn times to Cts (figure 1b). The threshold Ct for erythema varied in this study from 10-6100 mg.min.m^{-3}. These figures relate to the "white" subjects in Marshall's studies. Of those exposed to vapour, 10 were coloured and showed a much reduced sensitivity to HD, all with reaction times of 600 seconds.

The same authors also report some exposures to solutions to HD in paraffin (1, 0.1 & 0.01% w/v) solutions of HD. These were much larger scale tests (n=1629 whites and 84 coloured) and showed that overall, coloured individuals were less sensitive than whites. No coloured subject reacted to the 0.1% solution whereas 7.5% of the whites tested did and only 15% of the coloureds reacted to the 1% but 68% of the whites did. This supports the view that coloured skin is less sensitive but does not permit the difference to be quantified.

These figures must be interpreted with some caution. The authors comment on differences in the vesicating potency of different "batches" of HD, probably due to differences in purity of the agent. Though at this time they did not have the analytical capabilities of modern laboratories, they quote the physicochemical properties of the agent they used for the vapour studies (bp217°C, mp14.5°C, vp0.06mmHg, density 1.274 @ 20°C) which compare well with the pure material ($bp_{(760)}$ 215-217°C, mp 14.4°C, density 1.278; *Somani SM. In: Chemical Warfare Agents pp16 Acad. Press, 1992*).

Assuming the HD used by Marshall and his co-workers is pure, their data support the conclusion that a Ct of 50 mg.min.m^{-3} on cool dry forearm skin would cause erythema in only 7% of the white population. A smaller percentage of the coloured population would react to this Ct but Marshall's data does not permit an accurate estimate of this percentage. This figure is lower than would be expected from the limited whole body studies reported in the PCS and by Heinen et al (1945). Possible explanations for this are the difference in the conditions (i.e. cool dry skin in the Marshall study and hot wet skin in the whole body exposures), or that exposure of the whole body reduces the threshold for effects.

The observation that vesication occurs at lower Cts when the whole body is exposed rather than when small areas (i.e. a few cm^{-2} of the forearm) are exposed in isolation, appears valid. Erythema and blistering of the arms for instance was observed at between 100-200 mg.min.m^{-3} after the whole body exposures carried out in the US and AU. However the vapor train studies of Black et al (1944), and the studies where small areas of the arm were exposed in isolation (Marshall et al 1918), identified higher figures for both threshold erythema (6-6000 mg.min.m^{-3}) and blistering (2200 mg.min.m^{-3}). The possibility that small areas of skin could resist higher Cts of HD than the whole body, cannot therefore be excluded.

Black et al (1944) performed a series of experiments with a more sophisticated "vapor train" apparatus. It was concluded that the median blistering dose for cool dry skin was 2500 mg.min.m^{-3} (for exposure times of 30 – 60 mins) and 2200 mg.min.m^{-3} for sweating skin. However, erythema was induced at the lowest Ct of 410 mg.min.m^{-3} as illustrated by the number of volunteers with hot sweating skin reacting (table 4.1).

Table 4.1 - Response of sweating men to HD vapour. (32-34°C; 80-95% RH, 10 minute exposure)

Ct (mg.min.m^{-3})	Number showing erythema	Number showing blister formation	Size of blister (MM)
420	4/4	0/4	
715	12/12	0/12	
1160	12/12	0/12	
1450	12/12	2/12	2
1690	12/12	4/12	8.5
1940	12/12	7/12	7.7
2050	4/4	1/4	10
2350	12/12	7/12	8.9
2430	12/12	7/12	6.7

Black et al's (1944) median blister Ct is consistent with Marshall et al (1918) but erythema was clearly demonstrated at much lower Cts.

The results presented by Nagy et al (1946), though not seeking to define threshold, are consistent with Marshall's work. Using a sophisticated vapour-generating device, Nagy and his co-workers measured the amount of HD penetrating the skin and related this to degree of injury at 48 hours. Ct's of 2300-7700 mg.min.m^{-3} (T = 21-23°C; t = 3-10 mins) and 2900-8800 mg.min.m^{-3} (T = 30.6°C; t = 2-6 mins) all produced erythema or vesication on the forearms of 6 to 12 volunteers. This report does not however describe the time course of the lesion and the reader is left to assume that the 48 hour lesion is the most severe reaction. Given the descriptions of time courses after whole body exposures described elsewhere (PCS, 1946; Heinen et al 1947) this seems unlikely.

4.2 Whole body exposures

The "Technical aspects of Chemical Warfare in the field" compiled by the US Project Coordination Staffs and published in 1946 (PCS 1946) is an assessment of the use of chemicals as offensive weapons. The estimates given in the report are probably not therefore suitable for the defensive purposes of the TOE. However, appendix 2 of the second volume gives an extensive list of the data from which these estimates were derived, including exposure conditions, Cts, numbers of men exposed and the effects recorded. The sources from which these data are drawn are US and UK chamber and field trials carried out during WWII. Most of the UK trials were carried out in Australia and India. Though most of the reports reviewed by the PCS have now been located and the relevant ones are reviewed herein, the PCS review is a good overview of the available data and is summarized as a starting point for this review.

The PCS divided data by exposure conditions into hot and humid (25-34°C, 42-89% RH), warm (21-27°C, 19-95% RH) and cool (14.4°C, 59% RH) weather. The hot and humid weather conditions are most relevant to the conditions under CB protective clothing. The state of dress of the volunteers was either normal combat clothing, KD shorts and tee shirts or in some of the higher Cts, impregnated protective shorts. For the purposes of this review it is assumed that volunteers wearing protective shorts and showing erythema or worse on unprotected areas, would have shown injuries to the genitalia if not protected.

In summary, 19 individuals were recorded as having been exposed to Cts of between 50 and 113 mg.min.m^{-3} (times between 5 and 63 minutes), 133 between 125 and 200 mg.min.m^{-3}, 27 between 220 and 300 mg.min.m^{-3} and 13 between 480 and 1040 mg.min.m^{-3}. Descriptions are given of most of the injuries produced and these are summarized in table 2.

Table 4.2 – Summary of effects of HD reviewed by the PCS (1946)

Ct (mg.min.m^{-3})	no effect	Erythema			Vesication
		Mild	Mod.	Severe	
50-113	2	12	3	2	0
125-200	0	13	86	8	26
220-300	0	0	0	1	26
480-1040	0	0	0	0	13

Between 50 and 113 mg.min.m^{-3} (C = 1-20 mg.m^{-3}; t = 5-63 mins) no volunteer showed any effect more severe than erythema and most of the reactions were mild. Ten of the volunteers were inactive during exposure and the remainder were exercising and wet with sweat. The more severe reactions were in the areas of the scrotum and axillae with only trace reactions of the chest, arms, back and legs.

Of the 133 volunteers exposed to Cts between 125 and 200 mg.min.m^{-3} (C = 2-27 mg/m^{-3}; t = 5 – 87 mins) all except 12 were exercising and wet with sweat when exposed. There was a single exposure of 82 men at 144 mg.min.m^{-3}. All volunteers showed effects in areas, other than the crotch, ranging from generalized erythema to the formation of blisters.

Four of the 27 volunteers exposed to Cts of 220-300 mg.min.m^{-3} (C = 4-10 mg.m^{-3}; t = 30-60 mins) were protected with impregnated shorts. All of the remainder had reactions in the genital region, which should be classed as casualty producing, since all required medical attention. Many of these men however completed an assault course daily during the trial. Ten of the men were unable to carry out duties and/or required hospitalization at some point during the trial.

All of the men exposed to Cts between 480 and 1040 mg.min.m^{-3} (C = 16-47 mg.m^{-3}; t = 16-62 mins) were incapacitated by their injuries and experienced nausea or vomiting during the early stages of the trial. Seven of the men required hospital treatment.

4.2.1 Chamber Trials

In a series of chamber exposures carried out in India in 1942 (CDRE India Report 245), men were exposed to Cts of HD from 40.9 to 176 mg min.m^{-3} (C = 1.8 – 26 mg.m^{-3}; t = 5 – 63 mins). In the first series of exposures men were exposed in groups of three or four wearing light anti-gas suits and respirators. Two inch diameter holes were cut into the arms and legs of the suits and the right hand was exposed in order to establish the Ct for the next series of experiments. In the next series, men were exposed wearing oilskin trousers and open neck cotton shirts with the sleeves rolled up (plus respirators and rubber boots). In the third series of experiments, the men wore ordinary KD and open necked shirts but oilskin underpants to protect the genital region. In the final series of experiments the men wore ordinary tropical battle dress. The exposures are summarized in table 4.3.

Table 4.3 – Summary of exposures to HD vapour carried out at Rawalpindi, India (1942)

Exp No	Date	Mean Temp (°F)	Min RH %	Conc^{n}. (mg.m^{-3})	Duration (mins)	Ct (mg.min.m^{-3})	No of subjects
SERIES 1							
1	23/7/42	89	49	6.25	7.5	40.9	4
2	27/7/42	91	51	7	10	70	4
3	29/7/42	95	44	6.8	10	68	2
4	31/7/42	89	51	10.6	11	116.6	3
5	18/8/42	80	56	9	15	135	4
6	19/8/42	84	62	6	26	156	4
SERIES 2							
7	2/9/42	86	51	10.3	13.3	137	3

SERIES 3							
8	3/9/42	85	64	12	12	144	3
SERIES 4							
9	7/9/42	86	57	13.8	5	69	3
10	8/9/42	86	50	20	5.5	110	3
11	16/9/42	92	39	1.8	63	113.4	3
12	17/9/42	92	42	2.7	60	162	3
13	18/9/42	86	46	5	25	125	3
14	24/9/42	73	57	3.2	35	112	3
15	28/9/42	80	31	8.85	15	132	4
16	30/9/42	80	19	8.4	21	176	3

All those exposed in exposure 1 (40.9 mg.min.m^{-3}) showed no reaction, the most severe reaction in exposures 2 and 3 (70 & 68 mg.min.m^{-3}) was a raised erythema and some pigmentation whilst exposure 4 (116.6 mg.min.m^{-3}) produced nothing more than a trace erythema of the hand.

The exposure which formed series two (137 mg.min.m^{-3}) produced a erythema of the chest, back and shoulders in one of the subjects but nothing more than a trace erythema of the upper arms and chest in the other two.

Exposure 8 (series 3; 144 mg.min.m^{-3}) produced a generalized low-grade erythema over the chest, back, arms and legs of all three subjects with the areas protected by the respirator and protective shorts delineated in some. Some of the areas showing pigmented erythema in one subject. The exposures of unprotected men carried out in series 4 produced more marked effects.

Exposure 9 (69 mg.min.m^{-3}) produced irritable crutch and scrotal erythema in 1 of the 3 subjects, itching of the knee flexures I of 3 and trace erythema of the chest, back and arms or thighs in 2 0f 3. Similar reactions were produced by exposure 10 (110 mg.min.m^{-3}) except that one subject developed a “papular erythema” of the chest and back. No reaction in the crutch is mentioned in the other two. Exposure 11 (113.4 mg.min.m^{-3}) produced little visible effects with trace erythema on the back and scrotum of one subject and complaints of irritation in the other two. Exposure 12, to 162 mg.min.m^{-3} , produced a generalized erythema with vesication of the arms and torso of some subjects. The genitalia were erythematous and desquamated in two of the three and were irritated in the

third. The injuries were considered of casualty severity in all three subjects. Exposure 13 (125 mg.min.m^{-3}) produced similar but less severe injuries. A widespread erythema involved the arms, neck, chest, back and flanks, and the flexures of the limbs were irritated. In two of the three the scrotum was irritated and in one case desquamated. The clothing was worn for four hours after exposure 14 (112 mg.min.m^{-3}) with no more effect that a mild irritation of the torso, and an irritable crutch in 2 of 3 subjects. At the end of the last two exposures (15, 16) the subjects are recorded as leaving the chamber with cool dry skin. Exposure 15 (132 mg.min.m^{-3}) produced mild to moderate erythema and irritation of the axillae and scrotum in the two subjects who wore their clothes for four hours after exposure. The two subjects who bathed and changed immediately after exposure experienced very little effect. Exposure 16 (176 mg.min.m^{-3}) produced similar results with the two subjects who bathed and changed immediately after exposure experiencing little effect other than an irritable crutch. One subject who remained in his clothes for four hours after exposure developing erythema of the back and irritation of the scrotum with dry desquamation.

The racial origin of the subjects in the Indian studies is not clear from these reports. They are quoted as being drawn from the “British Army” but photographs of subjects published in the reports show them to be of Asian (possibly Indian) origin.

Two British subjects were exposed to a Ct of 750 mg.min.m^{-3} during a period of 16 minutes at 87°F and 84% RH (CDRE India Report 285). These men were protected with CC-2 impregnated drawers but were badly burned over the rest of their bodies. They were hospitalized for 19 and 28 days respectively.

In a study to evaluate the value of shorts impregnated with CC2, using various methods in protecting personnel, men were exposed to HD vapour (Freeman & Rollins 1943). It is clear that even the ordinary clothing worn reduced the effectiveness of the HD when compared to the Australian results (Gorrill 1943). Similar injuries to those produced by Australian workers with Cts of 50-59 mg.min.m^{-3} were sustained in Freeman and Rollin’s study after analytical Cts of 135 mg.min.m^{-3}. Men were dressed in herringbone twill suits with light wool socks, service shoes, gas masks and steel helmets. They were exercised so that they were sweating profusely before entering the chamber, exercised during exposure and for 4 ½ hours afterwards. For three days after the exposure they were exercised daily. Men were exposed in groups of four or six, of whom normally only two did not wear protective underpants. After an exposure of 135 mg.min.m^{-3} the two unprotected volunteers showed only very minor erythema of the neck and trunk, one man had some pin head vesicles. At 270 mg.min.m^{-3} the two unprotected men had mild erythema of the scrotum and one desquamated, there was some erythema of the trunk, neck, arms and legs and one man’s arms and legs vesicated. At 300 and 570 mg.min.m^{-3} all four volunteers were extensively erythematous with vesication occurring of the arms, legs, trunk and neck. The scrotum was erythematous and desquamated or vesicated. These data are summarised in the table below:

AEP-52
(Edition 1)

(US) Table 4.4 - Summary of effects on subjects without protective shorts from Freeman and Rollins (1943)

Ct (analytical)	C (analytical)	Time (mins)	Temp (° C)	RH (%)	n	scrotum	axilla	Trunk	neck	arms	Legs	head	vomit	Battle Casualty
135	27	5	30	78	2			2E-, 1PV	2E-					
270	9	30	28	73	2	2E-, 1D		1E, 1D	1E, 1D	1E, 1D, 1V	1E,1 D, 1V			
180	9	30	28	73	0									
300	10	30	27	79	2	2E, 1V, 1D		2E,	2E,	2E, 2D	2E, 2D			2
570	19	30	30	66	2	2E, 2D	2E,	2E,	2E, 2V	2E, 2V	2E, 2V	2E,	2	2
480	16	30	29	69	0									

KEY (After Sinclair, 1944)

E-	Just perceptible erythema
E	Definite erythema
E+	Erythema with oedema
D	Desquamation
PHV	Pin Head Vesicles
V	Frank vesication
M	Moist desquamation

In a series of trials carried out between in 1944-1945 Heinen and his co-workers (Heinen et al 1945) exposed a total of 212 men to HD in 33 separate tests. These trials were not reported in the PCS review. Men were exposed in the chamber described by Freeman and Rollins (NRLR P-2208) to Cts of 54-695 $mg.min.m^{-3}$ (C = 1.6-12 $mg.m^{-3}$, t = 30-60 mins, RH = 35-86%). Men were dressed in skivvy shirts, Nainsbrook shorts, watch caps, blue denim shirts, dungaree pants, standard socks and shoes. In some tests the Nainsbrook shorts were replaced by CC-2 impregnated shorts of the rib-knit variety impregnated by the aqueous process (05 mg $Cl.cm^{-2}$). In others carbon coated cloth suspenders were worn. No mention is made in the report of the length of time the men wore their clothes after exposure. The men did not exercise during exposure and led sedentary lives before and after exposure with occasional mild athletics.

Heinen made the general observation that the mild erythema he recorded was not a good measure of a threshold effect because it could be easily confused with erythema resulting from causes other than the HD challenge. Moreover, none of the men exposed during

these tests showed “actual bleb formation” which was observed in the Australian studies (see below).
When tests where carried out under similar conditions of relative humidity and temperature there was a dose dependent increase in severity and extent of the lesions produced by Cts of between 50 and 600 mg.min.m^{-3}.

There was also a temperature dependent change in the relative potency of mustard. HD vapour produced a severe generalized erythema at a Ct of 200 mg.min.m^{-3} at 100°F (38°C). A similar erythema was produced by 200-300 mg.min.m^{-3} at 90°F (32°C) but at a temperature of 70°F (21°C) this reaction was not achieved even by a Ct of 500-600 mg.min.m^{-3}. Moreover, a Ct of 600 mg.min.m^{-3} produced such severe reactions in the genitalia and the axillae at low temperatures (60-70°F, 16-21°C) that the sensitivity of the general body surface at low temperature could not be determined.

The data on the effect of increasing relative humidity are less convincing but do show that increasing the RH at constant temperature does seem to increase the severity and extent of the resulting lesion. Increasing the temperature seemed to decrease this effect. Identical exposures carried out in spring and summer showed that above a Ct of 150 mg.min.m^{-3} there was an appreciable increase in the severity of the skin damage in the summer. It is not clear whether the men changed their clothing soon after exposure. If, as was common practice in other studies, they remained in the clothes they were exposed in for several hours after exposure the possibility that they continued to absorb vapour from their clothes could not be excluded. The ambient temperature and humidity would influence such post exposure absorption.

Heinen et al’s (1945) observation, that the injuries to the penis and scrotum produce the casualties and that the neck is a sensitive area, is consistent with most other studies.

In a series of “special tests” Heinen et al (1945) investigated the effect of sweating, drying the skin and lanolin on the response to HD vapour. The results were consistent with the hypothesis that the degree of sweating determines the severity and extent of the lesions. Cooling of the subjects prior to exposure reduced the severity of the injury, as did drying the skin with aluminum chloride powder, which was graphically demonstrated by drying one side of the scrotum in some subjects.

Heinen et al (1945) concluded that whilst temperature did not change the severity of the burns of the genitalia and axillae, increasing the temperature did change the configuration of the axilla injury from a central lesion at low temperatures, to a more generalized injury which spared the central area at 90°F and above. There was a generalized intense erythema produced by a Ct of 250 mg.min.m^{-3} at 90°F and by 200 mg.min.m^{-3} at 100°F (37.7°C). Moreover, a Ct of 500 mg.min.m^{-3} at 70°F (21°C) and one of 600 mg.min.m^{-3} at 60°F (16°C) only produced moderate erythema over most of the body but more severe lesions in the genitalia and axillae.

Concurrently with the studies being performed in the US a series of studies was carried out in Australia by British scientists. The portable exposure chamber used in the Australian

chamber trials of 1943-44 and subsequently, has been described in a separate report (CD (Australia) report 13), as have the details of sampling and anlaysis used under tropical conditions (CD (Australia) report 26). Details of methods of maintaining chamber temperature, humidity and exposure concentration are given.

The tests carried out in Townsville at the beginning of 1943 are reported in a Progress Report (Gorrill, 1943) which details chamber trials with unprotected and protected men. The unprotected men wore standard tropical battle dress – shirts with long sleeves and open necks, underpants, long trousers, socks, boots and anklets. All wore respirators and fur felt hats (Australian pattern). Chamber exposures of unprotected volunteers were carried out at 80-100°F (27-38°C) and 70-90% RH to Cts of 56-660 mg.min.m^{-3} (C = 106-15.9 mg.m^{-3} t = 12-60 mins); chamber concentrations were sampled at the time of exposure and agent estimated by the iodoplatinate method (details of analysis methods in CD (Aus) Report No. 26). In all 9 groups of men were exposed, one group of six and eight groups of four. Men exposed to Ct's of 56 and 59 mg.min.m^{-3} developed erythema of the sensitive areas.

An attempt to determine the effect of temperature and humidity on HD injury is reported in CD (Australia) report 40. Exposures of six groups of between 6 and 10 men to Ct's of 150-220 mg.min. m^{-3} (t=57-70 mins) are described. The exposures were carried out at two temperatures 70°F (21°C) and 90°F (32.2°C) and at relative humidity between 62 and 95 percent. Men were dressed in Australian tropical battle dress and exercised (by marching around the chamber and carrying weighted ammunition boxes) during exposure. The results support the authors conclusions that relative humidity did not affect injury whereas increasing temperature increased the severity of injury in this Ct range. It is important to note that those exposed and 90°F (32.2°C) were recorded as profusely sweating whilst those exposed at 70°F (21°C) were "skin damp – dry clothing".

In another study five groups of five men were exposed to HD vapour to determine the effects of exercise on HD injury (CD (Australia) report 41). The first three groups were field trials and the last two chamber trials. The two groups exposed in the chamber were exposed to a Ct = 170 over 87 minutes at 77°F and 83% humidity, one of the group was exposed at rest and one during exercise (marching around the chamber and carrying a weighted ammunition box). The group exposed at rest developed scrotal erythema (4/5) and minor erythema in other parts of the body. Those exercising, developed generalized erythema (3/5) scrotal erythema (5/5) and oedema (2/5) with, in one case, breakdown of the scrotal epithelium; one subject developed oedema of the axillae and gluteal folds. Clothing was worn for a period of 4 hours after the exposure. The results support the authors conclusions that exercise increases the severity of lesions produced by HD vapour though it must be assumed that the exercise resulted in some sweating which was not necessarily present in the men who were exposed at rest.
Gorrill and his co-workers also sought to establish the relationship between Ct and physiological effect reported in CD (Australia) Note 50. Four groups of men were exposed to Cts of 50, 125, 120 and 220 mg.min.m^{-3} (t=55-63 mins, T = 90°F (32.2°C), RH =85%).

The first two groups were exposed during the Australian summer and the second two, four months later when the weather was cooler. The exposure of the second group was described as “anomalous” because the chamber mechanism exposed the group to the half the Ct in the last 15 minutes of exposure. Most of the group (10 men) exposed to 50 mg.min.m^{-3} developed erythema of the scrotum, neck, forearm and trunk with some other areas affected in some of the men. This erythema was not reported as severe and was scored as ‘E’ and ‘E-‘ on the scale used. One of the group develop pin-head vesicles on his forearm. The group exposed to 125 mg.min.m^{-3} (8 men, half Ct given in last 15 minutes) all developed a generalized erythema and most desquamated or vesicated in the sensitive areas. The group exposed to 120 mg.min.m^{-3} (8 men) in the cooler weather developed erythema of the sensitive areas (scrotum, neck) and some less sensitive (thighs and trunk) with some showing this effect in other areas. All those exposed to a Ct of 220 mg.min.m^{-3} (10 men) developed generalized erythema and most desquamated or vesicated. The descriptions of the injuries sustained by group four indicate that they would have required treatment and three of the ten were hospitalized at the time.

The effects of repeated Cts of HD (T = 85-86°F (29.4-30°C); t = 15-51 mins) was also investigated (CD (Australia) Note 56). Men were exposed dressed in KD shorts and trousers, gaiters, fur felt hats, webbing, light haversack and respirators, they kept their clothing on for four hours after exposure. The first group (4 men) was exposed to four divided Cts separated by four days each to a total of 280 mg.min.m^{-3}. The second group (5 men) were exposed to 300 mg.min.m^{-3} in four divided Cts of 75 mg.min.m^{-3} separated by two days each and the third group (8 men) were exposed in two divided doses separated by one day to a total Ct of 270 mg.min.m^{-3}. Four of the men in the last group were exposed at rest, all the other volunteers were exercised by marching and carrying a weighted ammunition box during exposure. A fourth group (8 men) was exposed to 100 mg.min.m^{-3} in a single exposure, four men at rest and four exercising. The results support the conclusion that the effects of HD are less severe if given in divided Cts.
In a trial designed to test the effect of exposure time on response to HD under tropical conditions four groups of subjects were exposed to a Ct of 1250 mg.min.m^{-3}. Exposure times were 40 mins (97°F, 90%RH), 2 hours (88°F, 84%RH), 4 hours (87°F, 75-85%RH) or 4 hours (92°F, 90%RH). The men were protected with impregnated long underwear, shirts, trousers, hoods & socks, boots and respirators. Concentrations in the chamber were confirmed by analysis using the iodo-platinate method. All of the subjects vesicated on the arms and legs within 72 hours.

Quantification of skin burns
There have been a number of attempts to devise methods of quantifying skin burns in terms of severity and extent. The two most useful appear to be that developed at Porton and used by Sinclair (1949) to report on his own studies and on the clinical aspects of the Australian trials (CD (Australia) report 42 and 55) and that used by Heinen et al (1944). Both systems are essentially subjective scoring systems based upon mild, moderate and severe erythema and oedema, desquamation and frank vesication. In order to make a more informed assessment of the data from the various chamber trials reviewed here those studies which present data in this form, or in a form readily converted to it, have been combined and are presented at Annex A.

4.2.2 Field Trials

A number of field trials of HD generating weapons systems were made during the period 1940-45. During these trials men were exposed to clouds of agent produced in a variety of ways with the intention of demonstrating the physiological effects of the clouds.

All the field trial reports reviewed give Ct values and details of the methods of sampling and analysis by which these were estimated. However, current knowledge of concentration changes within clouds of vapour indicates that such Cts may not be reliable estimates of the true Ct to which each man was exposed, when compared to controlled chamber trials. Moreover, it is apparent from the analysis of clouds produced during the testing of delivery systems that they were a mixture of vapour and aerosolized droplets. Since HD will be present under protective clothing as a vapour such challenges are not relevant to the current problem. The field trial reports(Nos 50-58 Annex E) were assessed during the preparation of this report and found to be consistent with the conclusions of the chamber trials.

C5. The lethality of cutaneously absorbed HD vapour

In order to estimate the lethal cutaneous Ct of HD in man the lethality of the agent to animals must be considered and compared to highest Ct a human has survived. A number of animal studies were conducted during WWII in the US where the LCt_{50} of HD, after body only exposure, was estimated in a variety of species. In some cases the data which support the estimates is weak and does not support probit analysis. Nevertheless broad estimates of the range of Cts causing death may be made.

(US) Table 5.1 Estimates of LCt50 for body only exposure to HD in animals:

Species	Range of LCt_{50}	Source	Comments	Exposure time (mins)
Mouse	2000-5000	NDRC (May 1943) OSRD 1391 NDRC (July 1943) NDRC (October 1943)	4 Cts, n = 10 at each 5 Cts, n = 7-10 at each (2 flow rates)	10 10
Monkey	10,000-20,000	NDRC (November 1943) NDRC (January 1944)	3 Cts, n = 1 at each - no deaths. 3 Cts, n = 1 at each – 2 deaths.	20-60 45-100
Cat	6,000-16,000	NDRC (July 1943) NDRC (October 1943)	1 Ct, n=1 !!!!	78
Dog	6,000-11,000	NDRC (July 1943)		

Guinea pig	24,000	NDRC (October 1943)	4 Cts, n=4 at each	32-80
Rabbit	4,000-7,000	NDRC (October 1943) NDRC (November 1943)	4 Cts, n=1 at each 2 Cts, n = 1 at each – no deaths	32-80 13.5-18
Rat	2,200-6,500	NDRC (July 1943) NDRC (October 1943)	3 Cts, n=5-6 at each	10-30

Estimating an LCt_{50} for man from these figures is difficult. The confidence in the animal estimates is low and the skins of all the species tested are very different from man. All the animals are fur bearing and none of them produce eccrine sweat. Given the apparent high sensitivity of human skin for injury by HD the most sensitive species should be given weight. Therefore the best conclusion which can be drawn is that the LCt_{50} for man will be in the range 2,000-10,000 mg.min.m^{-3}, with a best estimate of 5,000 mg.min.m^{-3} for exposure times of 10 to 60 minutes. A low confidence can be attached to this estimate.

C6. General discussion

There are two factors which are critical to interpreting the effects of Cts in human exposures in the chamber or in the field, 1 – The degree of confidence in the accuracy and precision of the measurement of exposure concentration and 2 – the clothing worn by the volunteers during exposure.

6.1 Analytical considerations.

Most reports identify the analytical methods used to measure concentrations in chambers and in the field. The US reports give good details of the concentrations achieved in their chambers within the individual experimental reports. In addition the performance of the analytical methods in common use by each nation at the time that these studies were conducted are summarized in:

UK - Porton Memorandum 19.
AU – CD (Australia) 26
US – TDMR 731 and NRLR P-2208

The two methods which were in routine use were the iodoplatinate method and the bromine method. The performance of these in detecting HD and its breakdown products is summarized in Porton Report 2377 and Porton Memorandum 19.

6.2 Clothing

Some authors have concluded that ordinary clothing does not affect HD injury, but comparison of the results reported by Gorrill (1943) and Freeman and Rollins (1943) shows that this is probably not a valid conclusion.

Gorrill's volunteers were dressed in tropical dress and sustained injuries at lower Cts than those in Freeman and Rollin's study who were dressed in dungarees, shirts and helmets. Moreover, most reports highlight the injuries to neck and wrists and pictures presented in TDMR 731 and P-2579 delineate the neck -line of the clothing, consistent with the clothes worn protecting the skin of the shoulders and torso but not the neck. The clothing status of volunteers in the studies quoted by the PCS are detailed below as taken, where available, from the original reports.

Table 6.1 - Summary of clothing worn in chamber trials

Report	**Clothing worn during exposure**	**Duration of wear after exposure**
Freeman & Rollins 1943	Herringbone twill suits with light wool socks, service shoes, gas masks and steel helmets plus service respirators.	4 ½ hours
Heinen et al 1944	skivvy shirts, Nainsbrook shorts, watch caps, blue denim shirts, dungaree pants, standard socks and shoes and service respirators.	Not specified
CDRE India reports 245	Series 1 light anti-gas suits and respirators, holes cut into arms and legs of the suits, right hand exposed Series 2 oilskin trousers, open neck cotton shirts with sleeves rolled up, plus respirators and bubber boots. Series 3 ordinary KD and open necked shirts but oilskin underpants to protect the genital region Series 4 Ordinary tropical battle dress.	4 hours except 1 exposure wear 2 subjects changed and bathed immediately
CDRE India reports 285	CC-2 impregnated protective drawers, unimpregnated tropical battle dress, socks, ankle boots, anklets, and light respirators	2 hours
Gorrill 1943 (Townsville progress report)	Standard tropical battle dress – shirts with long sleeves and open necks, underpants, long trousers, socks, boots, anklets, respirators and fur felt hats (Australian pattern).	Half changed immediately, half after 4 hours
CD (Australia) Report 40	Fur felt hats, khaki trousers, drill shirts, with sleeves rolled up, boots, sock, full webbing equipment and respirators (two groups wore impregnated underpants)	4 hours

CD (Australia) Report 41	Ordinary Australian summer battle dress (without underclothes). Shirt sleeves rolled up full webbing equipment and packs.	4 hours
CD (Australia) Notes 50	KD shirts and KD or JG trousers, fur felt hats, socks, boots and gaiters, respirators and full webbing equipment.	4 hours
CD (Australia) Note 56	KD shorts and trousers, gaiters, fur felt hats, webbing, light haversack and respirators	4 hours

The length of time for which the clothes were worn after exposure may also be important. This was shown by the trials at Rawalpindi (CDRE Report No 245) where the removal of clothing and bathing immediately after exposure, eliminated injury sustained by subjects exposed concurrently but continuing to wear their clothing for four hours after exposure. This is probably due to the HD absorbed by the clothing continuing to be delivered to the skin after the exposure was complete. The effect of ambient weather conditions on injuries observed in the US (Freeman & Rollins 1943, Heinen et al 1944) and AU (CD (Australia) Report Nos 40, 41and Notes 50 and 56) trials may have been due to this effect. In hot summer weather the rate of evaporation of HD off the clothing would be greater than in cool spring or winter temperatures and would result in more HD penetrating the skin in hours after exposure until the clothing was changed.

6.3 Effects of increased temperature on HD injury

There are no reports available to the authors that quantify the effect of increasing temperature alone on the Ct of HD required to produce effects. However, all the studies, which have compared the effects of mustard in a variety of conditions, have shown that effects are produced at lower Cts on hot wet skin than on cool dry skin. Studies published by Renshaw in 1947 indicate that this is due to the layer of water on the skin. Renshaw concluded, from a limited number of exposures, that it was the layer of water which increased the vesicating potential of HD. The presence of NaCl in the water, or a filter paper on the surface of the skin made no difference, provided a continuous layer of water was present on the surface. As with Nagy et al's (1946) experiments this was based upon the lesions measured at 48 hours after challenge and no descriptions of lesion progression are given. The data supports the conclusion that "A layer of water, whether containing NaCl or not, enhances the vesicating potential of HD".

6.4 Reviews

The extensive article published in the 1946 NDRC Summary Technical Report of Division 9 by Birdsay Renshaw, continues to shape attitudes towards HD today. Though no data on effective Cts are given, Renshaw's comments on exposure time are pertinent to this assessment. He states that there is clear evidence that the LCt_{50} for HD and nitrogen mustards deviates from Haber's law between exposure times of 10 and 240 minutes, but does not give quantitative details. However, the bulk of evidence available to Renshaw

indicated that for exposures of between 5 and 240 minutes there was little effect of exposure time on the Ct required to cause blistering.

C7. Conclusions and Recommendations

Data from chamber trials support the following conclusions which are consistent with observations made during field trials.

The percutaneous toxicity of HD in man is enhanced by the presence of a layer of water on the skin surface. Thus an actively sweating man will be injured at lower doses than a man with cool dry skin. Increasing temperature above the level necessary to induce sweating or exercising will enhance the injuries produced by HD vapour. Since personnel will probably be working in IPE for some time they have been assumed to be actively sweating for the purposes of interpreting the human data reviewed above and recommended Cts are for these conditions. Personnel exposed under cooler conditions who are not actively sweating at the time of exposure may be exposed to higher Cts before effects are observed. There is also some indication that skin may be less sensitive when small areas are exposed than when the whole body is exposed, though there is no rationale for this conclusion. Recommended values are for whole body exposure.

The prevailing meteorological conditions immediately after exposure can also affect the severity of the resulting burn. Burns can be expected to be worse in hotter weather particularly if the clothes worn during exposure are not changed.

Personnel will show minor effects of HD, minor erythema and irritation of sensitive areas, at a dose of 50 $mg.min.m^{-3}$. From studies reviewed here, there is no data to establish a no effect dose for whole body however from the evidence available, the reviewers consider this is likely to be in the range of 10-40 $mg.min.m^{-3}$. Up to a Ct of 100 $mg.min.m^{-3,}$ personnel will show effects of HD which will not be life threatening or impair the performance of military tasks.

A range of 10-100 $mg.min.m^{-3}$ is therefore recommended within which exposed personnel will show minor symptoms of HD exposure which will not impair military performance. The recommended value is 50 $mg.min.m^{-3}$.

Above a whole body Ct of 100 $mg.min.m^{-3}$ casualties will be produced and may require hospitalization. Above a Ct of 400 $mg.min.m^{-3}$ hospitalization will be necessary for most casualties. In most studies reviewed a value of 200 $mg.min.m^{-3}$ was identified as the value at which subjects would show generalized erythema and start to vesicate on their arms, legs and torso.

A value of 200 $mg.min.m^{-3}$ is therefore recommended as the best estimate within the range of 100-400 $mg.min.m^{-3}$ where men will be incapacitated by their injuries and require medical aid.

The extrapolation of animal lethality data to man for percutaneous toxicants is confounded by the differences in the skin of virtually all animals and man. The species discussed herein are all fur bearing and this complicates interpretation. Human skin appears to be particularly sensitive to HD so increasing the estimate of the human LCt_{50} on the basis of increased body weight and surface area would not be a valid approach. Though a limited sample, the two individuals reported in CDRE (India) Report 285 who were exposed to a Ct of 750 mg.min.m^{-3} sustained serious burns which required intensive medical aid. These two individuals also contracted secondary infections which required treatment. Without such treatment it is possible that one or both of these subjects would have died.

A Ct of 750 mg.min.m^{-3} should be adopted as a lower limit of the range where there is danger of death. This is consistent with the other human exposures reviewed here and animal studies of lethality. Given that the lowest LCt_{50} measured in any species reviewed herein was 2000 mg.min.m^{-3} a value of 1500 mg.min.m^{-3} should be adopted as the upper limit.

Table 7.1 Recommended levels for hazard of HD vapour on the human skin.

	Range	**Recommended value**
Minor symptoms	**10-100 mg.min.m^{-3}**	**50 mg.min.m^{-3}**
Incapacitation	**100-400 mg.min.m^{-3}**	**200 mg.min.m^{-3}**
Danger of Death	**750-1500 mg.min.m^{-3}**	

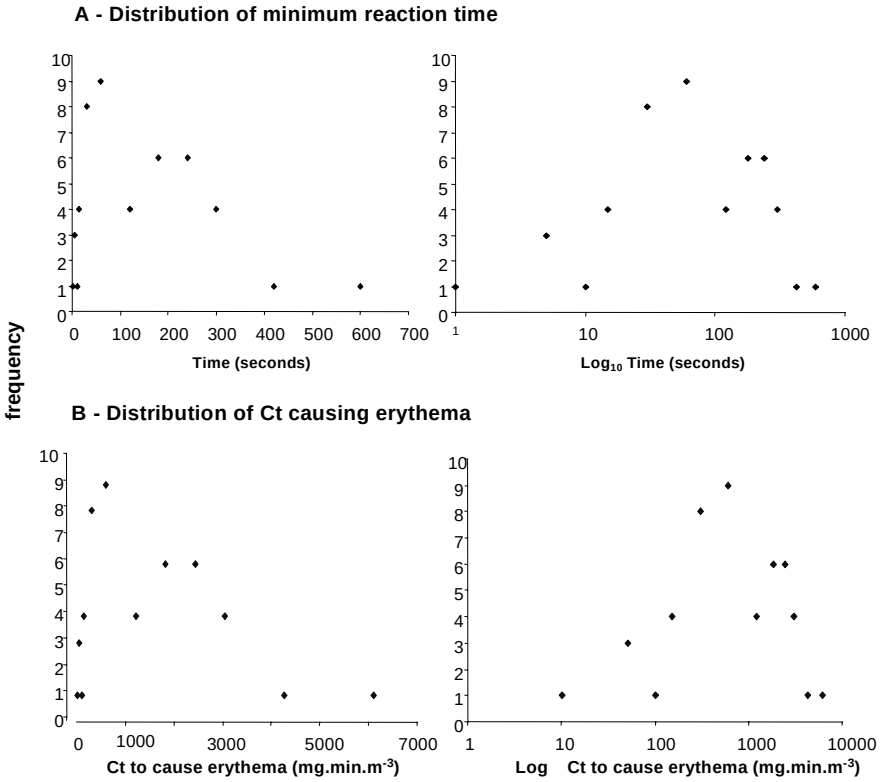


Figure 1

Frequency distributions of minimum time to erythema for HD vapour on the forearm (Marshall et al 1918). The graphs show the minimum exposure time to required to produce erythema (A) and the Ct causing erythema (B).

C8. ASSESSMENT OF THE HAZARD FROM PERCUTANEOUS LIQUID MUSTARD (H)

General Approach

1. The hazard from skin contact with liquid mustard is high since only very small quantities are required to produce quite severe injuries. Penetration of protective clothing by more than a few grams of H will cause incapacitating injury rapidly. Moreover, small quantities of liquid H penetrate protective clothing to produce vapour in the space between the clothing and the skin, as opposed to producing skin contact with liquid. Thence the hazard assessment for vapour is more relevant to pass/fail criteria for protective clothing and an assessment of liquid is included here for completeness.

2. Only data from human exposures has been used to derive the sub-lethal effects of H because the large differences between human and animal skin, and the existence of good studies in man.

Sub-lethal effects

Very small quantities of liquid H are required to cause injury to the skin. Due to the small volumes which need to be dispensed in order to establish the threshold for injury there have been very few studies where the relationship between dose of liquid H and injury have been investigated. One extensive study in humans was conducted in the US during WWII and reported by Bloom et al [95] using an application system capable of delivering quantities as small as 2.5 µg (2.5 nL) reproducibly – the "Benesh Micropippette", the results of which were subject to further mathematical analysis by Landahl et al [96]. Inevitably at this low volume the amount of liquid can only be delivered to the skin by "touching off" which introduces errors, though in trained hands, and used with the knowledge of the possibility of "touching off" errors, the Benesh micropipette appeared to deliver such small amounts reproducibly. Bloom et al [95] report studies at 15 doses between 2.5 µg and 70 µg applied to the forearms of human volunteers. The source material was either thiodiglycol or redistilled Levenstien H and the original experiments were reported in the informal Monthly progress reports of the University of Chigaco Toxicology Laboratories (NDRC 9-4-1 Nos 11-17) [97-100,95]. The groups sizes at each dose were between 40 and 1153 and the percentage of men responding by the formation of erythema and/or blisters were recorded (figure C8.1). Though this range of doses only encompasses part of the log dose response curves for erythema and blistering, it is clear that the linear sections of the two curves are almost parallel; and the dose necessary to produce blisters in any given percentage of a group was ten times that necessary to produce erythema. The threshold for injury (the level at which only 10% of the group would respond) can be estimated at 10 µg for blistering and 1.0 µg for erythema. The latter figure is estimated on the assumption that the low end of the dose response curve for erythema is the same shape as that for blistering and is ten times lower.

Clearly, these injuries were produced on very small areas and an estimate of contamination density needs to be made in order to assess the amount of H which would cause incapacitation. Bloom (1944) also records the average diameter of the injury produced, erythema or blister, enabling an estimate of the contamination density to be made (figure C8.2). For blistering, the contamination density at 10% incidence of injury is 276.27 µg.cm-2 and the equation for the straight line fitted to the first four points on the graph of the erythema response indicates that an equivalent dose for erythema is 22.144 µg.cm-2. To scale these up to a whole body dose certain assumptions must be made.

1. Estimate is made for a 70kg adult of height 6' with a body surface area of 1.91 m^2 (see normogram in figure C8.3)
2. An H burn is the same as a thermal burn in that 15% of the body surface area is must be burned before treatment is required. This would be defined as a casualty, since the treatment required would include intravenous fluids and bed rest.

Given that 15% of 1.91 m² is 0.2865 m² or 2865 cm² the following figures for the whole body liquid H dose necessary to produce erythema or blistering in 10, 50 and 80% of the population based on Bloom (1944) are:

	Erythema			Blistering		
	µg/cm²	µg/2865cm²	g/man	µg/cm²	µg/2865cm²	g/man
10%	27.92	80,004	0.08	276.27	791,514	0.79
50%	62.67	179,560	0.18	292.26	837,325	0.84
90%	97.41	279,092	0.28	370	1,060,050	1.06

If it is assumed that the first noticable effect will be erythema and actual vesication will be disabling at 15% body surface area, the WG recommends that a range of 0.08 g/man to 0.28 g/man for the "negligible military impact" hazard classification. A range of 0.79 to 1.06 g/man would have a significant effect upon military impact and is recommended for this hazard classification.

Lethal effects

It is well documented that H is not a lethal agent. Estimates are based upon animal studies of about 7g per man have been made.

Mustard casualties can die by two mechanisms, systemic poisoning by the agent, or sepsis following infection of skin burns which does not receive the appropriate treatment. For sepsis to be a cause of death the casualty would need to have a large area of the body burned, probably greater than 80%, and not be given appropriate anti-biotic therapy. Though absence of therapy of any kind is unlikely, and is consistent with WWI reports that casualties did not die of skin burns alone, there is "Danger of Death" from sepsis of left untreated. In order to vesicate 80% of the body surface of a 6', 70 kg man an H dose of greater than 1g would be required from the figure published by Bloom [95].

Available animal lethality data is consistent with this figure resulting in LD_{50} estimates of between 5 $mg.kg^{-1}$ and 100 $mg.kg^{-1}$ (350 mg /man to 7000 mg/man). These figures appear to have been extrapolated directly from animal data accounting only for difference in body mass, though in some cases the source of the estimate is not clear (e.g. Wood,[101]). There is also some evidence that some animals, notably the rabbit on which many early studies were carried out, is less sensitive to the effects of H than man making it difficult to say with any degree of confidence what value should be adopted for the LD50 of liquid H in man. However, a value of 1g/man as the value above which there is a danger of death from H contamination of the skin is consistent with both the local effects and possible systemic toxicity of the agent.

RECOMMENDED VALUES

	Range (g/man)	Recommended value (g/man)
Danger of Death	0.1-0.3	0.2
Significant decrement of military performance	0.8-1.0	0.8
Negligible military impact	Available data does not permit a reliable range to be estimated	>1

Inspection of figure 2 shows a very steep dose response relationship between contamination density necessary to cause blister formation and percent group reacting. The lower boundary of the range for Significant effect upon military performance has therefore been selected as the recommended value.

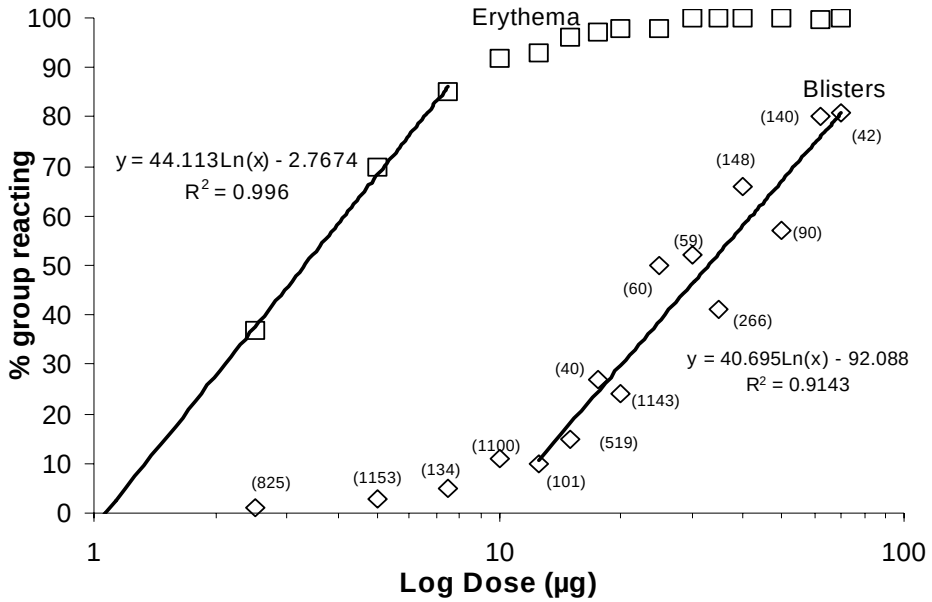


Figure C8.1

Relationship between dose of liquid and the percentage of the treated group responding by the production of erythema (open squares) or blistering (open diamonds) for liquid thiodiglycol of redistilled levenstein H on human forearm skin. The numbers in each treatment group are given in parenthesis. Lines are fitted to the “linear” portion of the log dose:response curve by regression (from Bloom 1944 [95])

AEP-52
(Edition 1)

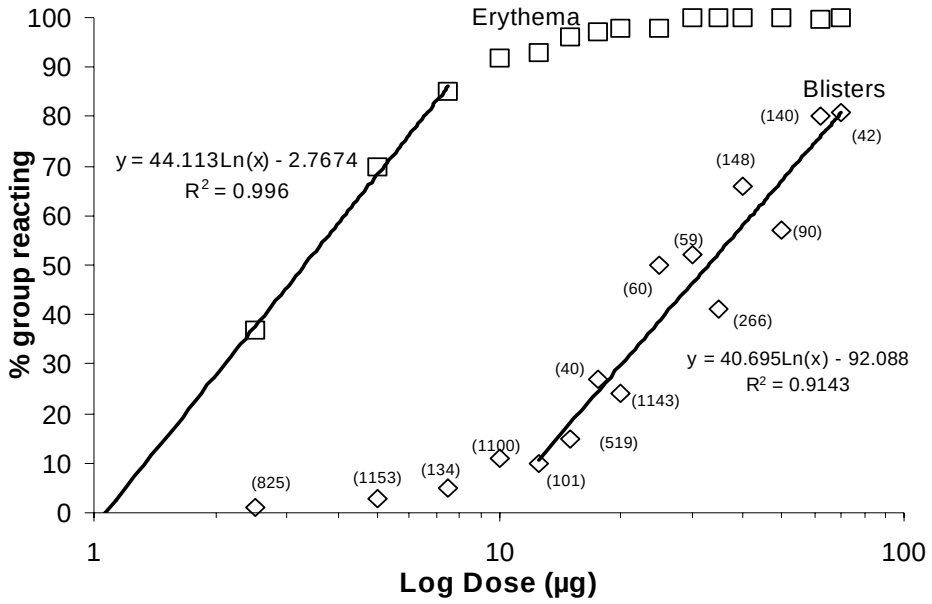
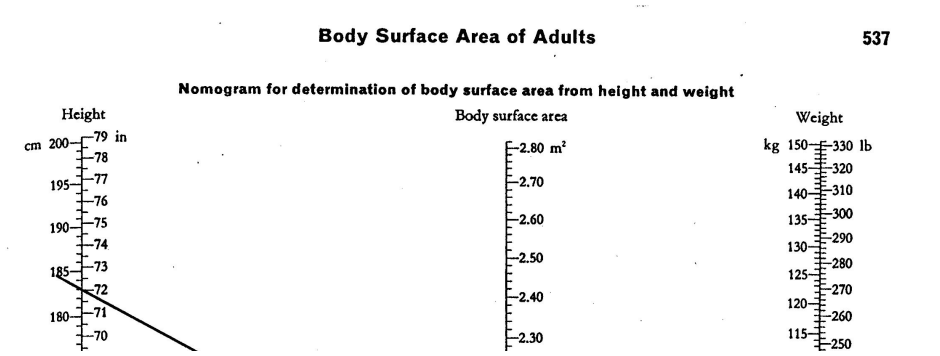
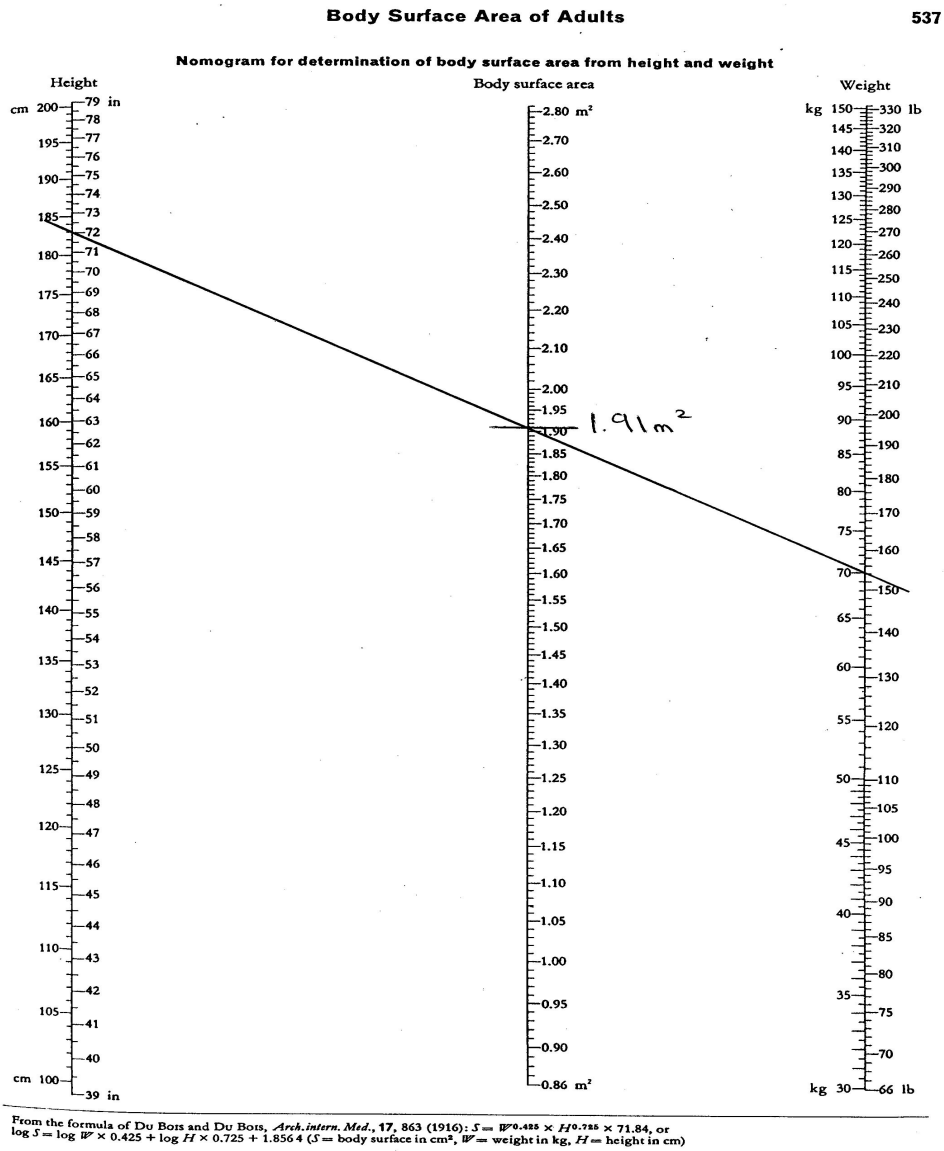


Figure C8.2

Relationship between contamination density of liquid and the percentage of the treated group responding by the production of erythema (open squares) or blistering (open diamonds) for liquid thiodiglycol or redistilled levenstein H on human forearm skin. The lines is fitted to the “linear” portion of the log dose:response curve by regression (from Bloom et al [95]).





From the formula of Du Bois and Du Bois, *Arch. intern. Med.*, 17, 863 (1916): $S = W^{0.425} \times H^{0.725} \times 71.84$, or $\log S = \log W \times 0.425 + \log H \times 0.725 + 1.8564$ (S = body surface in cm², W = weight in kg, H = height in cm)

Figure C8. 3

Normogram used to calculate body surface area

AEP-52
(Edition 1)

Appendix A to ANNEX C

Tabular summaries of data from US and AU chamber trials

Subjective Scoring System of Heinen et al (1943)

Score			Description
0			No effect
1			Mild erythema
2			Moderate erythema
3			Severe erythema
4	either	a -	Erythema with oedema
		b -	Maceration of the axillary skin
		c -	Dry scaling of the scrotum
5	either	a -	Vesicle
		b -	Numerous pinpoint vesicles
		c -	Crusting and ulceration of the scrotum and axilla

Subjective Scoring System of Sinclair (1944)

E-	Just perceptible erythema
E	Definate erythema
E+	Erythema with oedema
D	Desquamation
PHV	Pin Head Vesicles
V	Frank vesication
M	Moist desquamation

CT	**49-59**																													
Source	Heinen 1945					Source	Heinen 1945					Source	Gorrill 1943					Source	Gorrill 1943					Source	CD (Au) Note 50				
Country	US					Country	US					Country	AU					Country	AU					Country	AU				
Test No	1					Test No	2					Test No	1					Test No	1A					Test No	1				
Date	28/03/44					Date	25/07/44					Date	Jan-Feb 1943					Date	Jan-Feb 1943					Date	08/12/43				
C	1.8	mg.m^{-3}				C	1.6	mg.m^{-3}				C	1.6	mg.m^{-3}				C	1.18	mg.m^{-3}				C	0.9	mg.m^{-3}			
t	30	mins				t	30	mins				t	35	mins				t	50	mins				T	55	mins			
CT	54	mg.min.m^{-3}				CT	49	mg.min.m^{-3}				CT	56	mg.min.m^{-3}				CT	59	mg.min.m^{-3}				CT	50	mg.min.m^{-3}			
Temp	90	°F	32.2	°C		Temp	90	°F	32.2	°C		Temp	101	°F	38.3	°C		Temp	87	°F	30.5	°C		Temp	90	°F	32.1	°C	
RH(%)	65					RH(%)	65					RH(%)	80					RH(%)	90					RH(%)	84.4				

No of men	6						No of men	5						No of men	6						No of men	4						No of men	10				
severity	1	2	3	4	5			1	2	3	4	5		severity	1	2	3	4	5			1	2	3	4	5			1	2	3	4	5
neck	2						neck	2	2					neck	5	1					neck	1	1		1			neck		10			
ax							ax			1				ax	1						ax	1						ax		1			
pen							pen							pen							pen	1						pen					
scr							scr	1						scr	3		1	1			scr	2	1		1			scr		8			
cf							cf	4	1					cf							cf	2						cf					
pcp							pcp	1						pcp		1					pcp	2						pcp					
sh							sh	2						sh							sh	1						sh					
sc	1						sc	1						sc	1	1					sc	1						sc					
vth	1						vth	4						vth							vth	2						vth		8			
lth							lth							lth							lth	2						lth					
dth	1						dth	4	1					dth							dth	2						dth					
abd							abd	1	1					abd	1						abd	2						abd					
ing							ing							ing	2						ing	1						ing		4			
bt							bt	2						bt	1						bt	2						bt		3			
arm	1						arm	1						arm	1						arm	2						arm	2	5		1	
wr	1						wr							wr					1		wr	2						wr			1		1
thi							thi	2						thi	1						thi	1						thi		7			
leg							leg	2						leg							leg	2						leg		1			

CT	81-136					
Source	Gorrill 1943					
Country	AU					
Test No	2					
Date	Jan-Feb 1943					
C	1.6	mg.m^{-3}				
t	51	mins				
CT	81	mg.min.m^{-3}				
Temp	101	°F	38	°C		
RH(%)	80					
No of men	4					
severity	**1**	**2**	**3**	**4**	**5**	
neck	3					
ax						
pen						
scr	2			1		
cf						
pcp						
sh					1	
sc						
vth	2					
lth	1					
dth	1					
abd	1					
ing	1					
bt						
arm	2				1	
wr					3	
thi					1	
leg						

Source	Heinen et al 1945					
Country	US					
Test No	3					
Date	30/03/44					
C	1.65	mg.m^{-3}				
t	60	mins				
CT	99	mg.min.m^{-3}				
Temp	90	°F	32	°C		
RH(%)	65					
No of men	6					
	1	**2**	**3**	**4**	**5**	
neck	3	2				
ax		1				
pen		1				
scr	3	2				
cf	1	2				
pcp						
sh	1					
sc	1	2				
vth	2	1				
lth		2				
dth	3	2				
abd		1				
ing						
bt	2					
arm	2					
wr	3	1				
thi	4	2				
leg	5					

Source	Heinen et al 1945					
Country	US					
Test No	4					
Date	25/07/44					
C	1.6	mg.m^{-3}				
t	60	mins				
CT	97	mg.min.m^{-3}				
Temp	90	°F	32	°C		
RH(%)	65					
No of men	5					
	1	**2**	**3**	**4**	**5**	
neck		4				
ax	2	2	1			
pen						
scr	1		1			
cf	2	1	2			
pcp	2					
sh	1	1				
sc	2		1			
vth	1	3	1			
lth	2					
dth	4	1				
abd	1	3	1			
ing						
bt	2					
arm	2	1				
wr						
thi	2					
leg						

Source	Heinen et al 1945					
Country	US					
Test No	5					
Date	25/01/44					
C	1.7	mg.m^{-3}				
t	60	mins				
CT	102	mg.min.m^{-3}				
Temp	100	°F	38	°C		
RH(%)	35					
No of men	6					
	1	**2**	**3**	**4**	**5**	
neck	3	3				
ax	1					
pen						
scr	4			1		
cf	6					
pcp	2					
sh	5	1				
sc	5					
vth	3					
lth	3					
dth	5					
abd	1					
ing						
bt	6					
arm	2					
wr	6					
thi	5					
leg	1					

Source	Heinen et al 1945					
Country	US					
Test No	6					
Date	25/01/44					
C	1.65	mg.m^{-3}				
t	60	mins				
CT	99	mg.min.m^{-3}				
Temp	100	°F	38	°C		
RH(%)	67					
No of men	6					
	1	**2**	**3**	**4**	**5**	
neck	4	1	1			
ax						
pen						
scr	2					
cf	6					
pcp	2					
sh	5	1				
sc	5	1				
vth	1					
lth	1					
dth	2	1				
abd						
ing						
bt	6					
arm	1	1				
wr	4	1				
thi	3					
leg	2					

Source	Gorrill 1943					
Country	AU					
Test No	3A					
Date	Jan-Feb 1943					
C	5.4	mg.m^{-3}				
t	25	mins				
CT	136	mg.min.m^{-3}				
Temp	90	°F	32	°C		
RH(%)	80					
No of men	4					
	1	**2**	**3**	**4**	**5**	
neck	1				1	
ax						
pen						
scr	2	2				
cf						
pcp						
sh						
sc						
vth						
lth						
dth						
abd	1					
ing						
bt						
arm	2					
wr						
thi						
leg	1					

Source	Gorrill 1943					
Country	AU					
Test No	3					
Date	Jan-Feb 1943					
C	11.2	mg.m^{-3}				
t	12	mins				
CT	134	mg.min.m^{-3}				
Temp	94	°F	34	°C		
RH(%)	75					
No of men	4					
	1	**2**	**3**	**4**	**5**	
neck	1					
ax						
pen						
scr	1	2				
cf						
pcp						
sh	2					
sc						
vth		1				
lth						
dth		1				
abd						
ing						
bt						
arm	1	1				
wr						
thi	1	1				
leg						

Source	CD (Australia) Note 50					
Country	AU					
Test No	3					
Date	04/04/44					
C	1.9	mg.m^{-3}				
t	63	mins				
CT	120	mg.min.m^{-3}				
Temp	89					
RH(%)	85					
No of men	8					
	1	**2**	**3**	**4**	**5**	
neck	1	5				
ax	1	1				
pen		3				
scr		9		1		
cf						
pcp						
sh						
sc						
vth	4	3				
lth						
dth						
abd						
ing						
bt	1	3	1			
arm	1	4	1			
wr		2			1	
thi						
leg						

ing	1					ing						ing						ing						ing						ing						ing						ing					
bt						bt	2					bt	2					bt	6					bt	6					bt						bt						bt	1	3	1				
arm	2				1		arm	2						arm	2	1				arm	2					arm	1	1				arm	2					arm	1	1				arm	1	4	1				
wr					3		wr	3	1					wr						wr	6					wr	4	1				wr						wr						wr		2			1		
thi					1		thi	4	2					thi	2					thi	5					thi	3					thi						thi	1	1				thi							
leg							leg	5						leg						leg	1					leg	2					leg	1					leg						leg							

AEP-52
(Edition 1)

C	2.5	mg.m^{-3}	C	3.35	mg.m^{-3}	C	3.3	mg.m^{-3}	C	3.31	mg.m^{-3}	C	3.31	mg.m^{-3}	C	3.31	mg.m^{-3}
t	60	mins	t	60	mins	t	60	mins	t	60	mins	t	60	mins	t	60	mins
CT	150	mg.min.m^{-3}	CT	201	mg.min.m^{-3}	CT	198	mg.min.m^{-3}	CT	199	mg.min.m^{-3}	CT	199	mg.min.m^{-3}	CT	199	mg.min.m^{-3}
Temp	90	°F 32 °C	Temp	70	°F 21 °C	Temp	80	°F 26 °C	Temp	80	°F 26 °C	Temp	90	°F 32 °C	Temp	100	°F 37 °C
RH(%)	65		RH(%)	53		RH(%)	46		RH(%)	86		RH(%)	65		RH(%)	66	
No of men	10		No of men	7		No of men	6		No of men	5		No of men	10		No of men	4	
severity	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
neck	2	6	2			neck	3	2				neck	3	2	1			neck	2	3				neck		8	2			neck			1	2	1
ax		1				ax	4		2			ax	3					ax	2					ax	1	3	2			ax		1	3		
pen	2					pen						pen						pen						pen	3					pen					
scr	3					scr	3					scr	2	1			1	scr						scr	3	2	5			scr				1	3
cf	3	4				cf			1			cf	3	1				cf	3	1				cf		5	4	1		cf			3	1	
pcp						pcp	3					pcp	4					pcp	2					pcp	2					pcp	1	1	2		
sh		3				sh	3	1				sh	4					sh	2					sh	1	7	1			sh			4		
sc	7	1			1	sc	3	1				sc	4					sc	2					sc	3	4	1			sc			4		
vth	3	3				vth	1					vth						vth						vth	3	2	2			vth	1	1	2		
lth		1				lth						lth						lth						lth		2				lth		1	3		
dth	6	4				dth	3	1				dth	3	1				dth	2					dth	2	4	3			dth		1	3		
abd		1				abd						abd						abd						abd	4	3				abd	1		3		
ing						ing						ing						ing						ing						ing			1		
bt	2	1				bt						bt	6					bt	4					bt	5	2				bt		2	2		
arm	4	3				arm						arm	1					arm	2					arm	3	3	2	1		arm			4		
wr						wr						wr	2	2				wr	5					wr	2	4				wr		1	3		
thi	5	1				thi	2					thi	3					thi	2					thi	3	2				thi		1	3		
leg	5	1				leg						leg	2					leg						leg	3	2				leg	2	1	1		

Ct 150-200								**Summer**					
Source	Heinen et al 1945						Source	Heinen 1945					
Country	US						Country	US					
Test No	8						Test No	13					
Date	26/07/44						Date	27/07/44					
C	2.5	mg.m^{-3}					C	3.33	mg.m^{-3}				
t	60	mins					t	60	mins				
CT	150	mg.min.m^{-3}					CT	200					
Temp	90	°F	32	°C			Temp	90	°F	32	°C		
RH(%)	65	%					RH(%)	65	%				
No of men	6						No of men	6					
severity	**1**	**2**	**3**	**4**	**5**			**1**	**2**	**3**	**4**	**5**	
neck			6				neck			2	2	2	
ax			6				ax			4		1	
pen	3						pen		1				
scr	2	1	3				scr		3	1		2	
cf			6				cf			6			
pcp	1	3	1				pcp	2	2				
sh		1	1				sh		2	1	1	1	
sc		2	4				sc			5	1		
vth			6				vth		1	4		1	
lth	1	1	3				lth		1	3			
dth		2	4				dth			4	1	1	
abd			5	1			abd			6			
ing		1					ing						
bt	2	2	1				bt	1	3	2			
arm	1	2	3				arm		1	4		1	
wr	1		1				wr	1		1		1	
thi	4	2					thi	3	3				
leg	4	2					leg	4	1				

Ct	218-250					
Source	Heinen et al 1945					
Country	US					
Test No	15					
Date	17-22/04/44					
C	4.16	$mg.m^{-3}$				
t	60	mins				
CT	250	$mg.min.m^{-3}$				
Temp	90	°F	32	°C		
RH(%)	65					
No of men	15					
severity	**1**	**2**	**3**	**4**	**5**	
neck	1	7	7			
ax	1	8	1		2	
pen	6	6	1			
scr	2	5	2		6	
cf	3	8	1	3		
pcp	2	6				
sh	4	8	2			
sc	1	5	3			
vth	4	6	3			
lth	1	7	2			
dth	2	8	5			
abd	3	3	3			
ing		1	1			
bt	4	2	3	1		
arm	3	7	4			
wr	7	6	2			
thi	10	3	1			
leg	8	4	1			

Source	Heinen et al 1945					
Country	US					
Test No	16					
Date	28/07/44					
C	4.15	$mg.m^{-3}$				
t	60	mins				
CT	249	$mg.min.m^{-3}$				
Temp	90	°F	32	°C		
RH(%)	65					
No of men	6					
	1	**2**	**3**	**4**	**5**	
neck			1	3	2	
ax			5	1		
pen		1	3			
scr		1	2	1	2	
cf			3	2	1	
pcp	1	2	3			
sh			2	1	2	
sc		1	5			
vth			4	2		
lth			4	1		
dth		1	5			
abd			4	1	1	
ing			4			
bt		2	4			
arm		3	1	4	2	
wr		1			2	
thi		1	3			
leg	1	2	2			

Source	Gorrill 1943					
Country	AU					
Test No	4					
Date	Jan-Feb 1943					
C	15.9	$mg.m^{-3}$				
t	13.75		mins			
CT	218	$mg.min.m^{-3}$				
Temp	97	°F	36	°C		
RH(%)	75.5					
No of men	4					
	1	**2**	**3**	**4**	**5**	
neck	4					
ax	1					
pen						
scr	2	1	1			
cf	1			1		
pcp						
sh						
sc						
vth						
lth						
dth						
abd						
ing						
bt		1				
arm						
wr						
thi						
leg	1					

Source	Gorrill 1943					
Country	AU					
Test No	5					
Date	Jan-Feb 1943					
C	7	$mg.m^{-3}$				
t	35	mins				
CT	245	$mg.min.m^{-3}$				
Temp	98	°F	36	°C		
RH(%)	80					
No of men	4					
	1	**2**	**3**	**4**	**5**	
neck	1	2				
ax	1					
pen	1	2				
scr				2	2	
cf	1					
pcp	1					
sh	1					
sc	1					
vth	1	3				
lth	1	1				
dth	1	3				
abd	1					
ing	1	1				
bt	1	1				
arm	1	1			2	
wr	1	1				
thi					2	
leg	1					

Source	CD (Australia) Note 50						
Country	AU						
Test No	4						
Date	04/04/44						
	3.92	$mg.m^{-3}$					
	56	mins					
CT	220	$mg.min.m^{-3}$					
Temp	89.9	°F	32	°C			
RH(%)	85						
No of men	10						
	1	**2**	**3**	**4**	**5**		
neck		4		6			
ax				10			
ah							
sc							
vth		2		5	3		
lth							
dth							
abd							
ing		4		6			
pen		3			7		
scr					10		
bt				7	3		
arm		1		6	3		
cf							
wr		9		1			
thi		2		7	1		
pcp							
leg	2	7		1			

Ct	**300**							Source	Heinen et al 1945						
Source	Heinen et al 1945							Country	US						
Country	US							Test No	17						
Test No	17							Date	28/11/44						
Date	28/11/44							C	4.98	mg.m-3					
C	4.98	mg.m-3						t	60	mins					
t	60	mins						Ct	299						
Ct	299							Temp	70	°F	21	°C			
Temp	70	°F	21	°C				RH(%)	60						
RH(%)	60							No of men	6						
No of men	6														
									1	**2**	**3**	**4**	**5**		
	1	**2**	**3**	**4**	**5**			neck	2	4					
neck	2	4						ax		1	5				
ax		1	5					pen	1	1					
pen	1	1						scr	2	1	1		1		
scr	2	1	1		1			cf	6						
cf	6							pcp	3	3					
pcp	3	3						sh	5	1					
sh	5	1						sc	6						
sc	6							vth	1	1					
vth	1	1						lth		1					
lth		1						dth	6						
dth	6							abd							
abd								ing			1				
ing			1					bt	1						
bt	1							arm	4						
arm	4							wr							
wr								thi		3					
thi		3						leg		2					
leg		2													

Ct	**396-397**							Source	Gorrill 1943						
Source	Heinen et al 1945							Country	AU						
Country	US							Test No	6						
Test No	26							Date	Jan-Feb 1943						
Date	01/12/44							C	11	mg.m-3					
C	6.62	mg.m-3						t	36	mins					
t	60	mins						Ct	396						
Ct	397							Temp	97	°F	36	°C			
Temp	70	°F	21	°C				RH(%)	86						
RH(%)	62							No of men	4						
No of men	6														
									1	**2**	**3**	**4**	**5**		
	1	**2**	**3**	**4**	**5**			neck			2	1			
neck	1	3	2					ax	1	1					
ax			5	1				pen	1			1			
pen	1							scr	1			2	1		
scr	2		1		2			cf	2						
cf	4	2						pcp	1		1				
pcp	1	3	1					sh	1						
sh	2	2	2					sc	1						
sc	1	3	2					vth	1		1				
vth	3							lth	1				1		
lth	2							dth	1				1		
dth	3	2	1					abd	1		1		1		
abd	1	1						ing	1		1				
ing								bt	1		1				
bt	2	2						arm			1		1		
arm	1	4						wr	1				1		
wr	4	1	1					thi	1				2		
thi	2	2						leg	1		1				
leg	1														

AEP-52
(Edition 1)

Ct	**506**											
Source	Heinen et al 1945						Source	Heinen et al 1945					
Country	US						Country	US					
Test No	27						Test No	28					
Date	09/01/45						Date	08/01/45					
C	8.4	$mg.m^{-3}$					C	8.4	$mg.m^{-3}$				
t	60	mins					t	60	mins				
Ct	506						Ct	506					
Temp	60	°F	16	°C			Temp	70	°F	21	°C		
RH(%)	48						RH(%)	48					
No of men	8						No of men	8					
	1	**2**	**3**	**4**	**5**			**1**	**2**	**3**	**4**	**5**	
neck		1	7				neck		1	5	1	1	
ax				2	2		ax		1	3	3	1	
pen	2	1			4		pen				1	7	
scr		1			7		scr					8	
cf	2	4	2				cf	1	3	4			
pcp	1	4	2				pcp	1	6	1			
sh	4	2	1				sh	3	3	2			
sc	3	2	2				sc	4	3	1			
vth	3	1	2				vth	3	2	2			
lth	2	1	2				lth	2	1	4			
dth	5		2				dth	6	1	1			
abd	4	1	2				abd	5	1	1			
ing		2	2				ing		1	4	1		
bt	1	1	4				bt	3	4				
arm	4	2	1				arm	3	2	3			
wr	3	4					wr	1	2	4			
thi	3	2	2				thi	4	2	1	1		
leg	3		2				leg	7	1				

CT	**596-660**												
Source	Heinen et al 1945						Source	Gorrill 1943					
Country	US						Country	AU					
Test No	30						Test No	7					
Date	12/01/45						Date	Jan-Feb 1943					
C	9.93	mg.m^{-3}					C	11	mg.m^{-3}				
t	60	mins					t	60	mins				
Ct	596						Ct	660					
Temp	60	°F	16	°C			Temp	94	°F	34	°C		
RH(%)	62						RH(%)	85					
No of men	8						No of men	4					
	1	2	3	4	5			1	2	3	4	5	
neck		1	7				neck					3	
ax				3	5		ax	1		1			
pen		1			7		pen				3	1	
scr					8		scr				1	3	
pcp	4	3	1				pcp	1		1	1		
cf	2	3	3				cf	1			1	2	
sh	4	2	2				sh			1		1	
sc	4	1	2				sc	1		1			
vth	1	1	4				vth	2		1			
lth		2	4				lth	2	1	1			
dth	4	1	2				dth	2	1	1			
abd		2	3				abd	2	1	1			
ing			5				ing	1		1			
bt	4		3		1		bt	1		1			
arm	6	1					arm					4	
wr	6	2					wr	1				1	
thi	4	2	2				thi			1		1	
leg	5						leg	1		1	1	1	

ANNEX D

COMMENTS AND NOTES ON L TOXICITY DATA

D1. L PERCUTANEOUS TOXICITY

1.1. The contents of this annex represent notes based on the discussion of the group and are not precisely structured documents.

1.2. For assumptions made, see chapter 2 and annex E for references considered

D2. SUMMARY

The literature concerning Lewisite (L) is filled with conflicting data, statements and conclusions. From the time the US weaponized L in 1918 until the late 1930s, it was regarded as having great potential as a chemical warfare (CW) agent (65) due to its extreme toxicity in comparison to sulphur mustard (H). By the 1940s, the toxicity of L was downgraded to being only slightly more toxic than H in liquid form and equitoxic by vapour exposure. Furthermore, its utility as a CW agent was said to be further compromised by the technical difficulties of putting up and maintaining adequately lethal vapour concentrations, as well as the ease with which protective clothing could protect against vapour challenges. Relatively little experimental work has been carried out on this agent during the last fifty years. However, while recent reviews of L still contain conflicting statements, the toxicological estimates of this compound with respect to man remain similar to those made in the 1940s. Further confounding an assessment of the human toxicity of L, most recent experimental data have tended to confirm its extreme toxicity in several different non-human animal species. Although the historical literature suggests that humans are especially resistant to the cutaneous effects of L, confirmation of this is now ethically untenable. It is therefore recommended that the human toxicity estimates documented by Gates (66) in 1946, be accepted for L. However, since the validity of these values cannot be rigorously supported by human data, it is recommended that when there is the possibility of liquid L contamination, that a safety factor of at least ten, be applied to these figures.

D3. INTRODUCTION

This assessment of the human toxicity of L differs from those previously carried out for the classical nerve agents and H, in that it has not thus far been possible to access most of the primary literature sources. Instead, the present report has relied heavily on two reviews completed in 1945 and 1946 (65,66). It is felt that it is unlikely that any uncertainties concerning the human toxicity of L will be further clarified by acquisition and subsequent examination of the primary literature. Compared to other classical agents, very few human exposures were carried out, and even in 1945/46 review authors expressed reservations with respect to the actual quantitation of L vapour exposures, as

well as its human toxicity. In addition, as has been the experience of this group with previous assessments, the older literature will often not contain enough experimental detail to enable critical analyses.

Lewisite was developed and suggested as a CW agent by W. Lee Lewis in 1917, and by 1918 150 tons were en route to Europe from the US, when the armistice was signed. The shipment was dumped at sea and the use of this agent on the battlefield has never been conclusively recorded. Its utility as a CW agent, even at this time, was unclear. In 1919 a US report stated that “We regard the laboratory data as offering strong support for the probability that L will prove to have great military value 67, cited in 66). The Germans were of the belief that the Americans were spared a great disappointment by being unable to use L in World War I (68 cited in 66), while the French thought that the vesicant arsines were "capable of playing eventually a military role of the first order (69, cited in 70). As late as 1937 Prentiss still expressed the view that “On the whole, we are inclined to believe that lewisite must be taken into serious consideration in any chemical warfare estimate of the future.”(70). With the advent of World War II, interest in the military value of the arsenicals spurred a flurry of research in both the UK and the US, and by 1946, although L was still thought to be the best arsenical for gas warfare, the opinion was held that this class of agents, including L, did not offer much promise of success in battle (65). This was due to 1) the difficulties of placing sufficiently lethal dosages in the field, 2) the rapid hydrolysis of L in the presence of moisture and 3) the ease of protection against L.

D4. CHEMICAL IDENTIFICATION OF LEWISITE

Munitions grade L is a mixture of 2-chlorovinyldichloroarsine (cis and trans, L-1, Lewisite), di-(2-chlorovinyl)-chloroarsine (L-2), tris-(2-chlorovinyl)-arsine (L-3) and additional impurities of minor toxicities. Cis and trans L are equitoxic (66), and are presumably at least as toxic, if not moreso, then L-2 and L-3, although no reference material was located to support this assumption. A complicating factor when assessing the effects of L therefore, is the determination of the true identity of the test substance used in the studies. Few reports, most particularly the older ones, identify whether the test L is munitions grade or distilled, by what process the L was prepared by, or what the constituents of the test article were. The original US procedure for preparation of this agent involved the acetylation of arsenic trichloride using aluminum chloride as a catalyst. The optimum yield of L-1 in this scheme was only about 20 % (71- 74, cited in 66) and one would assume that the L used in studies up until 1938 utilized test agent generated by this process. In 1938 the UK demonstrated that replacement of the original catalyst with mercuric chloride could improve the yield of L-1 to 75-85 %. Thereafter, plants in both the UK and the US produced L using mercuric chloride as the catalyst (66) and it seems likely that most studies after 1938 would use L produced by this method. Clearly, the chemical, physical and toxicological properties of test L will vary according to its chemical composition.

D5. TOXICITY OF LEWISITE IN HUMANS

There are a limited number of reports on the human toxicity of L and these variously describe it as being more toxic than H, equitoxic to H or less toxic than H. As described above, these reports suffer the drawback of not adequately describing the actual make-up of the test article used. In 1919, two US reports summarized the state of knowledge of this agent at that time (67, 75, cited in 66). Although no systematic studies on the effect of liquid L were carried out on human skin, it was stated that "Laboratory workers who have been accidentally burned with liquid L have given strong evidence for the greater effectiveness of this substance in man than liquid H. The L lesions develop with extreme rapidity, are painful and associated with constitutional symptoms. The lesion is not confined to the skin but extends to the deeper tissues." (67, cited in 66). However, Gates (66) dismissed these findings by stating that "In view of the divergence of these views from those currently accepted, it is well to bear in mind that these were accidental burns and hence were probably treated, that the accepted treatment at the time was application of 5 per cent sodium hydroxide to the lesion for a period of 30 minutes, and that sodium hydroxide itself in that strength produces a very strong destructive skin effect." The effects of liquid L were studied in rabbits and dogs and it was concluded that, if man were as susceptible to L as dogs, then the minimum lethal dose for man would be 1.4 ml (~ 2.6 g) distributed over an area of 5 square inches (75, cited in 66). German studies refuted this assessment, however, and stated that doses of 1.4 ml could be applied repeatedly to men without eliciting any clear-cut symptoms of poisoning (76, cited in 66). Support for this latter observation was described in the case of a worker suffering accidental L burns to over 20 % of his body (77, cited in 66). Although he showed signs of anemia 10 to 15 days after intoxication, no signs of systemic arsenic poisoning were evident. Gates (66) concluded that "man is not nearly so susceptible to systemic arsenical poisoning from skin contamination as was originally believed."

Liquid L has been cited as being a very potent vesicant as compared to H, with the median threshold blistering dose for man being 14 μg for L versus 32 μg for H (78, cited in 66). However, German literature (76, cited in 66), states that "in quantities up to 1 mg, either agent give rise to lesions of similar severity and that at higher doses, the lesions caused by L are less severe than those produced by H and heal much more quickly", findings that were also reproduced in the US where 1 mg doses of L gave rise to lesions of less severity than did those resulting from 1 mg H application (79, cited in 66). The pain associated with L skin exposure has been described as a stinging sensation that occurs immediately, with blistering occurring within 24 hours. In contrast, Rovida (80, 81, cited in 66) used relatively large amounts of "pure" L, but described the development of the resultant lesions as occurring very slowly, with erythema appearing after 11 hours and mild blisters forming only after nine days. No pain due to L was mentioned in these reports, and at least one recent review describes L as causing little pain (83). A constant in most reports is that lesions caused by L tend to heal more rapidly than do lesions of similar severity produced by H.

The effects of H and L vapour on the skin and eyes were studied in rabbits, dogs and man in experiments prior to 1919. As shown in Table 1, the rabbit was much more sensitive to the effects of L than to H, the dog was equally sensitive to the effects of both agents and man was much less sensitive to the effects of L versus H, at least with respect to the skin. The difficulties that persist to this day with the chemical analysis of L were discussed at length by Wardell (84, cited in 65). He concluded that the considerable variation seen in much of the early experimental work (pre-1941) was due to the technical difficulties of generating uniform and predictable vapour concentrations, since L was prone to rapid hydrolysis to lewisite oxide in the presence of moisture and that this hydrolysis product would then adsorb onto the walls of the exposure chamber and surfaces of the animals inside. Thus, the vesicant concentrations of L vapour in dry air on the skin of a man determined by Eldridge (85, cited in 65) in 1923, which ranged from 10,450 to 4716 $mg.min.m^{-3}$ (5 to 180 min exposures), were judged as likely being far too high, and the nominal concentrations likely to be much greater than the concentrations that made contact with the skin (65). Studies in which analytical concentrations of L vapour were determined report the vesicant LCT_{50} in man as being from 1000 $mg.min.m^{-3}$ (86, cited in 85) to 1800 $mg.min.m^{-3}$ (87, cited in 65). In the final analysis, the table presented by Gates (66) in 1946 probably represents the best attempt at assigning human toxicity values to L (Table 2). He had broad access to primary source material that still represents the only documentation of human studies with this agent.

TOXICITY OF LEWISITE IN ANIMALS

Table 3 shows a compilation of H and L toxicity in a variety of animal species as assessed in 1946. A cursory examination of this table shows that, except for the mouse and rabbit, L and H have similar cutaneous toxicities. Although the modern literature contains very few studies of L, a recent report (88) confirms the toxicity of L (95% L-1, 5% L-2) in male Dutch rabbits and quotes a value of 5.3 mg/kg when it is applied over a 2 cm^2 area. However, due to dissimilarities in the development and healing of L lesions between the human and rabbit, Gates (66) dismissed the predictive value of the rabbit with respect to human toxicity by stating that “The reaction of rabbit skin toward L, is therefore, not characteristic of the reaction of human skin.”

In the modern era, the two species that have been touted as being the best with which to study the vesicant action of H, are the hairless guinea pig and the domestic swine. These animal models are among the very few which, when challenged with H, exhibit similar skin pathology at the microscopic level to humans. In a model which has been used extensively by the UK to examine the effects of different treatment modalities on the healing of vesicant induced lesions, swine were used to assess the development and subsequent healing of lesions induced by both H and L (89-92). The dorsal skin of female domestic white pigs (20-32 kg, strain not mentioned) was gently wet shaved and a 40 cm^2 total area was exposed to vapour cups containing either H or L. The exposures were allowed to continue for six hours, using the animal’s body heat to evaporate the agent off the glass fibre disc in the vapour cups. The quantities of agent used were 12 mg L and 76.4 mg H (both > 96% pure), and were quoted as being the minimum necessary to cause

a reproducible skin injury (91). The injuries produced by both agents were assessed as being similar to those produced in humans. Both agents produced skin injuries that were similar to each other, except that L injury occurred in a much compressed time-frame compared to H and healed much faster.

Recent studies at Defence Research Establishment Suffield have used haired guinea pigs (92, 93). Male Hartley guinea pigs were shaved and then depilated 24 hours before use. On the day of experimentation the animals were restrained and 0.8 – 2.4 μL drops of H or L of greater than 95% purity were applied to their backs. Animals challenged with H developed erythema within 4 hours and blood scabs were maximally developed by 72 hours. In contrast, L produced erythema within minutes, which progressed to lesions of very much greater severity than those produced by H. These were fully formed by 24 – 48 hours. Similar results, and significantly more lethality, have been obtained in recent Dutch studies using hairless guinea pigs and topical liquid L treatments.

Vapour-cup studies using hairless guinea pigs and vapour cup challenges (94) also showed that the lesions produced by similar exposure times of the two agents differ significantly in their severity. L exposures produced erythema by the end of a six minute exposure whereas erythema resulting from H did not occur until 3 – 4 hours post-exposure. Two minute exposures to L resulted in more severe lesions than did six minute exposures to H.

Finally, in light of historical studies that suggest that compared to other species, man is particularly resistant to L, it is of interest to note recent studies carried out in cell cultures derived from four different species, including man (94). In every case L was at least 80 times more toxic than H, with human skin keratinocyte cultures being over 200 times more sensitive to the toxicity of L than to H.

CONCLUSION

The literature contains inconsistencies with respect to the toxicity of L and reviews dating back to the 1940s express reservations as to its true human toxicity and utility as a CW agent. More current literature also contains contradictions, but still shows that L is considerably more toxic than H in several different animal species. However, historical work tends to suggest that man, for some reason, is particularly resistant to the effects of L. Although this type of species specific phenomenon is not without precedent in cutaneous toxicology, the extent of the difference is unusual, especially since tissue culture studies show L to be at least two orders of magnitude more toxic than H in human skin cells. Ethical considerations preclude additional human studies on L and there is no rational way to refute the human toxicity estimates of L determined in the 1940s. It is therefore recommended that the human toxicity estimates documented by Gates (Table 2), be accepted for L. However, in light of the fact that even in 1946, these numbers were acknowledged as being based on only very sparse data with humans (66), and recent work that demonstrates that liquid L readily defeats a number of currently in-service NATO

CPMs, it is also highly recommended that when there is the possibility of liquid L contamination, that a safety factor of at least ten, be applied to these figures.

Table 1: Approximate Concentration of Lewisite to Produce Skin or Eye Lesions in 30 Minute Exposures

	Rabbit $mg.min.m^{-3}$	Dog $mg.min.m^{-3}$	Man $mg.min.m^{-3}$
Lewisite (Eye)	30	600	--
Sulphur Mustard (Eye)	1500	600	30
Lewisite (Skin)	750	1500	6000
Sulphur Mustard (Skin)	6000	1500	750

Values taken from reference 66

Table 2: Estimated Toxicities of Lewisite for Humans

	Vapor LCT_{50} ($mg.min.m^{-3}$)	Liquid Dose (mg)*
Death by inhalation	1200 – 1500	
Death (by body exposure)	100000	2800
Vesication of skin	1200 – 1500	0.014
Serious corneal damage	1500	0.1

Values taken from reference 66. Exposure expression times not noted.
*Not noted whether these values represent minimum, or median effective doses.

Table 3: Toxicity of Lewisite and Sulphur Mustard by Topical Application

Animal	***Lewisite*** LD_{50} (mg/kg)	Sulphur Mustard LD_{50} (mg/kg)
Mouse	15	92
Rat	15-2	5-20
Rabbit	5-6	40-100
Guinea Pig	12	20-25
Dog	38-70	20
Goat	10-24	50

Lewisite values taken from reference 66, sulphur mustard values taken from reference 94.

AEP-52
(Edition 1)

ANNEX E

REFERENCES CONSULTED

TOPIC	REFERENCES	TEXT SECTION
GB vapour	1 to 15 and 102 to 103	A2
GA vapour	16 to 18 & 8,13, 14	A3
GD vapour	19 to 22 & 6,8,14,18	A4
GF vapour	23 to 26 & 6,8,20	A5
VX vapour	27 to 31	B2
VX liquid	32 to 33 & 30,31	B3
GB liquid	8 and 6	A6
GA liquid	16	
GF liquid	6,26,23,24,25,26,34	A7
Mustard	35 to 63 (vap) and 95 – 101(liq)	Annex C
[lewisite]	64 to 94	Annex D

[1], "*Absorption of GB vapour through the skin of rabbits*", M. Ainsworth, Porton Technical Paper PTP172, (1950)

[2], "*Classified Title*", F. N. Marzulli, R. G. Horton, H. Elrod, M. R. Williams, R. Oliver and J. Wiles, Medical Laboratories Research Report 119, (1952)

[3], "*A comparison between depilated and clipped skin in the percutaneous toxicity of nerve gases to rabbits.*" A. Muir and S. Callaway, Porton Technical Paper PTP134, (1950)

[4], "*Observations on percutaneous absorption of liquid GB*", J. D. Cruickshank, Porton Technical Paper PTP396, (1954)

[5], "*Penetration of clothing by liquid GB and GF*", M. Ainsworth and T. W. N. Truckle, Porton Technical Paper PTP134, (1950)

[6], "*The percutaneous toxicity of the G-compounds*", H. Cullumbine, S. Callaway, W. K. Berry, J. W. Blackburn and J. Rutland, Porton Technical Paper PTP399, (1954)

[7], "*The toxicity of the G-compounds. Part V. The toxicity of GA, GB, GD and GE by the percutaneous route.*" A. Muir, S. Callaway and F. Burgess, Porton Technical Paper PTP90, (1948)

[8], "*The toxicity of the G-compounds. Part XI. The toxicity to rabbits of GA, GB, GD and GE by the percutaneous route*", A. Muir, S. Callaway and F. Burgess, Porton Technical Paper PTP215, (1950)

[8], "*Review of acute human-toxicity estimates for selected chemical warfare agent. Subcommittee on Toxicity Values for Selected Nerve Agents and Vesicant Agents. National Research Council.*" NRC, (1997)

[9], "*The toxicity of the G compounds. Part II. The toxicity to rats of GA, GB, GD, GE by the percutaneous route.*" A. Muir, S. Callaway and F. Burgess, Porton Tehnical Paper PTP 82, (1948)

[10], "*Protective action of standard clothing materials against percutaneous exposure of monkeys to GB vapor.*" F. W. Oberst, F. P. McGrath and I. A. DeArmon, Medical Laboratories Research Report 147, (1952)

[11], "*Toxicity of GA vapour by cutaneous absorption for monkey and man*", E. H. Krackow and I. Fuhr, MDR179, (1949)

[12], "*The toxicity of GA to dogs and rabbits by body exposure*", C. B. Marquand and T. W. Kethley, MDRR 88, (1946)

[13], "*Chemical warfare agents, toxicological and medical consideration*", J. R. Wood, MDR 201, (1949)

[14], "*Toxicity Review*", R. A. Howd, C. E. Green and A. J. Valdes, CRDEC-CR-86001, (1986)

[15], "*The toxicity of the G compounds. Part IX. The toxicity to guinea pigs of GA, GB, GD, GE and GF by the subcutaneous route*", M. Muir, S. Callaway and F. Burgess, Porton Technical Paper PTP163, (1950)

[18], "*Enemy CW and smoke intelligence summary*", R. E. Evans, CDR 5 No. 89, (1946)

[19], "*An old demonstration with GD*", W. S. S. Ladell, Porton Note PN212, (1961)

[20], "*The fate of G and V agents on the skin*", R. T. Tregear and P. Dirnhuber, PTP 756, (1961)

[21], "*Summary Report*", S. D. Silver, CRDL-SR-4-54, (1964)

[22], "*A comparison between depilated and clipped skin in the percutaneous toxicity of nerve agents to rabbits*", M. Muir and S. Callaway, PTP 215, (1955)

[23], "*The percutaneous LCt50 of GF vapour to monkeys by permeable clothing materials*", F. P. McGrath, V. J. Von Berg, F. W. Oberst, J. N. Carter and N. G. Marius, MLRR 214, (1953)

[24], "*Relative percutaneous toxicities of liquid GB and GF*", F. N. Marzulli, R. G. Horton, H. Elrod, M. R. Williams, R. Oliver and J. Wiles, MLRR 119, (1952)

[25], "*Toxicity and perception of GF vapour*", F. P. McGrath, V. J. Von Berg and J. N. Oberst, MLRR 185, (1953)

[26], " *The toxicity of G compounds. Part VIII. Studies on the toxicity of GF*", A. Muir, S. Callaway and F. Burgess, PTP 130, (1949)

[27], "*Human exposure to VX vapour*", E. C. B. Bramwell, PTP 830, (1963)

[28], "*Percutaneous exposure of the arm or forearm of man to VX vapor*", P. Cresthull, W. S. Koon, N. P. Musselman, M. Bowers and F. W. Oberst, US Army Chemical Research and development Laboratories Technical Report 3176, (1963)

[29], "*Human Responses to intravenous VX*", F. R. Sidell, Edgewood Arsenal: MRL US Army No. 4082, (1967)

[30], "*Variability of different human intact skin sites to the penetration of VX*", V. M. Sim, Edgewood Arsenal: US Army No 3122, (1962)

[31], "*VX percutaneous studies in man*", V. M. Sim and J. L. Stubbs, Edgewood Arsenal US Army No 3015, (1960)

[32], "*Penetration of VX applied to the forearm at environmental temperatures of 65^{O}F and 115^{O}F*", F. N. Craig, E. G. Cummings, L. A. Mounter, B. R. Tharp and F. J. Vocci, US Army Edgewood Arsenal Technical Report No 4064, (1967)

[33], "*Effects of environmental temperature on the penetration of VX applied to the cheek*", E. G. Cummings and F. N. Craig, US Army Edgewood Arsenal CRDL Technical Report No 3256, (1965)

[34], "*The fate of GF applied to clothed pigs*", P. Dirnhuber, A. C. Allenby and R. T. Tregear, PTP 849, (1963)

[35], "*Chamber tests with human subjects IX, basic tests with H vapor*", J. H. Heinen, H. W. Carhart, W. H. Taylor, B. N. Stolp, J. C. Connor and N. M. Clauson, NRL Washington Report 2579, (1945)

[36], "*The value of permeable protective shorts as a means of reducing the number of casualities from exposure to H vapour*", M. D. Freeman and J. H. Rollins, TDMR 731, (1943)

[37], "*The penetration of vesicant vapors into human skin*", M. Bergmann, J. S. Fruton, C. Golumbic, S. M. Nagy, M. A. Stahmann and W. H. Stein, OSRD 4855, (1945)

[38], "*Chamber tests with human subjects I. Design and operation of chamber. II. Initial tests of navy issue protective clothing against H vapor*", W. H. Taylor, H. W. Carhart and L. E. Daily, NRL Washington Report No P-2208, (1943)

[39], "*Mechanisms in Production of cutaneous injuries by sulfur and nitrogen mustards. In: Chemical Warfare agents and related chemical problems - Parts III-IV*", B. Renshaw, Summary Technical Report of Division 9, NDRC of the OSRD, (1946)

[40], "*Toxic effects of compounds related to mustard 1. Toxic effects of mustard, mustard sulfone, sesquimustard and sesquimustard analogues*", E. M. K. Geiling and F. C. McLean, OSRD 1391, (1943)

[41], "*A vapor train study of the comparative vesicancy of mustard and several related amines ans sulfides on human skin*", S. Black, K. P. Dubois and M. A. Lipton, CTLR 34, (1944)

[42], "*Florida trials with experimental thermal generator*", anon, Dugway Proving Ground Special Report No 34, (1944)

[43], "*Performance of the 50lb LC/AC bomb in wooded terrain*", anon, Dugway Proving GFround Field Progress Report No 4, (1944)

[44], "*National Defence Research Committee: Informal monthly report on toxicity of chemical warfare agents*", anon, Informal report No 9-4-1-9 October, (1943)

[47], "*National Defence Research Committee: Informal monthly report on toxicity of chemical warfare agents*", anon, Informal report No 9-4-1-17 June, (1944)

[48], "*The relative insensitivity to mustard gas of the skin on the hand*", H. Cullumbine, Porton report 2429, (1942)

[49], "*Vesicant prophylaxis and decontamination*", R. P. Chilcott, Ph.D. Thesis, University of London, (2000)

[50], "*The casualty producing effect of a company shoot of rockets. 5-inch charged mustard gas*", anon, Porton report 2379, (1942)

[51], "*Sampling and analysis of initial clouds*", anon, Porton Memorandum 19, (1943)

[52], "*Chemical sampling of CW agents in field experiments*", anon, Porton Memorandum 19, (1942)

[53], "*Trials to determine the casualty producing value of initial clouds of mustard gas*", anon, Porton Report 2313, (1941)

[54], "*Area shoot with 5.5 inch BE/Chem shell charged HTV plus 0.1% perspex*", anon, Suffield Report No 98, (1943)

[55], "*Vapoour danger from gross mustard contamination*", anon, Suffield Report No 92, (1943)

[56], "*Thge physiological activity of the cloud produced by Coming's thermal generator*", anon, Suffield Report No 98, (1943)

[57], "*Effectiveness of given Cts of mustard gas vapour for long exposure periods under tropical conditions*", anon, Suffield Technical Minute 103,
[58], "*The effect of mustard vapour on the skin under hot weather conditions*", anon, CDRE India Report 245, (1942)

[59], "*Report on two doses of severe skin burns from mustard gas vapour under tropical conditions in India*", anon, CDRE India Report 285, (1944)

[60], "*On dichloroethtylsulphide (mustard gas)*", E. K. Marshall, V. Lynch and H. W. Smith, J.Pharmacol.Exp.Therap. 12 291-301, (1918)

[61], "*Observations on the role of water in the susceptibility of human skin to injury by vesicant vapors*", B. Renshaw, J.Invest.Dermatol. 75-85, (1947)

[62], "*The penetration of vesicant vapors into human skin*", S. M. Nagy, C. Golumbic, W. H. Stein, J. S. Fruton and M. Bergmann, J.Ge n.Physiol. 29 441-467, (1946)

[63], "*The clinical reaction of the skin to mustard gas vapour*", D. C. Sinclair, Brit.J.Dermatol. & Syphillis 61 113-125, (1949)

[64], "*Inhalation Toxicology of Aerosolised Nerve Agents. 1. VX Revisited*", R. W. Bide and D. J. Risk, DRES TR 2000-063, (2000)

[65], "*Fasciculus on chemical warfare medicine. Vol. III. Skin and systemic poisons*", H. E. Harrison, Committee on treatment of gas casualties, division of the National Research Council, (1945)

[66], "*Chemical warfare agents and related chemical problems*", M. Gates, J. W. Williams and J. A. Zapp, Summary Technical Report of Div 9, NRDC, Vol 1, (1946)

[67], "*The relative military value of mustard-1 and G-34*", J. A. E. Eyster and A. S. Loevenhart, US Report AM538, (1919)

[68], "*Die Chemische Waffe*", W. Mueller, second edition, Berlin, (1932)

[69], "*L'arme chimique et ses blessures*", C. Hederer and M. Istin, J.B.Bailliere et fils, Paris, (1935)

[70], "*Chemicals in war. A treatise on chemical warfare*", A. M. Prentiss, McGraw-Hill Inc, NY and London, (1937)

[71], "*An experimental study mapharsen (meta-amino para-hydroxyphenyl arsine oxide) as an antisyphilitic agent*", A. L. Tatum and G. A. Cooper, J.Pharmacol.Exp.Therapeut. 50., (1934)

[72], "*The beta-chlorovinylchloroarsines*", W. L. Lewis and G. A. Perkins, Ind.Eng.Chem., 15, 290-295, (1923)

[73], "*The beta-chlorovinylarsines and their derivatives*", W. L. Lewis and H. W. Steigler, J.Amer.Chem.Soc., 47, 2546-2556, (1925)

[74], "*The beta-chlorovinyl arsines*", F. Q. Mann and W. J. Pope, J.Chem.Soc., 121, 1754, (1922)

[75], "*Mustard-1, chemical, pathological and toxicological characteristics. Physiological action and comparison with other substances*", J. A. E. Eyster, US Report Ph. 237, (1919)

[76], "*Grun u gelbkreuz*", H. Buscher, Hamburg, (1932)

[77], "*Second conference of medical officers and consultants*", L. T. D. Williams, Pine Bluff Arsenal 16/17 Nov, US Report BCWR 2389, (1943)

[78], "*A formal analaysis of the action of liquid vesicants on the bare skin*", A. D. Landahl, W. Bloom, R. K. Cannon and E. M. K. Geiling, OSRD 5032, Univesity of Chicago, May 5. Div. 9-360-M2, (1945)

[79], "*Chamber tests with human subjects. VI. Arm chamber exposures to L vapour*", Anonymous, NRL Report P-2483, (1945)

[80], "*Lewisite II. Action on the skin of animals*", G. Rovida, Sperimentale, 83, 101-113, (1929)

[81], "*Lewisite III. Action on the human skin*", G. Rovida, Sperimentale, 83, 115-120, (1929)

[82], "*Lewisite: Its chemistry, toxicology and biological effects*", M. Goldman and J. C. Dacre, Rev.Environ.Contam.Toxicol., 110, 75-115, (1989)

[83], "*The prevention and treatment of cutaneous injury secondary to chemical warfare agents. Application of these findings to other dermatological conditions and wound healing*", K. J. Smith, Adv.Milit.Dermatol., 17, 41-60, (1999)

[84], "*Lewisite (M-1): 1940 summary of physiologic and toxicologic data*", E. L. Wardell, Edgewood Arsenal Technical Report 285, (1940)

[85], "*Blistering concentrations of M-1 vapors for exposures from 5 minutes to three hours*", W. A. Eldridge, Edgewood Arsenal Medical Research Division Report 8, (1923)

[86], "*The effect of lewisite vapour on small animals and man*", H. Cullumbine, Porton Report 2553, October 29, (1943)

[87], "*Lewisite. Determination of vesicant action on man by use of a continuous flow chamber*", S. D. Silver, Edgewood Arsenal Toxicity Research Laboratory Report 1, August 21, (1943)

[89], "*Efficacy of dimercapto chelating agents for the treatment of poisoning by percutaneously applied dichlor(2-chlorovinyl)arsine in rabbits*", R. H. Inns and P. Rice, Human Exp. Toxicol., 12, 241-246, (1993)

[89], "*The use of laser doppler imaging as an aid in clinical management decision making in the treatment of vesicant burns*", P. Rice, R. F. R. Brown and N. J. Bennett, Burns, 24, 692-698, (1998)

[90], "*The development of lewisite vapour induced lesions in the domestic white pig*", P. Rice and R. F. R. Brown, Int.J.Pathol., 80, 59-67, (1999)

[91], "*Non-invasive quantification of skin injury resulting from exposure to sulphur mustard and lewisite vapours*", R. P. Chilcott, R. F. R. Brown and P. Rice, Burns, 26, 245-250, (2000)

[92], "*Comparison of three skin decontamination systems for activity against G and H agents*", R. W. Bide, S. J. Armour, T. W. Sawyer, D. Parker and D. Risk, Suffield Memorandum 1265, (1989)

[93], "*Activity of the proposed Canadian reactive skin decontaminant lotion against lewisite*", R. W. Bide and D. Risk, Suffield Memorandum 1301, (1991)

[94], "*Minutes of NATO/LG7/WG1, TNO/PML, 5-7 January*", Anonymous, (2001)

[95], "*The Benesh Micropipette*", W. Bloom, J. F. Thomson, E. Goldwaser, J. Savit and P. Debruyn, OSRD 4230, (1944)

[96], "*A formal analysis of the action of liquid vesicants on the bare skin*", A. D. Landahl, W. Bloom, R. K. Cannon and E. M. K. Geiling, OSRD 5032, (1945)

[97], "*National Defence Research Committee:Informal monthly progress report on toxicity of chemical warfare agents*", Anonymous, NDRC 9-4-1-11 (10-12-43), (1943)

[98], "*National Defence Research Committee:Informal monthly progress report on toxicity of chemical warfare agents*", Anonymous, NDRC 9-4-1-12 (10-1-44), (1944)

[101], "*Chemical Warfare Agents. Toxicological and Medical Considerations*", J. R. Wood, Medical Division Report 201, (1949)

[102], "*The toxicity of liquid GB applied to the skin of man.*" G. Freeman, F. N. Marzulli, A. B. Craig, J. R. Trimble and M. R. Williams, Medical Laboratories Research Report (MLRR) 217, (1953)

[103], "*Toxicity of GB vapor by cutaneous adsorption in monkey and man.*" F. P. McGrath, C. W. Dutreau and E. H. Bray, MLRR 49, (1951)