Review of the U.S. Army's Health Risk Assessments for Oral Exposure to Six Chemical-Warfare Agents

Subcommittee on Chronic Reference Doses for Selected Chemical Warfare Agents, National Research Council

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Subcommittee on Chronic Reference Doses For Selected Chemical-Warfare Agents
Committee on Toxicology
Board on Environmental Studies and Toxicology
Commission on Life Sciences
National Research Council

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Preface

Several military bases contaminated with chemical-warfare agents as a result of storage and past disposal practices are slated to be closed pursuant to the Base Realignment and Closure Act. Before those military bases can be transferred to civilian use, contaminated soil and water must be cleaned to levels that are considered safe. To help make decisions on restoration required at contaminated sites and on the potential uses of the former military installations (e.g., for housing, occupational, or wildlife purposes), the U.S. Army developed interim chronic oral reference doses and, where appropriate, oral slope factors for six chemical-warfare agents that are likely to be encountered at contaminated sites. Similar information for inhalation exposure is under development.

In this report, the Subcommittee on Chronic Reference Doses for Selected Chemical-Warfare Agents of the National Research Council's (NRC's) Committee on Toxicology reviews the scientific validity of the Army's interim values for the six chemical-warfare agents—GA, GB, GD, VX, sulfur mustard, and lewisite. The NRC report is intended to be useful to the Army in making site-specific cleanup decisions.

This report has been reviewed in draft form by individuals chosen for their technical expertise and diverse perspectives in accordance with procedures approved by the NRC's Report Review Committee for reviewing NRC and Institute of Medicine reports. The purpose of that independent review was to provide candid and critical comments to assist the NRC in making the published report as sound as possible and to ensure

that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals, who are neither officials nor employees of the NRC, for their participation in the review of this report: Joseph Borzelleca, Virginia Commonwealth University; John Doull, University of Kansas Medical Center; Ronald W. Estabrook (report review committee monitor), University of Texas Southwestern Medical Center; Florence Kinoshita, Hercules Inc.; Loren Koller (report review coordinator), Oregon State University; John O'Donoghue, Eastman Kodak Company; and Joseph Rodricks, Life Sciences Trust.

The individuals listed above have provided many constructive comments and suggestions. It must be emphasized, however, that responsibility for the final content of this report rests entirely with the authoring committee and the NRC.

We gratefully acknowledge Veronique Hauschild, Joe King, and Steve Kistner (all of the U.S. Army Center for Health Promotion and Preventive Medicine) and Dennis Opresko, Robert Ross, Annetta Watson, and Robert Young (all of Oak Ridge National Laboratory) for providing background information and for making presentations to the subcommittee.

We are grateful for the assistance of the NRC staff for preparing the report. Staff members who contributed to this effort are James J. Reisa, director of the Board on Environmental Studies and Toxicology; Carol A, Maczka, senior program director for toxicology and risk assessment; Ruth E. Crossgrove, editor; and Linda Leonard, senior project assistant. We especially wish to recognize the major contributions of the project director, Kulbir S. Bakshi, and the program officer, Susan N.J. Pang, who directed the preparation of the subcommittee's report. Their knowledge of the scientific and technical literature and their tireless effort to obtain information and to organize the subcommittee meetings and the report aided in the successful completion of the project.

Finally, we would like to thank all the members of the subcommittee for their dedicated efforts throughout the development of this report.

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Contents

Review of the U.S. Army's Health Risk Assessments For Oral Exposure to Six Chemical-Warfare Agents

Summary

The U.S. ARMY is under a congressional mandate and the Chemical Weapons Convention of January 1993 to destroy its entire stockpile of chemical munitions. In addition to stockpiled munitions, nonstockpile chemical materiel (NSCM) has been identified for destruction. NSCM includes a host of lethal wastes from past disposal efforts, unserviceable munitions, chemically contaminated containers, chemical-production facilities, newly located chemical munitions, known sites containing substantial quantities of buried chemical weapons and wastes, and binary weapons and components. There are eight stockpile sites located in the continental United States and one on an island in the Pacific Ocean, and 82 NSCM locations have been identified. There are concerns, based on storage and past disposal practices, about soil and groundwater contamination at those sites. Six of the most commonly found chemical-warfare agents at stockpile and NSCM sites are the nerve agents GA, GB, GD, and VX and the vesicating (blistering) agents sulfur mustard and lewisite.

To ensure that chemical contamination is reduced to safe concentrations at stockpile and NSCM sites before they are used for residential, occupational, or wildlife purposes, the U.S. Army requested that health-based exposure limits for GA, GB, GD, VX, sulfur mustard, and lewisite be developed to protect the public and the environment. Oak Ridge National Laboratory (ORNL) was asked to conduct the health risk assessments and propose chronic oral reference doses (RfDs) and, where

appropriate, oral slope factors (SFs) for the six agents. RfDs are toxicological values developed for noncancer effects and used as reference points to limit human oral exposure to potentially hazardous concentrations of chemicals thought to have thresholds for their effects. RfDs are estimates (with uncertainty spanning an order of magnitude or greater) of daily oral chemical exposures that are unlikely to have deleterious effects during a human lifetime. For chemicals identified as carcinogens (e.g., sulfur mustard), SFs are also calculated. SFs are estimates of upper-bound lifetime cancer risk from chronic exposure to an agent.

The Army's Surgeon General adopted the proposed RfDs and SFs developed by ORNL as interim values to ensure that consistent health-based criteria were applied in ongoing initiatives requiring decisions on the safety of contaminated sites. The Army's Surgeon General also requested that the NRC independently review the scientific validity of these values. The NRC assigned this task to the Committee on Toxicology (COT), and a multidisciplinary subcommittee of experts was convened to assess the scientific validity of the interim RfDs developed for GA, GB, GD, VX, sulfur mustard, and lewisite and the SF developed for sulfur mustard. Specifically, the subcommittee was asked to (1) determine whether all the relevant toxicity data were considered appropriately; (2) review the uncertainty, variability, and quality of the data; (3) determine the appropriateness of the assumptions used to derive the RfDs (e.g., the application of uncertainty factors); and (4) identify data gaps and make recommendations for future research.

Although multiple agents are present at stockpile and NSCM sites, the subcommittee was asked to evaluate the agents only on an individual basis. Furthermore, although the most likely routes of exposure to chemical-warfare agents at these sites are the inhalation and dermal routes, the subcommittee was only asked to evaluate toxicological risk from the oral route at this time. The Army is in the process of developing inhalation exposure guidelines. The subcommittee was also not asked to address issues related to risk management, such as technology, detection, and feasibility.

EVALUATION OF THE ARMY'S INTERIM RFDS AND SFS

Table S-1 presents the interim RfDs and SFs adopted by the Army for GA, GB, GD, VX, sulfur mustard, and lewisite, as well as the recommenda

tions of the subcommittee. The subcommittee found that the guidelines used to derive the Army's interim RfDs were consistent with guidelines used by the U.S. Environmental Protection Agency (EPA) and were appropriate. In general, the approach was to identify the no-observed-adverse-effect level (NOAEL) or the lowest-observed-adverse-effect level (LOAEL) from animal or human studies. The NOAEL or LOAEL was divided by an overall uncertainty factor that reflects the uncertainties associated with the types of data used and a professional judgment of the entire data base for the chemical. An SF for sulfur mustard was derived using a comparative potency method.

TABLE S-1 Reference Doses and Slope Factors for Six Chemical-Warfare Agents

Agent	Army's Interim RfDs (mg/kg/d)	NRC's Recommended RfDs (mg/kg/d)	Army's Interim SFs (per mg/kg/d)	NRC's Recommended SFs (per mg/kg/d)
GA	4×10^{-5}	4×10^{-5}	NA	NA
GB	2×10^{-5}	2×10^{-5}	NA	NA
GD	4×10^{-6}	4×10^{-6}	NA	NA
VX	6×10^{-7}	5×10^{-7}	NA	NA
Sulfur mustard	7×10^{-6}	7×10^{-6}	9.5	1.6
Lewisite	1×10^{-4}	1×10^{-5}	NA	NA

Abbreviations: RfDs, reference doses; SFs, slope factors; NA, not applicable.

The subcommittee determined that the Army's interim RfDs for GA, GB, GD, and sulfur mustard were scientifically valid but concluded that the RfDs for VX and lewisite and the SF for sulfur mustard were too high. The bases for those conclusions are described below. Research recommendations for filling major data gaps are also presented.

CONCLUSIONS AND RECOMMENDATIONS

GA

The Army's interim RfD of 4×10^{-5} mg/kg of body weight per day for GA was based on a subchronic intraperitoneal toxicity study in rats, in which depression in plasma-cholinesterase (ChE) activity was considered the

critical end point. Although that end point is considered a biomarker of exposure rather than an adverse effect, the subcommittee agrees that the study is the best available one to use for deriving the RfD for GA and concludes that the available data on GA support the proposed RfD.

The major gap in the available information on GA is the lack of either a subchronic or a chronic oral toxicity study from which to derive the RfD. The absence of oral data could be addressed by conducting a subchronic oral toxicity study that assesses anti-ChE activity in red blood cells (RBCs) and plasma in one or preferably two species. If further research reveals that significant toxic effects can be induced by any of the nerve agents at doses below those that cause significant ChE inhibition, additional studies should be conducted to reassess the safety of the recommended RfD for GA.

GB

The Army's interim RfD of 2×10^{-5} mg/kg per day for GB was based on a subchronic oral toxicity study in rats, in which depression in RBC-ChE activity was considered the critical end point. Although that end point is a biomarker of exposure rather than an adverse effect, the subcommittee believes that this study is the best available one from which to derive the RfD for GB and concludes that the proposed RfD is scientifically valid.

The major gap in the available information on GB is the lack of either a subchronic or a chronic oral toxicity study that demonstrates a clear LOAEL or NOAEL. The absence of that type of data could be addressed by conducting a subchronic oral toxicity study that assesses anti-ChE activity in RBCs and plasma in one or preferably two species. If further research reveals that significant toxic effects can be induced by any of the nerve agents at doses below those that cause significant ChE inhibition, additional studies should be conducted to reassess the safety of the recommended RfD for GB.

GD

The Army's interim RfD of 4×10^{-6} mg/kg per day for GD was based on a subchronic oral toxicity study in rats, in which depression of plasma-

ChE activity was observed. Although that end point is a biomarker of exposure rather than an adverse effect, the subcommittee believes that this study is the best available one from which to derive the RfD for GD and concludes that the proposed RfD is scientifically valid.

The major gap in the available information on GD is the lack of either a subchronic or a chronic oral toxicity study that demonstrates a clear dose-response relationship between GD exposure and ChE inhibition. The absence of that type of data could be addressed by conducting a subchronic oral toxicity study that assesses anti-ChE activity in RBCs and plasma in one or preferably two species. Range-finding studies focusing on ChE analytical methods offer the best possibility for filling the data gap. If further research reveals that significant toxic effects can be induced by any of the nerve agents at doses below those that cause significant ChE inhibition, additional studies should be conducted to reassess the safety of the recommended RfD for GD.

VX

The Army's interim RfD of 6×10^{-7} mg/kg per day for VX was based on an oral toxicity study in sheep, in which depression in blood-ChE activity was observed. After evaluating that study, the subcommittee concludes that uncertainties about the relevance of this animal model to humans and weaknesses in the study design undermine the use of the study for deriving the RfD. Instead, the subcommittee recommends using a 1964 study of human volunteers in whom depression in RBC ChE was observed after oral exposure to low concentrations of VX. Although that study also has weaknesses and involves a biomarker of exposure rather than an adverse effect, the subcommittee believes it is preferable to use human data rather than data from a questionable animal model, because the uncertainty associated with extrapolating from animals to humans is avoided. On the basis of the human study, the subcommittee concludes that the data on VX support an RfD of 5×10^{-7} mg/kg per day, which is slightly lower than the Army's interim RfD of 6×10^{-7} mg/kg per day.

The major gap in the available information on VX is the lack of either a subchronic or a chronic oral toxicity study that demonstrates a clear dose-response relationship between VX exposure and ChE inhibition. The absence of that type of data could be addressed by conducting a

subchronic oral toxicity study that assesses anti-ChE activity in RBCs and plasma in one or preferably two species. If further research reveals that significant toxic effects can be induced by any of the nerve agents at doses below those that cause significant ChE inhibition, additional studies should be conducted to reassess the safety of the recommended RfD for VX.

SULFUR MUSTARD

The Army's interim RfD of 7×10^{-6} mg/kg per day for sulfur mustard was based on an oral two-generation reproductive toxicity study in rats, in which thickening of the forestomach epithelium was observed. The subcommittee agrees that this study is the best available one from which to derive the RfD for sulfur mustard and concludes that the interim RfD for sulfur mustard is scientifically valid. However, the subcommittee recommends adjustments in two of the uncertainty factors used to derive that RfD. Although the adjustments do not change the RfD for sulfur mustard, the subcommittee believes that they are scientifically justified and should be reflected in the Army's supporting documentation for the RfD.

Sulfur mustard is the only agent in this report associated with sufficient evidence of carcinogenicity in animal studies and, therefore, is the only agent for which a carcinogenic SF was derived. The indirect approach used to estimate the SF involved comparing the carcinogenic potency of sulfur mustard to that of the well-known carcinogen benzo[α]pyrene (B[α]P). Although the subcommittee finds that approach to be scientifically valid, given the absence of either a epidemiological investigation or a chronic oral animal bioassay on sulfur mustard, it recommends the use of a more recent risk estimate of the carcinogenic potency of B[α]P. On the basis of that estimate, the subcommittee concludes that the Army's interim SF of 9.5 per milligram per kilogram per day should be lowered to 1.6 per milligram per kilogram per day. Thus, if the potential carcinogenic risk from ingestion of sulfur mustard is restricted to less than 1 in 100,000 persons, daily oral doses should be limited to 6×10^{-6} mg/kg per day, a value slightly lower than the Army's interim RfD of 7×10^{-6} mg/kg per day.

The major gap in the available information on sulfur mustard is the lack of a chronic oral animal bioassay from which to derive the RfD and

SF. Because of that deficiency, the RfD for sulfur mustard is estimated by extrapolating from a subchronic study in animals, and the SF is established by applying comparative carcinogenic potency methods. The absence of chronic oral toxicity data can be addressed by conducting a chronic oral animal bioassay. It is important that sulfur mustard be delivered to animals at a slow rate (i.e., in the diet) rather than by stomach tube, because it is corrosive at the point of entry.

LEWISITE

The Army's interim RfD of 1×10^{-4} mg/kg per day for lewisite was based on two oral studies: a two-generation reproductive study and a 90-day toxicity study in rats. In both studies, necrosis and hyperplasia of the forestomach were observed. After considering those studies and other potential studies, the subcommittee concludes that a 1987 teratogenicity study conducted in rabbits is more appropriate than the rat studies for deriving the RfD, because there is evidence that the rabbit might be more susceptible to lewisite than the rat. On the basis of the rabbit study, in which maternal mortality and gastric lesions were observed, the subcommittee believes that the RfD for lewisite should be lowered from 1×10^{-4} mg/kg per day to 1×10^{-5} mg/kg per day.

The major gaps in the available information on lewisite are the lack of information on the implications of administering lewisite directly to the stomach over a short time and the absence of chronic oral toxicity data from which to derive an RfD. Because of those deficiencies, the RfD for lewisite was estimated by extrapolating from a less-than-ideal animal study to humans. Confidence in the RfD can be increased if subchronic oral toxicity studies in rabbits and rats are conducted to compare the effects of chronic oral exposure to low concentrations of lewisite with the effects of short-term intragastric administration of small volumes of lewisite. Such studies will provide not only the data needed to better understand the implications of dosing techniques but also more pertinent information on whether the rabbit is more appropriate than the rat for deriving an RfD for lewisite.

1

Introduction

IN 1985, the U.S. Congress passed Public Law 99–145 requiring the destruction of the stockpile of lethal chemical-warfare agents and munitions in the United States. Two principal types of chemical-warfare agents are found at U.S. stockpile sites—nerve agents (e.g., GB and VX) and vesicating (blistering) agents (e.g., sulfur mustard agents). Chemical stockpile sites are located at nine sites: Umatilla Depot, Oregon; Tooele Army Depot, Utah; Pueblo Depot, Colorado; Newport Army Ammunition Plant, Indiana; Aberdeen Proving Ground, Maryland; Lexington-Blue Grass Army Depot, Kentucky; Anniston Army Depot, Alabama; Pine Bluff Arsenal, Arkansas; and Johnston Atoll in the Pacific Ocean.

Some chemical-warfare agents and related materiel, referred to as nonstockpile chemical materiel (NSCM), were not included in the 1985 law requiring destruction but were subsequently added to the chemical demilitarization program in the House Appropriations Report 101–822 that accompanied the fiscal year 1991 Defense Appropriations Act. NSCM includes lethal wastes from past disposal efforts, unserviceable munitions, chemically contaminated containers, chemical-production facilities, newly located chemical munitions, known sites containing significant quantities of buried chemical weapons and waste, and binary weapons and components. The U.S. Army has identified 82 NSCM locations in the United States, involving 33 states, the Virgin Islands, and the District of Columbia (Opresko et al. 1998). Table 1–1 presents a list

TABLE 1–1 Summary of Chemical Materiel Thought to Be Located at Nonstockpile Sites[a]

State—EPA Region	Site	Material of Concern[b]
Alabama—IV	Anniston Army Depot	GB, VX
	Ft. McClellan	GB, VX, mustard, HD, CK, CG, BZ, CX, AC
	Camp Sibert	Mustard degradation products
	Huntsville Arsenal	Mustard
	Redstone Arsenal	HD, L, uncharacterized rounds, GB, VX
	Theodore Naval Ammunition Magazine	Mustard and/or its degradation products
Alaska—X	Adak	Mustard, L
	Chicago Harbor	Mustard, L
	Gerstle River Test Center	Mustard, L, GB, GA, VX
	Unalaska Island	CAIS[c] vials
	Ft. Wainwright	CAIS[c]
Arizona—IX	Navajo Depot Activity	Mustard, white phosphorus, PWP
	Yuma Proving Ground	Mustard, GB, VX
Arkansas—VI	Ft. Chaffee	CAIS[c] residue
	Pine Bluff Arsenal	Mustard, HN, L, and degradation products, CAIS[c]
California—IX	Ft. Ord	Mustard, CAIS[c]
	Santa Rosa Army Airfield	CAIS[c]
	Edwards AFB	Mustard, GB, phosgene, CK, HCN
Colorado—VII	Rocky Mountain Arsenal	GB, mustard, CG, VX
	Pueblo Army Depot Activity	Mustard
District of Columbia—III	American University	L, adamsite
Florida—IV	Brooksville Army Air Base	Mustard
	Drew Field	Mustard, CAIS[c]
	MacDill AFB	Mustard
	Withlacoochee	Mustard (Levinstein)
	Dry Tortuga Keys	Mustard
	Zephyr Hills Gunner Range	Mustard
Georgia—IV	Ft. Gillem	Mustard
	Ft. Benning	G-agents
	Manchester	Mustard
Hawaii—IX	Kipapa Ammunition Storage Site	Mustard
	Schofield Barracks	H, L, CK, HCN, and residues, CAIS[c] GB, BZ
	Waiakea Forest Reserve	CAIS[c] GB, BZ
Idaho—X	Targhee National Forest	Phosgene, NO_2
Illinois—V	Savanna Army Depot Activity	Mustard and residue

State—EPA Region	Site	Material of Concern[b]
Indiana—V	Camp Atterbury	Mustard, CAIS[c]
	Naval Weapons Support Center	Mustard, CAIS[c]
	Newport Army Ammunition Plant	VX and residue
Kansas—VII	Marysville	Mustard
Kentucky—IV	Blue Grass Army Depot	Mustard
Louisiana—VI	England AFB	CAIS[c], phosgene
	Ft. Polk	CAIS[c] (mustard, L)
	Mississippi River near New Orleans	Bombs with unknown fill
	Concord Spur	Mustard
Maryland—III	Edgewood Area-APG	VX, mustard, GA, GB, white phosphorus, riot control agents; spectrum of US, foreign, and experimental CW
Mississippi—IV	Columbus Army Airfield	Mustard
	Horne Island	Mustard, arsenic-containing agents, unspecified others
	Camp Shelby	Mustard
Nebraska—VII	Nebraska Ordnance Plant	Mustard
Nevada—IX	Hawthorne Army Ammunition Plant	Mustard, phosgene, unspecified others
New Jersey—II	Lakehurst Naval Air Base	Unspecified "toxic agent shells"
	Raritan Arsenal	Mustard and residues
	Delaware Ordnance Depot	Phosgene
	Ft. Hancock	Unspecified "gas storage cylinders"
New Mexico—VI	Wingate Ordnance Depot	Mustard
New York—II	Mitchel Field	CAIS[c]
North	Camp LeJeune	CAIS[c], CN, unspecified others
Carolina—IV	Laurinburg-Maxton Army Air Base	Mustard
Ohio—V	Ravenna Army Ammunition Plant	Mustard
Oregon—X	Umatilla Depot Activity	Mustard, VX, other "mixed contamination"
Pennsylvania—III	Defense District Region East (formerly New Cumberland Army)	CAIS[c]
South	Charleston Army Depot	Mustard
Carolina—IV	Naval Weapons Center	Mustard

State—EPA Region	Site	Materiel of Concern[b]
South Dakota—VIII	Black Hills Ordnance Depot	Mustard, CG
Tennessee—IV	Defense Depot Memphis	Mustard, CAIS[c]
Texas—VI	San Jacinto Ordnance Depot	Phosgene, mustard
	Ft. Hood	Mustard, CN
	Camp Stanley Storage Activity	Mustard
	Camp Bullis	Mustard, CN, CS, phosgene, PS, white phosphorus
Utah—VIII	Dugway Proving Ground	VX, GA, GB, GD, CS, mustard, agent residues, foreign chemical munitions, unspecified others; biologicals
	Defense Depot Ogden	CAIS[c], mustard, phosgene, smoke bombs
	Tooele Army Depot	Mustard and residues, smoke pots, GA, incendiaries
Virginia—III	Ft. Belvoir	CAIS[c]
Washington—X	U.S. Naval Magazine	Phosgene
Virgin Islands—II	(Former) Ft. Segarra (St. Thomas, Water Island)	CG, CK, HCN, phosgene, H, HT, GA

[a] Data from USACMDA (1993a,b).

[b] GA, GB, GD, and VX are organophosphate nerve agents with anticholinesterase properties; H, HD, and HT are various formulations of sulfur mustard (vesicant); HN is nitrogen mustard (vesicant); L is the organic arsenical vesicant, lewisite. The following are less common: adamsite is an organic arsenical vomiting agent; AC is hydrogen cyanide (HCN); BZ is 3-quinuclidinyl benzilate, a hallucinogen; CK is the casualty agent cyanogen chloride; CG is phosgene (carbonyl chloride), a choking agent; CX is phosgene oxime (vesicant); CN is chloroacetophenone ("tear gas") and is used as a riot-control agent; CS is *o*-chlorobenzalmalononitrile ("tear gas") and is used as a riot-control agent.

[c] Chemical Agent Identification Set, a training aid containing vials of various chemical-warfare agents normally in dilute chloroform solution.
Source: Opresko et al. 1998.

of specific sites and the chemical materiel present at those sites. Sulfur mustard agents is the most frequently identified materiel. Historically, disposal of chemical-warfare agents was accomplished through burial, although some NSCM was placed in bodies of water. Thus, there is potential for soil and groundwater contamination at many of the NSCM sites.

In the past, the destruction of NSCM was not addressed as intensely as the disposal of existing chemical-weapon stockpiles, because most NSCM sites do not pose an immediate risk to public health or the environment. However, the urgency for destroying NSCM has recently increased because of the discovery of buried NSCM and the signing of the chemical-arms-control treaty (Convention on the Prohibition of the Development, Production, Stockpiling, and Use of the Chemical Weapons and on Their Destruction) by the United States and 150 other nations to eliminate chemical weapons from the inventories of all nations. The 1993 Defense Authorization Act (Section 176 of Public Law 102–484) directed the Army to examine the scale of effort and to consider plans needed to dispose of NSCM.

The NRC's Board on Army Science and Technology is involved in studies of the destruction of stockpile and nonstockpile chemical munitions. The Committee on Review and Evaluation of the Army Chemical Stockpile Disposal Program and the Committee on Review and Evaluation of the Army Chemical Non-Stockpile Materiel Disposal Program are reviewing the technical aspects of the Army's disposal methods on an ongoing basis (see NRC 1999a, b).

The U.S. Army Environmental Center (USAEC) serves as the program manager for the Army's Installation Restoration Program and is involved in the Army Base Realignment and Closure program. It has responsibilities for supporting installation-restoration (environmental cleanup) activities at Army installations and property nationwide. The USAEC and the Army Corps of Engineers investigate, characterize, and remediate sites where contamination is found. In recent years, there has been an increasing need for decision-making criteria to determine the scale of installation restoration required at active military installations and formerly used defense sites where chemical-warfare agent contamination has occurred. The goal of the restoration efforts is to ensure that chemical contamination is reduced to safe concentrations in these areas before they are used for residential, occupational, or wildlife purposes. Therefore, health-based exposure limits must be established to protect the public and the environment. Although people can be exposed to chemical-warfare agents in different ways, including ingestion of contaminated drinking water or soil, inhalation of vapors or contaminated dust, and dermal contact from contaminated soil, the subcommittee was asked to consider only the oral pathway.

Reference doses (RfDs) are toxicological values used as reference points to limit human oral exposure to potentially hazardous amounts of

chemicals that are thought to have thresholds for their effects. An RfD is an estimate (with uncertainty spanning an order of magnitude or greater) of the daily oral exposure to a potential toxicological hazard that is likely to have no risk of deleterious effects during a human lifetime (EPA 1989). The RfD is derived by identifying the no-observed-adverse-effect level or lowest-observed-adverse-effect level from animal and human studies, and dividing by uncertainty factors, that reflect the uncertainties associated with the types of data used, and a modifying factor, that is based on a professional judgment of additional uncertainties not addressed by the standard uncertainty factors.

For those chemicals identified as carcinogens (e.g., sulfur mustard), slope factors (SFs) are derived in addition to RfDs. An SF is the slope of the dose-response curve for an agent in the low-dose region. An SF is used to estimate the lifetime cancer risk from chronic exposure to an agent and is typically determined by modeling an agent's dose-response curve as the doses approach zero. An upper-bound on the slope is usually used instead of the slope itself. For agents that do not have an appropriate chronic toxicity study from which to model a dose-response curve, SFs can be determined by comparing the carcinogenic potency of an agent with that of a well-known carcinogen.

The Army uses RfDs and SFs to make site-specific decisions on cleanup of sites contaminated with chemical-warfare agents and to make decisions on the potential uses of military installations. Because RfDs and SFs are applicable to the oral exposure route, they are used to calculate exposure limits for drinking water, soil, and other media that have the potential to be ingested by persons at or near remediation sites. Although RfDs and SFs can be applied to more than one site, exposure limits are more appropriately determined on a site-specific basis. RfDs and SFs are not media (e.g., water and soil) standards for purposes of safe cleanup or decontamination goals. RfDs and SFs are translated into safe media concentrations by incorporating information on site-specific exposure variables, including exposure frequency; exposure duration; estimated amount of contaminated soil, water, or specific food ingested; ingestion rate; and body weight. The site-specific exposure variables and the RfD or SF are incorporated into a health risk assessment according to approved methods and calculations of the U.S. Environmental Protection Agency (EPA). Thus, safe public and environmental standards are calculated from the RfD or SF and are dependent on the situation.

At the request of the Army, Oak Ridge National Laboratory conducted

health risk assessments and derived RfDs for six chemical-warfare agents—GA, GB, GD, VX, sulfur mustard, and lewisite—and an SF for sulfur mustard (see Appendices A–F). These agents are considered priority chemicals, because they are the ones most commonly found at stockpile and NSCM sites. GA, GB, GD, and VX are nerve agents, and lewisite and sulfur mustard are vesicating (blistering) agents. Because immediate establishment of the RfDs and SFs for those agents was believed to be necessary to ensure that consistent, health-based criteria would be applied in ongoing initiatives requiring decisions on the safety of contaminated sites, the Army's Surgeon General accepted the proposed RfDs and SFs as interim values until an independent review of them was conducted by the National Research Council (NRC). In 1996, the Army requested that the NRC review the scientific validity of the RfDs and SFs for the six chemical-warfare agents. The NRC assigned this task to the Committee on Toxicology (COT), which assembled the Sub-committee on Chronic Reference Doses for Selected Chemical-Warfare Agents to review the scientific validity of the RfDs developed for GA, GB, GD, VX, lewisite, and sulfur mustard and the SF for sulfur mustard. The multidisiplinary subcommittee of experts was asked to (1) determine whether all the relevant toxicity data were appropriately considered; (2) review the uncertainty, variability, and quality of data; (3) determine the appropriateness of the assumptions used to derive RfDs (e.g., application of uncertainty factors); and (4) identify data gaps and make recommendations for future research.

To address its task, the subcommittee critically reviewed the health-risk-assessment documents on the individual chemical-warfare agents provided by the Army (see Appendices A–F, which were published in 1998 by Opresko et al.), published and unpublished studies cited in the Army's reports, and conducted its own literature search to identify any relevant data that were missing. Although the potential exists for multiple agents to be present at NSCM sites, the subcommittee was asked to evaluate the agents only on an individual basis. Furthermore, although the most likely routes of exposure to chemical-warfare agents at stockpile and NSCM sites are the inhalation and dermal routes, the subcommittee was only asked to evaluate toxicological risk from the oral route of exposure at this time. The Army informed the subcommittee that inhalation exposure guidelines are in development. The subcommittee was not asked to address issues related to risk management, such as technology, detection, and feasibility.

The results of the subcommittee's evaluations are presented in Chapters 2 through 9. Chapter 2 reviews the method used by the Army to derive RfDs, and also includes a discussion of the benchmark dose method as a point of departure for calculating RfDs. Chapters 3 through 6 evaluate the RfDs for the nerve agents GA, GB, GD, and VX. Chapter 7 evaluates the RfD and slope factor for sulfur mustard, and Chapter 8 provides an evaluation of the RfD for lewisite. Research recommendations are presented at the end of Chapters 3–8 for each of the specific chemical-warfare agents.

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2

Derivation of Reference Doses

THIS CHAPTER contains a brief description of the methods used by toxicologists at Oak Ridge National Laboratory (ORNL) to derive the U.S. Army's interim reference doses (RfDs) for GA, GB, GD, VX, sulfur mustard, and lewisite. Those methods were based on the procedures outlined by the U.S. Environmental Protection Agency for Superfund risk assessment guidelines (EPA 1989) and for reference concentrations (EPA 1994). An alternative method, the benchmark-dose (BD) approach (Crump 1984) is also described. Because uncertainty factors are integral to both approaches, further consideration is also given to the statistical distribution and confidence associated with them.

Because sulfur mustard is the only agent identified in this report as a carcinogen, a description of the derivation of the carcinogenic slope factor is presented in the chapter on sulfur mustard (see Chapter 7).

REFERENCE-DOSE CALCULATION

The RfD is an estimate (with uncertainty spanning perhaps an order of magnitude or greater) of a daily dose to the human population (including susceptible subpopulations) that is likely to be without an appreciable risk of deleterious health effects during a lifetime (EPA 1989). Numerical estimates of risk (probability of an adverse health effect) are not provided by the RfD process. The RfD process only describes the

exposure conditions that are unlikely to cause noncancer health effects, which are typically assumed to have a threshold dose above which deleterious health effects would be expected to occur. The major issues in the calculation of the RfD are identifying the most sensitive effects that are relevant to the human for selecting the no-observed-adverse-effect level (NOAEL) or the lowest-observed-adverse-effect level (LOAEL), the determination of the NOAEL or LOAEL from the most appropriate study, and the magnitudes of the uncertainty factors and the modifying factor used in the process.

ORNL has published a detailed description of the method for deriving the RfD (see Opresko et al. 1998). Briefly, the RfD is calculated by determining the most sensitive and significant NOAEL or LOAEL for noncancer effects and dividing that by the product of a series of uncertainty factors and a modifying factor:

$$\mathrm{RfD} = \frac{\mathrm{NOAEL\ \ or\ \ LOAEL}}{\mathrm{UF_A \bullet UF_H \bullet UF_L \bullet UF_S \bullet UF_D \bullet MF}},$$

where UF_A represents the uncertainty of using experimental animal data for human effects, UF_H represents the variable susceptibilities in the human population (e.g., genetic, nutritional, age), UF_L represents the expected ratio of the LOAEL to the NOAEL when a LOAEL is used instead of a NOAEL, UF_S represents the uncertainty of predicting chronic exposure effects on the basis of subchronic exposure studies, UF_D represents the uncertainty assigned to an inadequate data base, and MF is a modifying factor to account for any additional uncertainty not addressed by the standard uncertainty factors.

Typically, uncertainty factors are assigned values ranging from 1 to 10. If information concerning a factor is sparse and uncertainty is high, a default value of 10 generally is used. If information is available, the uncertainty factor might be reduced to 1. For example, UF_A would be 1 if the NOAEL or LOAEL is based on human data. A value of less than 1 for the UF_A would be used if the NOAEL for the biological end point of concern is more sensitive in animals than in humans. UF_H would be less than 10 if the NOAEL or LOAEL is based on a susceptible human subpopulation. UF_S would be 1 if adequate chronic exposure studies have been conducted or chronic effects are not expected. UF_L would be 1 if a NOAEL is available. UF_D would be 1 if an adequate array of human

and animal data is available for various biological effects. For an uncertainty factor that falls between 1 and 10, a factor of 3 is typically assigned, because 3 is the approximate logarithmic mean of 1 and 10, and the assumption is made that the uncertainty factor is distributed lognormally (EPA 1994).

A modifying factor between 1 and 10 is used when the five uncertainty factors do not explicitly account for all scientific uncertainties that exist. The default value for the modifying factor is 1.

The main shortcoming of the traditional RfD approach is the use of the NOAEL. Weaknesses with that use include the following: (1) the NOAEL does not incorporate information on the slope of the dose-response curve or the variability in the data, (2) the NOAEL is likely to be higher with smaller sample sizes or an inadequate study, (3) the NOAEL is limited to one of the experimental doses, (4) the number and spacing of doses in a study influence the dose chosen for the NOAEL, and (5) because the NOAEL is defined as a dose that does not produce an observed increase in adverse effects compared with control levels and is dependent on the power of the study, theoretically, the risk associated with the NOAEL might fall anywhere between zero and 10% for quantal data (EPA 1991). The true risk at a NOAEL can vary from zero to over 20% depending on the end point, spacing of doses, and numbers of animals used (Leisenring and Ryan 1992).

BENCHMARK DOSE

Because of shortcomings in the use of NOAELs to determine doses with low risk, Crump (1984) proposed that the NOAEL be replaced by a benchmark dose (BD) associated with a biological effect. The BD is a dose with a specified low level of excess health risk, generally in the range of 1% to 10%, that can be estimated from data with little or no extrapolation outside the experimental dose range. Specifically, the BD is derived by modeling the data in the observed experimental range, selecting an incidence level within or near the observed range (e.g., the effective dose producing a 10% increased incidence of response), and determining the upper confidence limit on the model. Because the method does not involve extrapolation far below the experimental range of doses, the BD is less dependent on the choice of the mathematical form of the dose-response model than it is on the choice of the uncer

tainty factors. To account for experimental variation, a lower confidence limit or uncertainty factors on the BD are recommended to assure that the specified excess risk (e.g., 10%) is not likely to be exceeded.

The BD approach uses more of the available toxicity data (such as the number of animals, dose-response data, and data variability) than the traditional RfD method and provides a consistent basis for calculating the RfD. However, selecting the magnitudes of the uncertainty factors remains an issue in the BD approach, as in the traditional RfD approach. Depending on the level of risk selected at the BD, the uncertainty factor would be comparable to the ratio of the LOAEL to the NOAEL (Dourson et al. 1985).

In the proposed cancer risk-assessment guidelines of the U.S. Environmental Protection Agency (EPA 1996), a BD approach based on uncertainty factors is one of the methods suggested when a nonlinear dose response is expected (e.g., for nongenotoxic carcinogens or tumor promoters). Low-dose linear extrapolation below the BD would still be used for direct-acting (genotoxic) carcinogens. For carcinogens that act indirectly (e.g., nongenotoxic carcinogens, such as tumor promoters), it might be possible to predict doses below which no appreciable risk is expected. However, it must be shown that exposure to such carcinogenic substances does not augment existing background processes that lead to low-dose linearity (Crawford and Wilson 1996). Hence, EPA (1996) is proposing that techniques for risk assessment be based upon the mode of action of the carcinogen. It has been suggested that the lower confidence limit on an excess risk of 10% or less for the BD is the point of departure (the point at which low-dose extrapolation occurs for either linear or nonlinear extrapolation) for low-risk assessment.

UNCERTAINTY FACTORS

Multiplying several uncertainty factors together possibly can lead to a large overall uncertainty factor that results in an unnecessarily small RfD. It is unlikely that each uncertainty factor simultaneously needs to be at its maximal value. Several investigators recently addressed the issue of compounding conservatism resulting from using the upper bounds on each of the uncertainty factors in the calculation of a RfD or reference concentration (e.g., Burmaster and Harris 1993; Calabrese and Gilbert 1993; Bogen 1994; Nair et al. 1995; Baird et al. 1996; Swartout

et al. 1998). EPA typically uses a maximum of 3,000 for the product of four uncertainty factors that individually are greater than 1, and a maximum of 10,000 with five uncertainty factors (Dourson 1994).

The product of the uncertainty factors ($U = UF_A \times UF_H \times UF_L \times UF_S \times UF_D$) must produce high confidence that the overall product (U) is large enough to protect susceptible subpopulations adequately from long-term exposures. In the absence of information to select a specific value for an uncertainty factor, default values of 10 generally are used. The value assigned to an uncertainty factor can be considered a random variable, the default value being larger for most end points and chemicals. For example, Swartout (1996) examined the ratio of doses that produced equivalent adverse effects, NOAELs or LOAELs, from subchronic and chronic exposures to about 100 substances. Swartout (1996) observed that the median ratio was 2. If data were available only from a subchronic study, on average, the NOAEL or LOAEL dose for chronic exposures should be reduced by a factor of 2. Swartout (1996) observed that the 95th percentile for the ratio of subchronic to chronic doses for NOAELs or LOAELs was 17. Hence, to cover only the uncertainty of estimating chronic effects from subchronic data, the UF_S should be 17 for 95% coverage. A different factor might be necessary for some classes of chemicals or specific end points. The currently used default factor of 10 provides about 89% coverage in general. If an adverse effect is proportional to the total dose (i.e., the dose rate times the duration of exposure), extrapolation of results from a 90-day subchronic exposure to a 2-year chronic exposure would use an average UF_S of 730 days ÷ 90 days = 8.

From selected databases providing the distributions of various uncertainty factors, Baird et al. (1996) used computer simulations to obtain the overall product of uncertainty factors required to achieve selected levels of confidence. They indicated that dividing the NOAEL from a chronic exposure study in animals by the product of default values of 10 for UF_A and UF_H ($10 \times 10 = 100$) gives about 95% confidence that RfD = NOAEL ÷ 100 is adequately conservative. Baird et al. (1996) found that a total uncertainty factor of 1,000 tends to provide about 99% confidence of adequate conservatism when three default values of 10 are used, and a total of 3,000 provides a similar result when four uncertainty factors are used. Thus, it appears that the conventional products of 10 for default values of uncertainty factors provide reasonable assurance of safety.

CONCLUSIONS

The subcommittee concludes that the method used by ORNL to derive the Army's interim RfDs is scientifically sound and is consistent with the guidelines and process used by EPA. It must be emphasized that scientific judgment is often a key overriding factor in that method. Because the process involves a series of extrapolations, each with its own degree of uncertainty, emphasis should be placed on establishing doses that are judged to be safe for human exposure based on the best scientific information. In addition, the subcommittee believes that the BD approach should also be considered in establishing RfDs in the future. Similar risk estimates from the conventional and the BD methods would provide greater confidence in the proposed RfDs.

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3

Evaluation of the Army's Interim Reference Dose for GA

THE CHEMICAL-WARFARE agent GA (also known as tabun) is an organophosphate nerve agent found at several stockpile and nonstockpile munition sites in the United States and its territories. At the request of the U.S. Army, Oak Ridge National Laboratory (ORNL) conducted a health risk assessment of GA. The assessment included a detailed analysis of GA's physical and chemical properties, environmental fate, mechanism of action, and animal and human toxicity data (see Appendix A, *Health Risk Assessment of GA*, ORNL 1996). On the basis of that assessment, ORNL proposed a reference dose (RfD) of 4×10^{-5} mg/kg of body weight per day for noncancer health effects of GA exposure. Because there was no evidence that GA is carcinogenic, a slope factor was not derived. The Army's Surgeon General accepted ORNL's proposed RfD as an interim exposure value until an independent evaluation of the proposed RfD was conducted by the National Research Council (NRC). This chapter contains the NRC's independent assessment of the scientific validity of the Army's interim RfD for GA.

DERIVATION OF THE ARMY'S INTERIM RFD

The Army's interim RfD for GA is 4×10^{-5} mg/kg per day (or 0.04 μg/kg per day). ORNL (1996) calculated that value on the basis of the highest intraperitoneal (i.p.) dose of GA that did not cause significant

depression in plasma cholinesterase (ChE) activity in rats. The no-observed-adverse-effect level (NOAEL) of GA was 28.13 μg/kg per day in a subchronic toxicity study (Bucci et al. 1992). In that study, male and female rats were injected i.p. with GA 5 days a week for 13 weeks. To estimate the equivalent oral NOAEL from the i.p. NOAEL, ORNL compared the oral and i.p. LD_{50} (lethal dose to 50% of test animals) values for the rat and assumed that the same ratio would apply for longer-term exposures. Rat studies reported an oral LD_{50} of 3,700 μg/kg (RTECS 1995) and i.p. LD_{50} of 490 μg/kg (RTECS 1995) and 800 μg/kg (U.S. Department of the Army 1974) (average 645 μg/kg). Thus, the equivalent oral NOAEL was calculated as follows:

$$28.13\ \mu g/kg\ per\ day \times (3{,}700\ \mu g/kg \div 645\ \mu g/kg) = 161\ \mu g/kg\ per\ day.$$

Because of the discontinuous exposure regimen, ORNL adjusted the equivalent oral $NOAEL_{adj}$ for continuous exposures by multiplying 161 μg/kg per day by a factor of 5/7 (i.e., 5 days/7days) to yield a $NOAEL_{adj}$ of 115 μg/kg per day (or 0.115 mg/kg per day). The RfD for GA was calculated to be 4×10^{-5} mg/kg per day by dividing the $NOAEL_{adj}$ by 2,700, the product of the uncertainty factors and the modifying factor selected by ORNL.

APPROPRIATENESS OF THE CRITICAL STUDY

The critical study used by ORNL for deriving the RfD for GA was a subchronic toxicity study (Bucci et al. 1992) in which Caesarian-derived Sprague-Dawley rats (12 males and 12 females per group) were injected i.p. with GA at doses of 28.13, 56.25, and 112.50 μg/kg per day for 5 days per week for 13 weeks and then sacrificed and necropsied. PlasmaChE and red-blood-cell (RBC)-acetylcholinesterase (AChE) measurements, as well as several other blood measurements, were taken before dosing and at the end of weeks 1, 3, 7, and 13. Considerable variability was observed in RBC-AChE concentrations. Significant depression in RBC AChE was observed in female rats in the mid-and high-dose groups at weeks 7 and 3, respectively, compared with control values and in male rats at all doses at week 1. Bucci et al. (1992) reported that mean baseline values (measurements taken before exposure) for RBC AChE

were substantially increased in male and female rats compared with control values recorded in previous nerve-agent studies conducted in the same laboratory. The increased baseline values were attributed to faulty reagents. The percentage reduction caused by exposure to GA was noted to be in the same range as in the earlier work. ORNL (1996) reanalyzed the data with analysis of variance (ANOVA) and Dunnett's comparison and reported significant depression in RBC AChE in females at all doses at weeks 1 and 7 and at the high dose at week 3 compared with baseline values but not compared with control values. For male rats, RBC-AChE concentrations were significantly reduced in the low- and mid-dose groups compared with baseline values but not with control values.

Plasma-ChE measurements were reported by Bucci et al. (1992) to be significantly decreased in females of the mid- and high-dose groups before exposure and at weeks 1,3, and 7 compared with controls but not at week 13. In males, significant decreases in plasma ChE were observed in the high-dose group at weeks 3 and 7. ORNL (1996) reanalyzed the data with ANOVA and Dunnett's comparison and reported a significant decrease in plasma ChE in females at all doses at weeks 1, 3, and 7 compared with control values but not with baseline values. At week 13, a significant increase in plasma ChE was observed in females in the low-dose group. This increase might be due to the compensatory increase in the synthesis of ChE to accommodate the losses due to the repeated exposure and irreversible inhibition AChE. For males, significant decreases in plasma ChE were observed in the mid- and high-dose groups at weeks 1, 3, and 7 compared with control and baseline values; in addition, significant decreases were observed in the mid-dose group at weeks 1 and 13 compared with baseline but not controls values.

ORNL noted that plasma-ChE values in male rats provided the least variable indicator of the lowest-observed-adverse-effect level (LOAEL) and NOAEL for GA and that there was evidence (based on mean plasma-ChE values) of a dose-response relationship. Therefore, ORNL used that data to determine the LOAEL and NOAEL for ChE inhibition by GA. ORNL considered 56.25 μg/kg per day to be the LOAEL because of the significant reduction in plasma-ChE concentrations observed in male rats at this dose (relative to controls and baseline values). Because of the lack of consistent change in plasma- and RBC-AChE values (relative to controls and baseline values), ORNL considered the low dose of 28.13 μg/kg per day to be the NOAEL for the study.

The subcommittee noted several weaknesses in using the Bucci et al.

(1992) study to determine a NOAEL or a LOAEL for GA. The study involved the i.p. exposure route, which is not a relevant route of exposure for determining an RfD, the study was subchronic in duration (13 weeks) rather than chronic (104 weeks), and ChE measurements varied and did not show a consistent dose-response relationship across ChE types and genders. In addition, the methods used to measure ChE were not ideal (see Appendix G).

The subcommittee considered other possible critical studies for the derivation of the RfD for GA. In a study by Dulaney et al. (1985), GA at 100 μg/kg was injected subcutaneously into rats daily for 85 days. Reduced growth rates were observed until day 38 of dosing, when rates returned to control levels. In addition, acetylcholinesterase (AChE) activity in the striatum and the remainder of the brain was 13% and 22%, respectively, of control values when measured 24 h after the last injection. The investigators also conducted a cumulative mortality study, in which rats were subcutaneously injected with GA at a dose of 70 or 100 μg/kg per day for 25 days. One animal from the low-dose group and two from the high-dose group died by day 20. The study was continued with the remaining rats for an additional 60 days, but no additional deaths occurred.

The subcommittee found the Dulaney et al. (1985) studies to be even weaker than the Bucci et al. (1992) study for deriving the RfD for GA. Only one dose was used in the 85-day study and the only toxicity end points evaluated were growth rate and AChE activity in the brain. Similarly, even though the 25-day study included two doses, the only effect considered was mortality, an inappropriate end point for deriving RfDs. The subcommittee found no oral studies that involved repeated exposure to GA and no other suitable toxicity studies for deriving the RfD for GA. Thus, the subcommittee agrees with ORNL that the study by Bucci et al. (1992) is the most appropriate of the available studies for derivation of the RfD for GA.

APPROPRIATENESS OF CRITICAL END POINT

The $NOAEL_{adj}$ (115 μg/kg per day) used by ORNL for derivation of the RfD for GA was based on the dose that did not cause a significant depression in plasma-ChE activity in rats (Bucci et al. 1992). The subcommittee notes that ChE inhibition is typically considered a biomarker of expo

sure to organophosphate agents rather than an adverse effect. However, it is generally agreed that inhibition of ChE contributes to the overall hazard identification of ChE-inhibiting agents. The U.S. Environmental Protection Agency (EPA) has used ChE inhibition to establish RfDs for several organophosphate pesticides, such as malathion (EPA 1992) and ethion (EPA 1989).

Although the subcommittee agrees with ORNL that ChE inhibition is a valid end point on which to base the RfD for GA, the subcommittee's confidence in the data is undermined by the increased baseline RBC-AChE concentrations, which were attributed to laboratory errors, in the critical study. Furthermore, confidence in the calculation of an equivalent oral NOAEL from an i.p. NOAEL is diminished because data from a secondary reference (i.e., RTECS 1995) were used to determine the ratio of the oral LD_{50} to the i.p. LD_{50}. The subcommittee suggests that the data be verified from the primary source and cited appropriately.

The subcommittee considered other possible toxicity end points, notably neurotoxicity, associated with GA exposure. Organophosphate compounds like GA may act directly on nerve cell receptors or, by inhibiting neural AChE, interfere with neuromuscular transmission and produce delayed-onset subjunctional muscle damage. In addition, some organophosphate compounds cause a neurotoxic effect (organophosphate-induced delayed neuropathy, or OPIDN) that is not associated with ChE inhibition. Emerging research in this area might indicate alternative toxicity end points to RBC-ChE inhibition that could be used to derive RfDs for nerve agents in the future.

The subcommittee also notes that additional human data available on anti-ChE agents were not included in ORNL's assessment. Data summaries from human experimentation conducted in the 1950s and 1960s were evaluated by the NRC in a series of reports titled *Possible Long-Term Health Effects of Short-Term Exposure to Chemical Agents* (NRC 1982, 1984, 1985). The reports include an evaluation of health records of volunteer soldiers who were exposed intravenously or intramuscularly to chemical-warfare agents, as well as a follow-up morbidity study conducted by the NRC in 1985. The NRC found no long-term health effects from short-term exposure to any specific chemical-warfare agent, but there was some evidence of an increase in malignant neoplasms among men exposed to anti-ChE agents. Although these studies are not directly applicable to deriving RfDs, they add to the completeness of the data base on GA.

Provided that appropriate assays were used, the subcommittee finds no

reason at this time to alter the practice of using RBC-ChE or plasma-ChE inhibition as the critical end point and agrees with ORNL that such inhibition is the best available critical noncancer end point on which to base the calculation of the RfD for GA.

APPROPRIATENESS OF UNCERTAINTY FACTORS

For GA, ORNL assigned values greater than 1 to four out of five uncertainty factors and to the modifying factor. The product of those factors was 2,700. The subcommittee evaluated each of the uncertainty factors and the modifying factor below.

EXTRAPOLATION FROM ANIMAL TO HUMAN

ORNL assigned a factor of 10 to the uncertainty factor for the extrapolation of data from animals to humans (UF_A) because no evidence suggests that humans are less susceptible than rats to GA. ORNL cited evidence that rodents have much lower RBC-AChE activity than humans (Ellin 1981), suggesting that rats might be more susceptible than humans to anti-ChE compounds, but also noted that the lower RBC-AChE activity might be offset by aliesterases (e.g., carbonyl esterase) present in the blood of rats. These enzymes, which are not found in humans (Cohen et al. 1971), are known to bind to and, therefore, reduce the toxicity of nerve agents (Fonnum and Sterri 1981). The subcommittee notes that rats have true ChE (AChE) in their plasma (Traina and Serpietri 1984), possibly reducing the toxicity of GA in rats. Furthermore, studies (Grob and Harvey 1958; Bucci and Parker 1992) with a similar nerve agent (GB) suggest that depression of RBC or plasma ChE with repeated oral administrations of nerve agents is much more difficult to induce in rats than in humans.

The available data on GA are insufficient to evaluate species differences fully with regard to ChE activity in humans and rats. Few human acute toxicity data can be compared with the available data on the rat. However, ORNL noted that acute toxicity data are available for comparisons with monkeys (see Appendix A, Table 2). Although the data suggest that monkeys are more susceptible than rats to GA, such an evaluation is weakened by the fact that it is based on data from a secondary reference (i.e., RTECS 1995). The subcommittee suggests that the

original data cited in the secondary reference be verified and the primary reference cited appropriately. Nevertheless, the subcommittee notes that limitations exist in drawing any conclusions from this type of comparison because the data from monkeys are not necessarily directly applicable to humans and because the studies did not involve the oral route of administration and involved only single exposures. However, given the enzyme differences between humans and rats described above and the available data on GB (see Chapter 4), a similar nerve agent, the subcommittee agrees with ORNL that a factor of 10 is appropriate for interspecies extrapolation.

PROTECTING SUSCEPTIBLE SUBPOPULATIONS

ORNL used a factor of 10 for the uncertainty factor to protect susceptible subpopulations (UF_H) because some individuals have a genetic polymorphism causing their serum-ChE activity to be abnormally low (Evans et al. 1952; Harris and Whittaker 1962). For homozygous individuals, the activity can be as low as 8–21% of the normal mean (Bonderman and Bonderman 1971). Genetic polymorphisms are also recognized for butyrylcholinesterase and paraoxonase, enzymes that might function in the sequestration and metabolism of organophosphate nerve agents (Loewenstein-Lichtenstein et al. 1995; Maekawa et al. 1997; Furlong et al. 1998). Individuals with those polymorphisms might be unusually susceptible to organophosphate anti-ChE compounds (Morgan 1989). The subcommittee agrees that a factor of 10 is appropriate for protecting this susceptible subpopulation.

EXTRAPOLATION FROM LOAEL TO NOAEL

The subcommittee agrees with the identification of the NOAEL by ORNL and concurs that a factor of 1 should be assigned to the uncertainty factor for the extrapolation from a LOAEL to a NOAEL (UF_L) because a NOAEL was used to calculate the RfD.

EXTRAPOLATION FROM SUBCHRONIC TO CHRONIC EXPOSURES

ORNL noted that in the derivation of RfDs for other organophosphate compounds, EPA (1989, 1992) used NOAELs for ChE inhibition that

were based on subchronic exposure data without adjustment for chronic exposures, because ChE inhibition is unlikely to change over time. Hence, a factor of 1 was used for the uncertainty factor for extrapolation from subchronic to chronic exposures (UF_S). For example, studies with the nerve agent VX indicate that maximal ChE inhibition occurs after 30-60 days of exposure and then levels off and sometimes shows signs of recovery (Goldman et al. 1988). However, because chronic exposure studies are not available to verify that additional effects would not occur from longer exposures to GA, ORNL assigned a factor of 3 to UF_S. The subcommittee agrees that a factor of 3 is appropriate.

DATA-BASE ADEQUACY

As noted by ORNL, the data base on GA lacks a multigeneration reproductive toxicity study, a standard toxicity study in a second species, and toxicity studies by the oral exposure route. In addition, the subcommittee notes that some evidence indicates that GA might be weakly genotoxic, but no chronic exposure studies are available to evaluate the carcinogenic potential of GA. Because of those deficiencies, a factor of 10 arguably could be assigned for the uncertainty factor for data-base adequacy (UF_D). Studies on other nerve agents, however, including a multigeneration study on VX (Goldman et al. 1988), two developmental toxicity studies on GB (Denk 1975; La Borde and Bates 1986), and a chronic inhalation study on GB (Weimer et al. 1979) indicate that reproductive, developmental, and carcinogenic effects are unlikely. Therefore, the subcommittee agrees with ORNL that a factor of 3 is adequate for UF_D to account for the incomplete data base.

MODIFYING FACTOR FOR ADDITIONAL UNCERTAINTY

ORNL used a modifying factor (MF) of 3 to compensate for the uncertainty associated with the extrapolation of data from the i.p. to the oral route. The subcommittee also believes that an MF is necessary. In the subcommittee's judgment, the uncertainty associated with the route-to-route extrapolation was not great enough to warrant the use of a factor of 10 and, therefore, agrees with ORNL that a factor of 3 (the approximate logarithmic mean of 1 and 10) is appropriate.

SUMMARY

Table 3-1 presents the values assigned to the uncertainty factors and the modifying factor by ORNL and those recommended by the subcommittee. The subcommittee's recommendations are the same as those of ORNL.

WEIGHT AND STRENGTH OF EVIDENCE

The subcommittee believes that the strength of evidence for the Army's interim RfD of 4×10^{-5} mg/kg per day is weak, because the critical study (Bucci et al. 1992) used to calculate the RfD involved a nonoral route of exposure.

CONCLUSIONS

The approach used by ORNL to calculate the RfD for GA is consistent with the guidelines of the EPA. On the basis of available toxicity and related data on GA, the subcommittee concludes that the Army's interim RfD for GA of 4×10^{-5} mg/kg per day is scientifically valid.

TABLE 3-1 Uncertainty Factors Used by ORNL and the NRC to Calculate the RfD for GA

Uncertainty Factor	Description	ORNL	NRC
UF_A	For animal-to-human extrapolation	10	10
UF_H	To protect susceptible subpopulations	10	10
UF_L	For LOAEL-to-NOAEL extrapolation	1	1
UF_S	For subchronic-to-chronic extrapolation	3	3
UF_D	For data-base adequacy	3	3
MF	Modifying factor for additional uncertainty	3	3
TOTAL UF		2,700	2,700

Abbreviations: LOAEL, lowest-observed-adverse-effect level; MF, modifying factor; NOAEL, no-observed-adverse-effect level; NRC, National Research Council; ORNL, Oak Ridge National Laboratory; RfD, reference dose; and UF, uncertainty factor.

DATA GAPS AND RESEARCH RECOMMENDATIONS

The major gap in the available information on GA is the lack of subchronic or chronic oral toxicity studies from which to directly derive the RfD. The absence of oral data could be addressed by conducting a subchronic oral toxicity study that assesses anti-ChE activity in RBCs and plasma. Study of two species would be preferable. If further research reveals that significant toxic effects can be induced by any of the nerve agents evaluated (i.e., GA, GB, GD, or VX) at doses below those that cause significant ChE inhibition, new studies should be conducted to reassess the safety of the recommended RfD for GA.

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4

Evaluation of the Army's Interim Reference Dose for GB

THE CHEMICAL-WARFARE agent GB (also known as sarin) is an organophosphate nerve agent found at several stockpile and nonstockpile munition sites in the United States. At the request of the U.S. Army, Oak Ridge National Laboratory (ORNL) conducted a health risk assessment of GB. The assessment comprised a detailed analysis of GB's physical and chemical properties, environmental fate, mechanism of action, and animal and human toxicity data (see Appendix B, *Health Risk Assessment of GB,* ORNL 1996). On the basis of that assessment, ORNL proposed a reference doses (RfD) of 2×10^{-5} mg/kg of body weight per day for noncancer health effects of GB exposure. Because there was no evidence that GB is carcinogenic, a slope factor was not derived. The Army's Surgeon General accepted ORNL's proposed RfD as an interim exposure value until an independent evaluation of the proposed RfD was conducted by the National Research Council (NRC). This chapter contains the NRC's independent assessment of the scientific validity of the Army's interim RfD for GB.

DERIVATION OF THE ARMY'S INTERIM RFD

The Army's interim RfD for GB is 2×10^{-5} mg/kg per day. ORNL (1996) calculated that value on the basis of the lowest oral dose of GB that caused significant depression in red-blood-cell (RBC)-cholinesterase

(ChE) activity in rats. The lowest-observed-adverse-effect level (LOAEL) of GB was 0.075 mg/kg per day in a subchronic toxicity study (Bucci and Parker 1992). In that study, male and female rats were administered GB by gavage for 5 days per week for 13 weeks. Because of the discontinuous exposure regimen, ORNL adjusted the LOAEL (LOAEL $_{adj}$ for continuous exposures by multiplying 0.075 mg/kg per day by a factor of 5/7 (i.e., 5 days/7 days) to yield a $LOAEL_{adj}$ of 0.054 mg/kg per day. The RfD for GB was calculated to be 2×10^{-5} mg/kg per day by dividing the $LOAEL_{adj}$ by 2,700, the product of the uncertainty factors and the modifying factor selected by ORNL.

APPROPRIATENESS OF THE CRITICAL STUDY

The critical study used by ORNL for deriving the RfD for GB was a subchronic toxicity study (Bucci and Parker 1992) in which Caesarian-derived Sprague-Dawley rats (12 males and 12 females per group) were administered GB with diisopropylcarbodiimide as a stabilizer (type II GB) by gavage at doses of 0.075, 0.15, and 0.3 mg/kg per day 5 days per week for 13 weeks. Plasma-ChE and RBC-acetylcholinesterase (AChE) measurements, as well as several other blood measurements, were taken before dosing and at the end of weeks 1, 3, 7, and 13. Plasma-ChE values in females of the mid-dose group were reported to be significantly lower than control values at weeks 1 and 7 and in the high-dose group at weeks 1, 3, and 7. In males, significant reduction in plasma ChE was observed in the low- and mid-dose groups only at week 1. ORNL reanalyzed the data using analysis of variance (ANOVA) and Dunnett's comparison and reported significant decreases in plasma ChE in females in the high-dose group at weeks 1, 3, and 7 and in the mid-dose group at weeks 1 and 3 compared with control and baseline values. A significant decrease was also observed in the mid-dose females at week 7 compared with control but not baseline values. For males, significant depression in plasma ChE was observed in the mid- and high-dose groups compared with control but baseline values. throughout the test period. At the low dose, significant decreases in ChE were observed at week 1 and 7 compared with control but not baseline values and at weeks 3 and 13 compared with baseline but not control values.

Bucci and Parker (1992) reported significant dose-related RBC-AChE depression in female rats in the mid- and high-dose groups and in male

rats in all dose groups compared with controls. By week 13, the degree of inhibition was diminished, suggesting a compensatory increase in the hepatic synthesis of ChE to accommodate for the losses due to the repeated exposure and irreversible inhibition of AChE. ORNL reanalyzed the data using ANOVA and Dunnett's comparison and reported significantly lower RBC-AChE values in males in all dose groups compared with baseline values at weeks 1,3, and 7 and for the two highest dose groups at week 13. The values for all dose groups were also significantly lower than control values at weeks 1, 3, and 7. Similar results were observed with female rats in the mid- and high-dose groups, but RBC-AChE concentrations were not reduced significantly in the low-dose group compared with control or baseline values. Because there was a statistically significant reduction in RBC AChE in male rats at the lowest dose of 0.075 mg/kg per day, ORNL considered that dose to be the LOAEL for the study.

The critical study (Bucci and Parker 1992) involved a relevant route of exposure (oral) for determining an RfD. Rats were administered GB by oral gavage, a route of administration that exaggerates the exposure that would normally occur from methods resulting in a slower rate of delivery (e.g., in feed or water). However, the study was subchronic in duration (13 weeks) rather than chronic (104 weeks), and ChE measurements varied and did not show a consistent dose-response relationship across ChE types and genders. Thus, the subcommittee believes that the study was too short in duration and that the results were too variable to form an ideal basis for determining a LOAEL. In addition, the methods used to measure ChE were not ideal (see Appendix G). However, in the absence of other well-conducted studies, the subcommittee agrees with ORNL that the study by Bucci and Parker (1992) is the most appropriate of the available studies for derivation of the RfD for GB.

APPROPRIATENESS OF CRITICAL END POINT

The $LOAEL_{adj}$ (0.054 mg/kg per day) used by ORNL for derivation of the RfD for GB was based on the lowest dose that caused a significant depression in RBC AChE activity in rats (Bucci and Parker 1992). The subcommittee notes that ChE inhibition is typically considered a biomarker of exposure to organophosphate agents rather than an adverse effect. However, it is generally agreed that inhibition of ChE contributes

to the overall hazard identification of ChE-inhibiting agents. The U.S. Environmental Protection Agency (EPA) has used ChE inhibition to establish RfDs for several organophosphate pesticides, such as malathion (EPA 1992) and ethion (EPA 1989).

The subcommittee considered other possible toxicity end points, notably neurotoxicity, associated with GB exposure. Organophosphate compounds like GB may act directly on nerve cell receptors or, by inhibiting neural AChE, interfere with neuromuscular transmission and produce delayed-onset subjunctional muscle damage. In addition, some organophosphate compounds cause a neurotoxic effect (organophosphate-induced delayed neuropathy, or OPIDN) that is not associated with ChE inhibition. OPIDN has not been observed in humans exposed to acutely toxic concentrations of GB (Munro et al. 1994), but some laboratory studies have suggested the OPIDN can be induced in mice (Husain et al. 1993) and chickens (Davies et al. 1960; Davies and Holland 1972; Gordon et al. 1983). However, other studies have shown conflicting results (Bucci et al. 1992).

The subcommittee notes that ORNL did not consider all of the available human data on GB. In 1994, GB was released by terrorists in Matsumoto City, Japan, where approximately 600 residents and rescue staff were exposed to the agent (Morita et al. 1995). Since then, several studies on the effects of the exposure have been published (Murata et al. 1997; Nakajima et al. 1997, 1998; Yokoyama et al. 1998a,b). There have also been unconfirmed reports of persisting neurological effects following low-dose exposures to GB. Emerging research in those areas might indicate alternative end points to ChE inhibition that could be used to derive RfDs for nerve agents in the future.

The subcommittee also notes that additional human data are available on anti-ChE agents, which were not included in ORNL's assessment. Data summaries from human experimentation conducted in the 1950s and 1960s were evaluated by the NRC in a series of reports titled *Possible Long-Term Health Effects of Short-Term Exposure to Chemical Agents* (NRC 1982, 1984, 1985). The reports include an evaluation of health records of volunteer soldiers who were exposed intravenously or intramuscularly to chemical-warfare agents, as well as a follow-up morbidity study conducted by the NRC in 1985. The NRC found no long-term health effects from short-term exposure to any specific chemical-warfare agent, but there was some evidence of an increase in malignant neoplasms among men exposed to anti-ChE agents. Although these studies are not

directly applicable to deriving RfDs, they add to the completeness of the data base on GB.

Provided that appropriate assays were used, the subcommittee finds no reason at this time to alter the practice of using RBC-ChE or plasma-ChE inhibition as the critical end point and agrees with ORNL that such inhibition is the best available critical noncancer end point on which to base the calculation of the RfD for GB.

APPROPRIATENESS OF UNCERTAINTY FACTORS

For GB, ORNL assigned values greater than 1 to five uncertainty factors and a value of 1 to the modifying factor. The product of those factors was 2,700. The subcommittee evaluated each of the uncertainty factors and the modifying factor below.

EXTRAPOLATION FROM ANIMAL TO HUMAN

ORNL assigned a factor of 10 to the uncertainty factor for the extrapolation of data from animals to humans (UF_A, citing evidence (Grob and Harvey 1958, Bucci and Parker 1992) that humans are more susceptible than rats to GB. Grob and Harvey (1958) reported that the single oral dose required to lower RBC AChE by 50% in humans was 0.01 mg/kg and that an average daily dose of 0.034 mg/kg for 3 days resulted in signs of moderate toxicity. In comparison, Bucci and Parker (1992) reported that GB administered to rats at a dose of 0.3 mg/kg per day for 90 days caused decreases in RBC-ChE concentrations but no signs of toxicity. Rats are known to have aliesterases (e.g., carbonyl esterase), which are enzymes in blood known to bind to and, therefore, reduce the toxicity of GB (Fonnum and Sterri 1981). Aliesterases are not present in humans (Cohen et al. 1971). The subcommittee also notes that rats have true ChE (AChE) in their plasma (Traina and Serpietri 1984), a factor that might reduce the toxicity of GB in rats.

ORNL also presented a comparison of acute toxicity values for GB (see Appendix B, Table 2). The data suggest that humans are more susceptible than rats to GB; however, such an evaluation is weakened by the fact that it is based on data from secondary references (i.e., RTECS 1995) and the human estimates are based on animal data (i.e., Somani et al. 1992). The subcommittee suggests that the original data cited in the

secondary reference be verified and the primary reference cited appropriately.

Given the available data on GB, the subcommittee agrees with ORNL that a factor of 10 is appropriate for interspecies extrapolation. The factor of 10 should be considered an estimate of the difference in sensitivity between humans and rats to GB and not a default value.

PROTECTING SUSCEPTIBLE SUBPOPULATIONS

ORNL used a factor of 10 for the uncertainty factor to protect susceptible subpopulations (UF_H because some individuals have a genetic polymorphism causing their serum-ChE activity to be abnormally low (Evans et al. 1952; Harris and Whittaker 1962). For homozygous individuals, the activity can be as low as 8–21% of the normal mean value (Bonderman and Bonderman 1971). Genetic polymorphisms are also recognized for butyrylcholinesterase and paraoxonase, enzymes that might function in the sequestration and metabolism of organophosphate nerve agents (Loewenstein-Lichtenstein et al. 1995; Maekawa et al. 1997; Furlong et al. 1998). Individuals with those polymorphisms might be unusually susceptible to organophosphate anti-ChE compounds (Morgan 1989). The subcommittee agrees with ORNL that a factor of 10 is appropriate for protecting this susceptible subpopulation.

EXTRAPOLATION FROM LOAEL TO NOAEL

ORNL assigned a factor of 3 rather than 10 to the uncertainty factor for extrapolation from a LOAEL to a NOAEL (UF_L) because ChE inhibition is a biomarker of exposure rather than a toxic effect. Although it could be argued that a dose of GB that significantly induces ChE inhibition in the absence of toxic effects is indicative of a NOAEL rather than a LOAEL, the subcommittee agrees that a factor of 3 is a conservative choice for UF_L.

EXTRAPOLATION FROM SUBCHRONIC TO CHRONIC EXPOSURES

In the derivation of RfDs for other organophosphate compounds, ORNL noted that EPA (1989, 1992) used NOAELs for ChE inhibition that were

based on subchronic exposure data without adjustment for chronic exposures, because ChE inhibition is unlikely to change over time. Hence, a factor of 1 was used for the uncertainty factor for extrapolation from subchronic to chronic exposures (UF_S). For example, studies with the nerve agent VX indicate that maximal ChE inhibition occurs after 30–60 days of exposure and then levels off and sometimes shows signs of recovery (Goldman et al. 1988). However, because chronic exposure studies are not available to verify that additional effects would not occur from longer exposures to GB, ORNL assigned a factor of 3 to UF_S. The subcommittee agrees that a factor of 3 is appropriate.

DATA-BASE ADEQUACY

As noted by ORNL, the most significant deficiency in the data base on GB is a multigeneration reproductive study. However, because two developmental toxicity studies on GB (Denk 1975; La Borde and Bates 1986) and a multigeneration reproductive study on VX (Goldman et al. 1988) indicate that reproductive effects are unlikely, the subcommittee agrees with ORNL that a factor of 3 for the uncertainty factor for data-base adequacy (UF_D) is appropriate.

MODIFYING FACTOR FOR ADDITIONAL UNCERTAINTY

The subcommittee considers the uncertainties of the data on GB to be represented adequately by the values assigned to the uncertainty factors above and agrees with ORNL that a modifying factor (MF) of 1 is appropriate.

SUMMARY

Table 4-1 presents the values assigned to the uncertainty factors and the modifying factor used by ORNL and those recommended by the subcommittee. The subcommittee's recommendations are the same as those of ORNL.

TABLE 4-1 Uncertainty Factors Used by ORNL and the NRC to Calculate the RfD for GB

Uncertainty Factor	Description	ORNL	NRC
UF_A	For animal-to-human extrapolation	10	10
UF_H	To protect susceptible subpopulations	10	10
UF_L	For LOAEL-to-NOAEL extrapolation	3	3
UF_S	For subchronic-to-chronic extrapolation	3	3
UF_D	For data-base adequacy	3	3
MF	Modifying factor for additional uncertainty	1	1
TOTAL UF		2,700	2,700

Abbreviations: LOAEL, lowest-observed-adverse-effect level; MF, modifying factor; NOAEL, no-observed-adverse-effect level; NRC, National Research Council; ORNL, Oak Ridge National Laboratory; RfD, reference dose; UF, uncertainty factor

WEIGHT AND STRENGTH OF EVIDENCE

The subcommittee believes that the strength of evidence for the Army's interim RfD of 2×10^{-5} mg/kg per day for GB is moderately good. There is a possibility that the LOAEL (0.075 mg/kg per day) used to calculate the RfD for GB is not accurate, because lower doses were not tested and variability in the RBC-ChE values was considerable. The subcommittee believes that because ChE inhibition is a biomarker of exposure rather than a toxic effect, use of this end point overestimates the oral toxicity of GB.

CONCLUSIONS

The approach used by ORNL to calculate the RfD for GB is consistent with the guidelines of the EPA. On the basis of available toxicity and related data on GB, the subcommittee concludes that the Army's interim RfD for GB of 2×10^{-5} mg/kg per day is scientifically valid.

DATA GAPS AND RESEARCH RECOMMENDATIONS

The major gap in the available information on GB is the lack of an oral subchronic or chronic toxicity study that demonstrates a clear LOAEL or NOAEL. The absence of that type of data could be addressed by conducting a subchronic oral toxicity study that assesses anti-ChE activity in RBCs and plasma in one or preferably two species. At least one dose between 0 and 0.075 mg/kg per day should be used. If further research reveals that significant toxic effects can be induced by any of the nerve agents evaluated (i.e., GA, GB, GD, or VX) at doses below those that cause significant ChE inhibition, new studies should be conducted reassess the safety of the recommended RfD for GB.

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5

EVALUATION OF THE ARMY'S INTERIM REFERENCE DOSE FOR GD

THE CHEMICAL-WARFARE agent GD (also known as soman) is an organophosphate nerve agent found at several stockpile and nonstockpile munition sites in the United States. At the request of the U.S. Army, Oak Ridge National Laboratory (ORNL) conducted a health risk assessment of GD. The assessment included a detailed analysis of GD's physical and chemical properties, environmental fate, mechanism of action, and animal and human toxicity data (see Appendix C, *Health Risk Assessment of GD,* ORNL 1996). On the basis of that assessment, ORNL proposed a reference dose (RfD) of 4×10^{-6} mg/kg of body weight per day for noncancer health effects of GD exposure. Because there was no evidence that GD is carcinogenic, a slope factor was not derived. The Army's Surgeon General accepted ORNL's proposed RfD as an interim exposure value until an independent evaluation of the proposed RfD was conducted by the National Research Council (NRC). This chapter contains the NRC's independent assessment of the scientific validity of the Army's interim RfD for GD.

DERIVATION OF THE ARMY'S INTERIM RFD

The Army's interim RfD for GD is 4×10^{-6} mg/kg per day. ORNL (1996) calculated that value on the basis of the lowest oral dose of GD that caused significant depression in plasma-cholinesterase (ChE) activity in

rats. The lowest-observed-adverse-effect level (LOAEL) of GD was 0.0175 mg/kg per day in a subchronic toxicity study (Bucci et al. 1992). In that study, male and female rats were administered GD by gavage 5 days a week for 13 weeks. Because of the discontinuous exposure regimen, ORNL adjusted the LOAEL ($LOAEL_{adj}$) for continuous exposures by multiplying 0.0175 mg/kg per day by a factor of 5/7 (i.e., 5 days/7 days) to yield a $LOAEL_{adj}$ of 0.0125 mg/kg per day. The RfD for GD was calculated to be 4×10^{-6} mg/kg per day by dividing the $LOAEL_{adj}$ by 2,700, the product of the uncertainty factors and the modifying factor selected by ORNL.

APPROPRIATENESS OF THE CRITICAL STUDY

The critical study used by ORNL for deriving the RfD for GD was a subchronic toxicity study (Bucci et al. 1992) in which Caesarian-derived Sprague-Dawley rats (12 males and 12 females per group) were administered GD by gavage at doses of 0.0175, 0.035, and 0.07 mg/kg per day for 5 days a week for 13 weeks and then sacrificed and necropsied. Plasma-ChE and red-blood-cell (RBC)-acetylcholinesterase (AChE) measurements, as well as several other blood measurements, were taken before dosing and at the end of weeks 1, 3, 7, and 13. Significant depression in plasma ChE was observed in male and female rats of the high-dose group at weeks 1 and 7 and in mid-dose males at week 7 compared with control values. No significant effect on RBC-AChE concentrations were observed. ORNL reanalyzed the data with analysis of variance and Dunnett's and Scheffe's comparisons and reported that at week 3 in females and week 7 in males, RBC-AChE concentrations of all dose groups were significantly lower than those of controls, but no dose-response relationship was found. In females during week 1, RBC-AChE concentrations in the controls and in all dose groups were inexplicably increased relative to baseline values (measurements taken before exposure). With regard to plasma ChE, a dose-related significant decrease relative to controls was observed at weeks 1 and 7 for male and female rats. In comparison with baseline values, plasma-ChE concentrations of the mid- and high-dose groups were significantly reduced at weeks 1, 3, 7, and 13 for males and females (with the exception of high-dose females at week 3). The lowest dose of 0.0175 mg/kg per day was considered by ORNL to be the LOAEL for the study because the reduction in plasma

ChE (relative to controls) at that dose was statistically significant and because the plasma-ChE activity during week 1 was reduced to 39% of baseline in males and 57% of baseline in females.

The critical study (Bucci et al. 1992) involved a relevant route of exposure (oral) for determining an RfD. Rats were administered GD by oral gavage, a route of administration that exaggerates the exposure that would normally occur from methods resulting in a slower rate of delivery (e.g., in feed or water). The study was subchronic in duration (13 weeks) rather than chronic (104 weeks), and ChE measurements varied and did not show a consistent dose-response relationship. Thus, the subcommittee believes that the study was too short in duration, and the results were too variable to form an ideal basis for determining a LOAEL. In addition, the methods used to analyze ChE were not ideal (see Appendix G). However, in the absence of other well-conducted studies, the sub-committee agrees with ORNL that the study by Bucci et al. (1992) is the most appropriate of the available studies for derivation of the RfD for GD.

APPROPRIATENESS OF CRITICAL END POINT

The $LOAEL_{adj}$ (0.0125 mg/kg per day) used by ORNL for derivation of the RfD for GD was based on the lowest dose that caused a significant depression in plasma-ChE activity in rats (Bucci et al. 1992). The sub-committee notes that ChE inhibition is typically considered a biomarker of exposure to organophosphate agents rather than an adverse effect. However, it is generally agreed that inhibition of ChE contributes to the overall hazard identification of ChE-inhibiting agents. The U.S. Environmental Protection Agency (EPA) has used ChE inhibition to establish RfDs for several organophosphate pesticides, such as malathion (EPA 1992) and ethion (EPA 1989).

The subcommittee considered other possible toxicity end points, notably neurotoxicity, associated with GD exposure. Organophosphate compounds like GD may act directly on nerve cell receptors or, by inhibiting neural AChE, interfere with neuromuscular transmission and produce delayed-onset subjunctional muscle damage. In addition, some organophosphate compounds cause a neurotoxic effect (organophosphate-induced delayed neuropathy, or OPIDN) that is not associated with ChE inhibition. Emerging research in this area might indicate alternative

end points to RBC-ChE inhibition that could be used to derive RfDs for nerve agents in the future.

The subcommittee also notes that additional human data are available on anti-ChE agents, which were not included in ORNL's assessment. Data summaries from human experimentation conducted in the 1950s and 1960s were evaluated by the NRC in a series of reports titled *Possible Long-Term Health Effects of Short-Term Exposure to Chemical Agents* (NRC 1982, 1984, 1985). The reports include an evaluation of health records of volunteer soldiers who were exposed intravenously or intramuscularly to chemical-warfare agents, as well as a followup morbidity study conducted by the NRC in 1985. The NRC found no long-term health effects from short-term exposure to any specific chemical-warfare agent, but there was some evidence of an increase in malignant neoplasms among men exposed to anti-ChE agents. While these studies are not directly applicable to deriving RfDs, the studies add to the completeness of the data base on GD.

Provided that appropriate assays were used, the subcommittee finds no reason at this time to alter the practice of using RBC-ChE or plasma-ChE inhibition as the critical toxicity end point, and agrees with ORNL that such inhibition is the best available critical noncancer end point on which to base the calculation of the RfD for GD.

APPROPRIATENESS OF UNCERTAINTY FACTORS

For GD, ORNL assigned values greater than 1 to five uncertainty factors and a value of 1 to the modifying factor. The product of those factors was 2,700. The subcommittee evaluated each of the uncertainty factors and the modifying factor below.

EXTRAPOLATION FROM ANIMAL TO HUMAN

ORNL assigned a factor of 10 for the uncertainty factor for the extrapolation of data from animals to humans (UF_A) because no evidence suggests that humans are less susceptible than rats to GD. ORNL cited the evidence that rodents have much lower RBC-AChE activity than humans (Ellin 1981), suggesting that rats might be more susceptible than humans to anti-ChE compounds, but also noted that the lower RBC-AChE

activity might be offset by aliesterases (e.g., carbonyl esterase) present in the blood of rats. These enzymes, which are not found in humans (Cohen et al. 1971), are known to bind to and, therefore, reduce the toxicity of nerve agents. Rats have true ChE (AChE) in their plasma (Traina and Serpietri 1984), which might reduce the toxicity of GD in rats. Furthermore, studies (Grob and Harvey 1958; Bucci and Parker 1992) with a similar nerve agent (GB) suggest that depression of RBC or plasma ChE with repeated oral administrations of nerve agents is much more difficult to induce in rats than in humans.

The available data on GD are insufficient to fully evaluate species differences with regard to ChE activity in humans and rats. Few human acute toxicity data can be compared with the available rat data. However, the subcommittee notes that acute toxicity data are available for comparisons with monkeys. For example, the intramuscular LD_{50} for GD is reported to be 9.5 µg/kg in monkeys (Lipp 1972) and 62 µg/kg in rats (Schoene et al. 1985), and the subcutaneous LD_{50} for GD is reported to be 13 µg/kg in monkeys (Fukuyama and Ashwick, unpublished material (1963), as cited in Dirnhubert et al. 1979) and 75 µg/kg in rats (Boškovi et al. 1984). Those data suggest that monkeys are approximately six times more susceptible than rats to GD. The subcommittee acknowledges the limitations of drawing any conclusions from this comparison because the data from monkeys are not necessarily directly applicable to humans and because the studies did not use the oral route of administration and involved only single exposures. However, given the enzyme differences between humans and rats described above and the available data on GB (see Chapter 4), a similar nerve agent, the subcommittee agrees with ORNL that a factor of 10 is appropriate for interspecies extrapolation. The factor of 10 should be considered an estimate of the difference in sensitivity between humans and rats to GD and not a default value.

PROTECTING SUSCEPTIBLE SUBPOPULATIONS

ORNL used a factor of 10 for the uncertainty factor to protect susceptible subpopulations (UF_H) because some individuals have a genetic polymorphism causing their serum-ChE activity to be abnormally low (Evans et al. 1952; Harris and Whittaker 1962). For homozygous individuals, the activity can be as low as 8–21% of the normal mean (Bonderman and Bonderman 1971). Genetic polymorphisms are also recognized for buty

rylcholinesterase and paraoxonase, enzymes that might function in the sequestration and metabolism of organophosphate nerve agents (Loewenstein-Lichtenstein et al. 1995; Maekawa et al. 1997; Furlong et al. 1998). Individuals with those polymorphisms might be unusually susceptible to organophosphate anti-ChE compounds (Morgan 1989). The subcommittee agrees that a factor of 10 is appropriate for protecting this susceptible subpopulation.

EXTRAPOLATION FROM LOAEL TO NOAEL

ORNL assigned a factor of 3 rather than 10 to the uncertainty factor for extrapolation from a LOAEL to a NOAEL (UF_L) because ChE inhibition is a biomarker of exposure rather than a toxic effect. Although it could be argued that a dose of GD that significantly induces ChE inhibition in the absence of toxic effects is indicative of a NOAEL rather than a LOAEL, the subcommittee agrees that a factor of 3 is a prudent choice for UF_L.

EXTRAPOLATION FROM SUBCHRONIC TO CHRONIC EXPOSURES

ORNL noted that in the derivation of RfDs for other organophosphate compounds, EPA (1989, 1992) used NOAELs for ChE inhibition that were based on subchronic data without adjustment for chronic exposures, because ChE inhibition is unlikely to change over time. Hence, a factor of 1 was used for the uncertainty factor for extrapolation from subchronic to chronic exposures (UF_S). For example, studies with the nerve agent VX indicate that maximal ChE inhibition occurs after 30–60 days of exposure and then levels off and sometimes shows signs of recovery (Goldman et al. 1988). However, because chronic exposure studies are not available to verify that additional effects would not occur from longer exposures to GD, ORNL assigned a factor of 3 to UF_S. The subcommittee agrees that a factor of 3 is appropriate.

DATA-BASE ADEQUACY

As noted by ORNL, the data base for GD lacks chronic oral studies in two species and reproductive and developmental toxicity studies. However, because studies on other nerve agents, including a multigeneration

reproductive study on VX (Goldman et al. 1988) and two developmental toxicity studies of GB (Denk 1974; La Borde and Bates 1986), indicate that reproductive and developmental effects are unlikely, the subcommittee agrees that a factor of 3 for the uncertainty factor for data-base adequacy (UF_D) is adequate to account for the incomplete data base.

MODIFYING FACTOR FOR ADDITIONAL UNCERTAINTY

The subcommittee considers the uncertainties of the data on GD to be represented adequately by the values assigned to the uncertainty factors above and agrees with ORNL that a modifying factor (MF) of 1 is appropriate.

SUMMARY

Table 5-1 presents the values assigned to the uncertainty factors and the modifying factor by ORNL and those recommended by the subcommittee. The subcommittee's recommendations are the same as those of ORNL.

TABLE 5-1 Uncertainty Factors Used by ORNL and the NRC to Calculate the RfD for GD

Uncertainty Factor	Description	ORNL	NRC
UF_A	For animal-to-human extrapolation	10	10
UF_H	To protect susceptible subpopulations	10	10
UF_L	For LOAEL-to-NOAEL extrapolation	3	3
UF_S	For subchronic-to-chronic extrapolation	3	3
UF_D	For data-base adequacy	3	3
MF	Modifying factor for additional uncertainty	1	1
TOTAL UF		2,700	2,700

Abbreviations: LOAEL, lowest-observed-adverse-effect level; MF, modifying factor; NOAEL, no-observed-adverse-effect level; NRC, National Research Council; ORNL, Oak Ridge National Laboratory; RfD, reference dose; UF, uncertainty factor

WEIGHT AND STRENGTH OF EVIDENCE

The subcommittee believes that the strength of evidence for the Army's interim RfD of 4×10^{-6} mg/kg per day is moderately good. There is a possibility that the LOAEL (0.0175 mg/kg per day) used to calculate the RfD for GD is not accurate, because lower doses were not tested and variability in the plasma-ChE values was considerable. The subcommittee believes that because ChE inhibition is a biomarker of exposure rather than a toxic effect, use of this end point overestimates the oral toxicity of GD.

CONCLUSIONS

The approach used by ORNL to calculate the RfD for GD is consistent with the guidelines of the EPA. On the basis of available toxicity and related data on GD, the subcommittee concludes that the Army's interim RfD for GD of 4×10^{-6} mg/kg per day is scientifically valid.

DATA GAPS AND RESEARCH RECOMMENDATIONS

The major gap in the available information on GD is the lack of an oral subchronic or chronic toxicity study that demonstrates a clear dose-response relationship between GD exposure and ChE inhibition. The absence of that type of data could be addressed by conducting a subchronic oral toxicity study that assesses anti-ChE activity in RBCs and plasma in one or preferably two species. At least one dose between 0 and 0.0175 mg/kg per day should be used. Range-finding studies focusing on ChE analytical methods offer the best possibility of filling the data gap. If further research reveals that significant toxic effects can be induced by any of the nerve agents evaluated (i.e., GA, GB, GD, or VX) at doses below those that cause significant ChE inhibition, new studies should be conducted to reassess the safety of the recommended RfD for GD.

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6

Evaluation of the Army's Interim Reference Dose for VX

THE CHEMICAL-WARFARE agent VX is an organophosphate nerve agent found at several stockpile and nonstockpile munition sites in the United States. At the request of the U.S. Army, Oak Ridge National Laboratory (ORNL) conducted a health risk assessment of VX. The assessment included a detailed analysis of VX's physical and chemical properties, environmental fate, mechanism of action, and animal and human toxicity data (see Appendix D, *Health Risk Assessment of VX,* ORNL 1996). On the basis of that assessment, ORNL proposed a reference dose (RfD) of 6×10^{-7} mg/kg of body weight per day for noncancer health effects of VX exposure. Because there was no evidence that VX is carcinogenic, a slope factor was not derived. The Army's Surgeon General accepted ORNL's proposed RfD as an interim exposure value until an independent evaluation of the proposed RfD was conducted by the National Research Council (NRC). This chapter contains the NRC's independent assessment of the scientific validity of the Army's interim RfD for VX.

DERIVATION OF THE ARMY'S INTERIM RFD

The Army's interim RfD for VX is 6×10^{-7} mg/kg per day (0.0006 µg/kg per day). ORNL (1996) calculated that value on the basis of the lowest oral dose of VX that caused significant depression in blood-cholinesterase (ChE) activity in sheep. The lowest-observed-adverse-effect level

(LOAEL) of VX was 3 μg per day in a subchronic toxicity study in which female sheep were fed VX daily for 55 days (Rice et al. 1971). The LOAEL was weight-normalized by dividing it by 52.7 kg (the average weight of the sheep) to yield a dose of 0.06 μg/kg per day. The RfD for VX was calculated to be 6×10^{-7} mg/kg per day by dividing the adjusted LOAEL by 90, the product of the uncertainty factors and the modifying factor selected by ORNL.

APPROPRIATENESS OF THE CRITICAL STUDY

The critical study used by ORNL for deriving the RfD for VX was a subchronic toxicity study with yearling sheep (Rice et al. 1971). Five females per group were hand-fed pellets treated with VX at doses of 3, 9, and 15 μg for 55 days. The control group comprised 10 sheep. Whole-blood-ChE determinations were made before dosing and 16 times during the exposure period. Significant depression of ChE was observed in all dose groups by day 21, and there were no signs of toxicity. ChE concentrations stabilized at about 60% of baseline values (measurements taken before exposure) by day 31 and remained at that level for the remainder of the testing period. ORNL considered the lowest dose of 3 μg per day to be the LOAEL for the study. As described earlier, that value was weight-adjusted to 0.06 μg/kg per day.

The subcommittee noted several weaknesses in the Rice et al. (1971) study, the most notable being the uncertainties about whether sheep are at least as susceptible as humans to VX. Sheep lack plasma-ChE and have lower red-blood-cell (RBC)-acetylcholinesterase (AChE) activity than humans (Ellin 1981); therefore, they might be more susceptible to VX toxicity than humans. In addition, sheep are ruminants, which means that ingested materials remain in their rumens and are later regurgitated for cud-chewing. It is unclear what effect this digestive process has on the absorption of VX. It is possible that VX is destroyed in the rumen, thereby decreasing the amount available for absorption. However, in vitro studies by Cook (1957) indicate that thiol isomers of organophosphate agents are not destroyed by rumen fluid; VX possesses a thiol group, suggesting that it would not be destroyed in the rumen. If that is the case, retention of VX in the rumen might allow increased absorption of VX through the epithelial surface of the rumen. However, there is no good evidence that shows that sheep are more sensitive than humans to VX.

The Rice et al. (1971) study used a small number of animals and the control animals were older than the experimental animals, but concern about those factors was lessened by a study showing no significant differences in blood-ChE activities among various age and gender groups of sheep (Halbrook et al. 1992). The exposure duration of the Rice et al. (1971) study was subchronic (8 weeks) rather than chronic (104 weeks), but the subcommittee believes that the data from other studies (Goldman et al. 1988, see below) indicate that significant effects are unlikely with longer exposures.

Other weaknesses in the Rice et al. (1971) study include lack of dose adjustments for body weight and large variation in animal weights (10-kg range). The subcommittee believes that it would be useful to review the raw data from the study to determine whether animal weight might explain an appreciable amount of the residual variance in ChE concentrations. The data could be evaluated with each animal serving as its own control, obviating potential problems with noncomparability because of the ages of test and control animals. Another weakness of the study was that the method used to measure ChE activity was not ideal (see Appendix G).

The subcommittee considered other possible critical studies for the derivation of the RfD for VX. In a study by Goldman et al. (1988), VX was administered to Sprague-Dawley rats (25 males and 25 females per group) by subcutaneous (s.c.) injection at doses of 0, 0.25, 1.0, and 4.0 μg/kg for 5 days per week for up to 90 days. A dose-dependent decrease in RBC-AChE concentrations was observed in male and female rats compared with controls. Plasma ChE was significantly depressed at day 30 in both sexes administered VX at a dose of 1.0 μg/kg per day, and at days 30, 60, and 90 in both sexes at a dose of 4.0 μg/kg per day. The data from rats exposed for 30 days was reanalyzed by ORNL using analysis of variance and Dunnett's and Scheffe's comparisons. ORNL reported that RBC-AChE activity was significantly lower in both sexes in all dose groups.

The subcommittee found several weaknesses in using the Goldman et al. (1988) study to identify a NOAEL or LOAEL. The study involved the s.c. route of exposure, which is not a relevant route of exposure for determining an RfD, and the study was subchronic in duration (exposures ranging from 4 to 13 weeks) rather than chronic (104 weeks). In addition, the methods used to measure ChE activity was not ideal (see Appendix G).

Another study the subcommittee considered that might serve as the

critical study involved exposure of humans to VX (Sim et al. 1964). Sixteen male volunteers were given drinking-water solutions containing VX at concentrations of 1.43 μg/kg daily for 7 days. Mean depression of RBC-AChE activity was 60% of baseline values, and no signs or symptoms of toxicity occurred. The weaknesses noted in the Sim et al. (1964) study include the small number of subjects and the much shorter exposure duration than is typical in subchronic or chronic exposure studies in animals. The data on GB (McNamara et al. 1973) indicate that the effects on ChE concentrations reach a plateau more quickly for VX than for GB, in part because the half-life for aging is longer for VX than for many other organophosphates and there is some reversibility of the binding of VX to ChE before aging occurs.

Although none of the three studies described above is sufficient to form an ideal basis for deriving the RfD for VX, the subcommittee believes that data from human studies should be used whenever possible. Thus, the subcommittee believes that the study of humans by Sim et al. (1964) is the most appropriate of the available studies for derivation of the RfD for VX.

APPROPRIATENESS OF CRITICAL END POINT

The LOAELs in the critical study used by ORNL (Rice et al. 1971) and the critical study recommended by the subcommittee (Sim et al. 1964) were based on the lowest dose of VX that caused a significant depression in RBC-ChE activity. The subcommittee notes that ChE inhibition is typically considered a biomarker of exposure to organophosphate agents rather than an adverse effect. However, it is generally agreed that inhibition of RBC and plasma ChE contributes to the overall hazard identification of ChE-inhibiting agents. The U.S. Environmental Protection Agency (EPA) has used ChE inhibition to establish RfDs for several organophosphate pesticides, such as malathion (EPA 1992) and ethion (EPA 1989). The subcommittee agrees with ORNL that ChE inhibition is a valid end point on which to base the RfD for VX but recommends that data on ChE inhibition be taken from the human study by Sim et al. (1964) rather than from the sheep study by Rice et al. (1971).

The subcommittee considered other possible critical end points, notably neurotoxicity, associated with VX exposure. Organophosphate compounds like VX might act directly on nerve cell receptors or, by inhibiting neural AChE, interfere with neuromuscular transmission and produce

delayed-onset subjunctional muscle damage. VX at concentrations of 10 picomolar has been shown to depress gabanergic transmission in the central nervous system (Rocha et al. 1998) and this could have profound implications for behavioral effects in laboratory animals and humans. Some organophosphate compounds cause a neurotoxic effect (organophosphate-induced delayed neuropathy, or OPIDN) that is not associated with ChE inhibition. However, OPIDN has not been observed in humans exposed to acutely toxic concentrations of VX (Munro et al. 1994).

The subcommittee notes that additional human data, which were not included in ORNL's assessment, are available. Data summaries from human experimentation conducted in the 1950s and 1960s were evaluated by the NRC in a series of reports titled *Possible Long-Term Health Effects of Short-Term Exposure to Chemical Agents* (NRC 1982, 1984, 1985). The reports include an evaluation of health records of volunteer soldiers who were exposed intravenously or intramuscularly to chemical-warfare agents, as well as a follow-up morbidity study conducted by the NRC in 1985. The NRC found no long-term health effects from short-term exposure to any specific chemical-warfare agent, but there was some evidence of an increase in malignant neoplasms among men exposed to anti-ChE agents. Although these studies are not directly applicable to deriving RfDs, they add to the completeness of the data base on VX.

Provided that appropriate assays were used, the subcommittee finds no reason at this time to alter the practice of using RBC-ChE or plasma-ChE inhibition as the critical toxicity end point and agrees with ORNL that such inhibition is the best available critical noncancer end point on which to base the calculation of the RfD for VX.

APPROPRIATENESS OF UNCERTAINTY FACTORS

Because the subcommittee recommends the use of the human study by Sim et al. (1964) as the basis for deriving the RfD for VX, it assigned values to the uncertainty factors and the modifying factor with respect to that study below.

EXTRAPOLATION FROM ANIMAL TO HUMAN

Because the Sim et al. (1964) study involved exposure to humans, the subcommittee assigned a factor of 1 to the uncertainty factor for extrapolation of data from animals to humans (UF_A)

PROTECTING SUSCEPTIBLE SUBPOPULATIONS

The subcommittee believes that a factor of 10 should be assigned to the uncertainty factor for protecting susceptible subpopulations (UF_H) because some individuals have a genetic polymorphism causing their serum-ChE activity to be abnormally low (Evans et al. 1952; Harris and Whittaker 1962). For homozygous individuals, the activity can be as low as 8–21% of the normal mean (Bonderman and Bonderman 1971). Genetic polymorphisms are also recognized for butyrylcholinesterase and paraoxonase, enzymes that might function in the sequestration and metabolism of organophosphate nerve agents (Loewenstein-Lichtenstein et al. 1995; Maekawa et al. 1997; Furlong et al. 1998). Individuals with those polymorphisms might be unusually susceptible to organophosphate anti-ChE compounds (Morgan 1989).

EXTRAPOLATION FROM LOAEL TO NOAEL

The subcommittee assigned a factor of 10 to the uncertainty factor for extrapolation from a LOAEL to a NOAEL (UF_L) because the 60% inhibition of ChE observed with the LOAEL was near levels where physical signs of clinical toxicity might occur. The subcommittee notes that ChE inhibition is a biomarker of exposure rather than a toxic effect. Although it could be argued that a dose of VX that significantly induces ChE inhibition in the absence of toxic effects is indicative of a NOAEL rather than a LOAEL, the subcommittee believes that given the 60% ChE inhibition observed at the LOAEL, a factor of 10 is a prudent choice for UF_L.

EXTRAPOLATION FROM SUBCHRONIC TO CHRONIC EXPOSURES

The subcommittee notes that in the derivation of RfDs for other organophosphate compounds, EPA (1989, 1992) used NOAELs for ChE inhibition that were based on subchronic data without adjustment for chronic exposures, because ChE inhibition is unlikely to change over time. Hence, a factor of 1 was used for the uncertainty factor for extrapolation from subchronic to chronic exposures (UF_S). For example, studies with VX indicate that maximal ChE inhibition occurs after 30–60 days of exposure and then levels off and sometimes shows signs of recovery

(Goldman et al. 1988). The subcommittee believes, however, that because the exposure duration of the critical study (7 days) is too short to be considered a subchronic exposure study, a factor of 10 should be assigned to UF_S to account for the greater uncertainty involved with extrapolating such data to chronic exposures.

DATA-BASE ADEQUACY

The subcommittee notes that with the exception of the Rice et al. (1971) study, subchronic or chronic toxicity studies of oral exposures to VX are lacking. Because it is unclear whether sheep (ruminants) are a relevant animal model for estimating the toxicity of VX to humans, the subcommittee believes that a factor of 3 should be assigned to the uncertainty factor for data-base adequacy (UF_D) to account for the absence of long-term oral studies of VX in humans or a relevant animal model.

MODIFYING FACTOR FOR ADDITIONAL UNCERTAINTY

The subcommittee considers the uncertainties of the data on VX to be represented adequately by the values assigned to the uncertainty factors above and believes a modifying factor (MF) of 1 is appropriate.

SUMMARY

Table 6-1 presents the uncertainty factors recommended by the subcommittee on the basis of the Sim et al. (1964) study. Using those uncertainty factors, and a LOAEL of 0.00143 mg/kg per day, the RfD for VX is 5×10^{-7} mg/kg per day (0.00143 mg/kg per day ÷ 3,000).

WEIGHT AND STRENGTH OF EVIDENCE

On the basis of the Sim et al. (1964) study, the subcommittee believes that the weight of evidence for an RfD of 5×10^{-7} mg/kg per day is moderately good. It is likely that the LOAEL (0.00143 mg/kg per day) for VX used to calculate the RfD might not be an accurate LOAEL for ChE

inhibition in humans, because 60% ChE inhibition was observed at that dose and lower doses were not tested. The subcommittee believes that because ChE inhibition is a biomarker of exposure rather than a toxic effect, use of this end point overestimates the oral toxicity of VX.

TABLE 6-1 Uncertainty Factors Used to Calculate the RfD for VX on the Basis of the Sim et al. (1964) Study

Uncertainty Factor	Description	NRC
UF_A	For animal-to-human extrapolation	1
UF_H	To protect susceptible subpopulations	10
UF_L	For LOAEL-to-NOAEL extrapolation	10
UF_S	For subchronic-to-chronic extrapolation	10
UF_D	For data-base adequacy	3
MF	Modifying factor for additional uncertainty	1
TOTAL UF		3,000

Abbreviations: LOAEL, lowest-observed-adverse-effect level; MF, modifying factor; NOAEL, no-observed-adverse-effect level; NRC, National Research Council; RfD, reference dose; UF, uncertainty factor

CONCLUSIONS

The subcommittee disagrees with ORNL that the Rice et al. (1971) study should be used as the basis for calculating an RfD for VX. The subcommittee found several weaknesses and uncertainties that undermined its confidence in this study. The subcommittee believes that data from human studies should be used to derive the RfD whenever possible; thus, the subcommittee recommends that the human study by Sim et al. (1964) be used instead. Although the Sim et al. (1964) study also had a number of weaknesses, the subcommittee prefers to use it rather than data from a questionable animal model. On the basis of the Sim et al. (1964) study, the RfD for VX is 5×10^{-7} mg/kg per day, which is similar to the Army's interim RfD of 6×10^{-7} mg/kg per day.

DATA GAPS AND RESEARCH RECOMMENDATIONS

The major gap in the available information on VX is the lack of an oral subchronic or chronic toxicity study that demonstrates a clear dose-

response relationship between VX exposure and ChE inhibition. The absence of that type of data could be addressed by conducting a subchronic oral toxicity study that assesses anti-ChE activity in RBCs and plasma in one or preferably two species. At least one dose between 0 and 0.00143 mg/kg per day should be used. If further research reveals that significant toxic effects can be induced by any of the nerve agents evaluated (i.e., GA, GB, GD, or VX) at doses below those that cause significant ChE inhibition, new studies should be conducted to reassess the safety of the recommended RfD for VX.

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7

Evaluation of the Army's Interim Reference Dose and Slope Factor For Sulfur Mustard

Sulfur mustard is a chemical-warfare agent. It is classified as a vesicating agent because of its ability to cause blisters on exposed skin. Sulfur mustard is the chemical-warfare agent present at most stockpile and nonstockpile munition sites in the United States and it territories. At the request of the U.S. Army, Oak Ridge National Laboratory (ORNL) conducted a health risk assessment of sulfur mustard. The assessment included a detailed analysis sulfur mustard's physical and chemical properties, environmental fate, toxicokinetics, mechanism of action, animal and human toxicity, and carcinogenicity (see Appendix E, *Health Risk Assessment of Sulfur Mustard,* ORNL 1996). On the basis of that assessment, ORNL proposed a reference dose (RfD) of 7×10^{-6} mg/kg of body weight per day for noncancer health effects and a slope factor (SF) of 9.5 per mg/kg per day for the carcinogenic potency of sulfur mustard. The Army's Surgeon General accepted ORNL's proposed RfD and SF as interim exposure values until an independent evaluation of the proposed RfD and SF was conducted by the National Research Council (NRC). This chapter contains the NRC's independent assessment of the scientific validity of the Army's interim RfD and SF for sulfur mustard.

EVALUATION OF THE ARMY'S INTERIM RFD

DERIVATION OF THE ARMY'S INTERIM RFD

The Army's interim RfD for sulfur mustard is 7×10^{-6} mg/kg per day. ORNL (1998) calculated that value on the basis of the lowest oral dose of sulfur mustard that produced forestomach lesions (epithelial acanthosis, which is an increase in the thickness of the stratum spinosum of the epithelial tissue of the forestomach) in rats. The lowest-observed-adverse-effect level (LOAEL) for that effect was 0.03 mg/kg per day in a two-generation reproductive study (Sasser et al. 1989a). In that study, male and female rats were administered sulfur mustard for 5 days per week for 15 weeks, daily for 3 weeks, and 4 days per week for 3 weeks. Because of this discontinuous dosing protocol, ORNL adjusted the LOAEL ($LOAEL_{adj}$) to calculate the doses for continuous exposures. That adjustment was done by calculating the total dose administered during the different exposure protocols:

0.03 mg/kg per day × 5 days × 15 weeks = 2.25 mg/kg.

0.03 mg/kg per day × 7 days × 3 weeks = 0.63 mg/kg.

0.03 mg/kg per day × 4 days × 3 weeks = 0.36 mg/kg.

The combined dose over the 21-week exposure period was 3.24 mg/kg. That value was divided by the total number of days (147 days) during the exposure period to yield a $LOAEL_{adj}$ of 0.022 mg/kg per day. The RfD for sulfur mustard was calculated to be 7×10^{-6} mg/kg per day by dividing the $LOAEL_{adj}$ by 3,000, the product of the uncertainty factors and the modifying factor selected by ORNL.

APPROPRIATENESS OF THE CRITICAL STUDY

The critical study used by ORNL for deriving the RfD for sulfur mustard was a two-generation reproductive study (Sasser et al. 1989a) in which Sprague-Dawley rats (20 males and 27 females per group) were intragastrically intubated with sulfur mustard dissolved in sesame oil at concentrations of 0.03, 0.1, and 0.4 mg/kg per day. Males and females were dosed for 5 days per week for 15 weeks, including 13 weeks before

and 2 weeks during the mating period. Female rats were also dosed for 7 days per week during the 3-week gestation period and 4 days per week during the 3-week lactation period. No significant adverse effects on reproductive performance or fertility were found at any dose through two consecutive generations. Dose-related epithelial acanthosis of the forestomach was observed in both sexes; the incidence of acanthosis was 0 of 94 in the controls, 71 of 94 in the low-dose group, 89 of 94 in the mid-dose group, and 94 of 94 in the high-dose group. In addition, benign forestomach lesions (squamous papillomas) were observed in 8 of 94 and 10 of 94 rats in the mid-dose and high-dose groups, respectively. Because acanthosis was described as mild at the lowest dose of 0.03 mg/kg per day, that dose was considered by ORNL to be the LOAEL for the study. As described earlier, that value was adjusted for a discontinuous exposure regimen to 0.022 mg/kg per day, and ORNL used the adjusted value to calculate the RfD.

The subcommittee considered other possible studies, such as those of Hackett et al. (1987) and McNamara et al. (1975). The study by Hackett et al. (1987) was a teratology study in which rats and rabbits were intragastrically intubated with sulfur mustard. For rats, maternal toxicity and teratogenic effects were observed at all doses tested (0.5, 1.0, and 2.0 mg/kg per day); for rabbits, there was no evidence of teratogenic effects at any of the doses tested (0.4, 0.6, and 0.8 mg/kg per day) but maternal toxicity was observed at the two highest doses. Dosing lasted only a few days (10 days for rats or 14 days for rabbits) in the Hackett et al. (1987) study compared with the 21 weeks in the Sasser et al. (1989a) study.

The study by McNamara et al. (1975) was an inhalation study using rats, mice, rabbits, guinea pigs, and dogs; therefore, equivalent oral doses could only be estimated from the data. Because of that, the subcommittee considered the study to be inappropriate for deriving the RfD for sulfur mustard. Furthermore, inhalation of sulfur mustard resulted in lesions to the skin and eyes, which would not be expected from oral exposure. The subcommittee also reviewed the Institute of Medicine's (IOM 1993) evaluation of the health effects of mustard gas and found no other relevant studies with respect to derivation of the RfD.

The subcommittee concurs with ORNL that the two-generation reproductive study (Sasser et al. 1989a) was the most appropriate of the available studies for calculating the RfD for sulfur mustard. Although

not a chronic exposure study, the Sasser et al. (1989a) study involved exposure to sulfur mustard for 21 weeks and involved oral exposure, the route of interest for deriving an RfD; the study included a range of doses and an adequate number of animals per group.

APPROPRIATENESS OF CRITICAL END POINT

The $LOAEL_{adj}$ (0.022 mg/kg per day) used by ORNL for derivation of the RfD for sulfur mustard was based on the dose that caused mild epithelial acanthosis in the forestomach of rats (Sasser et al. 1989a). The subcommittee believes that those lesions were probably a result of administering doses of sulfur mustard directly to the forestomach, which is typically more toxic than delivering doses at a slower rate throughout the day. If rats were administered sulfur mustard at a slower rate, in feed, for example, the daily dose required to induce the same mild lesions would likely be higher. Although humans do not have forestomachs, the subcommittee believes that the primary mechanism of toxicity of sulfur mustard is epithelial tissue damage from direct contact and agrees with ORNL that epithelial acanthosis of the forestomach in rats can be used as the critical noncancer toxicity end point for deriving the RfD. However, that end point resulting from direct administration to the forestomach is likely to overestimate the toxicity of sulfur mustard, resulting in an RfD that might be overprotective for noncancer health effects.

APPROPRIATENESS OF UNCERTAINTY FACTORS

For sulfur mustard, ORNL assigned values greater than 1 to four out of five uncertainty factors and a value of 1 to the modifying factor. The product of those factors was 3,000. The subcommittee evaluated each of the uncertainty factors and the modifying factor below.

Extrapolation from Animal to Human

Because sulfur mustard is a highly corrosive agent, the subcommittee believes that epithelial lesions at the point of entry into the stomach are

likely to occur across species. For that reason, the subcommittee considers the typical default value of 10 for the uncertainty factor for extrapolation of data from animals to humans (UF_A) to be too high and recommends a lower value of 3. The value of 3 is meant to indicate some similarity in action across species, while not excluding the possibility of some greater overall human susceptibility.

Protecting Susceptible Subpopulations

The subcommittee agrees with ORNL that a factor of 10 is appropriate for the uncertainty factor to protect susceptible subpopulations (UF_H).

Extrapolation from LOAEL to NOAEL

ORNL assigned a factor of 3 to the uncertainty factor for extrapolation from a LOAEL to a NOAEL (UF_L) to account for the corrosive action of sulfur mustard. The subcommittee believes, however, that action is best accounted for in the uncertainty factor for animal-to-human extrapolation (see UF_A above) and that a factor of 10 should be assigned to UF_L.

Extrapolation from Subchronic to Chronic Exposures

The subcommittee agrees with ORNL that a factor of 10 is appropriate for the uncertainty factor for extrapolation from subchronic to chronic exposures (UF_s) because the LOAEL was estimated from a subchronic exposure study. The subcommittee believes that a UF_s of 10 is conservative, because the subchronic exposure was for 21 weeks, which is one-fifth of the time of a chronic exposure.

Data-Base Adequacy

The subcommittee agrees with ORNL that a factor of 1 for the uncertainty factor for data-base adequacy (UF_D) is appropriate. Although no chronic oral exposure studies are available on sulfur mustard, several subchronic oral exposure studies are available, including two developmental toxicity studies in different species (Hackett et al. 1987), a two-

generation reproductive study (Sasser et al. 1989a), and a standard subchronic exposure study in one species (Sasser et al. 1989b). In addition, chronic inhalation exposure studies in five species (McNamara et al. 1975) are available as supporting information. The critical study (Sasser et al. 1989a) used by ORNL identifies a toxic effect (epithelial acanthosis of the forestomach) that is consistent with the vesicant properties of sulfur mustard. Exposure was exaggerated by the route of administration (gastric intubation). Because there is no evidence that any other experimental species might be more sensitive to sulfur mustard than rats, the subcommittee believes that additional oral toxicity studies in other species are not necessary.

Modifying Factor for Additional Uncertainty

The subcommittee considers the uncertainties of the data on sulfur mustard to be represented adequately by the values assigned to the uncertainty factors above and agrees with ORNL that a modifying factor (MF) of 1 is appropriate.

Summary

Table 7-1 presents the values assigned to the uncertainty factors by ORNL and those recommended by the subcommittee. The product of the factors for deriving the RfD for sulfur mustard is 3,000 for both sets of values. Thus, the subcommittee recommends the same RfD as ORNL for sulfur mustard (7×10^{-6} mg/kg per day) but notes that slightly different uncertainty factors were used in the calculation.

WEIGHT AND STRENGTH OF EVIDENCE

The subcommittee believes that the data on sulfur mustard support the interim RfD of 7×10^{-6} mg/kg per day. The strength of evidence for that RfD is moderately good but might lead to overestimation of the oral toxicity of sulfur mustard, because toxicity might have resulted from administration of sulfur mustard directly to the forestomach. Administration in feed, for example, over much greater time would be unlikely to have the same result.

TABLE 7-1 Uncertainty Factors Used by ORNL and the NRC to Calculate the RfD for Sulfur Mustard

Uncertainty Factor	Description	ORNL	NRC
UF_A	For animal- to-human extrapolation	10	3
UF_H	To protect susceptible subpopulations	10	10
UF_L	For LOAEL-to-NOAEL extrapolation	3	10
UF_S	For subchronic-to-chronic extrapolation	10	10
UF_D	For data-base adequacy	1	1
MF	Modifying factor for additional uncertainty	1	1
TOTAL UF		3,000	3,000

Abbreviations: LOAEL, lowest-observed-adverse-effect level; MF, modifying factor; NOAEL, no-observed-adverse-effect level; NRC, National Research Council; ORNL, Oak Ridge National Laboratory; RfD, reference dose; UF, uncertainty factor.

EVALUATION OF THE ARMY'S INTERIM CANCER SLOPE FACTOR

DERIVATION OF THE ARMY'S INTERIM CANCER SLOPE FACTOR

The carcinogenic potential of sulfur mustard administered orally has not been studied in either epidemiological or animals studies. In the absence of oral exposure studies, ORNL used the relative carcinogenic potency calculated by Watson et al. (1989) to estimate a slope factor (SF) for sulfur mustard. Watson et al. (1989) estimated the potency of sulfur mustard by an indirect method called the rapid screening of hazard (RASH) (Jones et al. 1988), which involved comparing the carcinogenicity of sulfur mustard (based upon intravenous (Heston 1950) and subcutaneous studies (Heston 1953) with that of the well-characterized carcinogen benzo[*a*]pyrene (B[*a*]P). They showed that the relative carcinogenic potency of sulfur mustard was approximately equivalent to that of B[*a*]P, having a best-estimate relative potency of 1.3. ORNL (1996) multiplied that value by the currently accepted SF for B[*a*]P of 7.3 per mg/kg per day (EPA 1992) to yield an SF for sulfur mustard of 9.5 per mg/kg per day.

APPROPRIATENESS OF METHOD USED

As described above, ORNL used the carcinogenic potency of sulfur mustard estimated by Watson et al. (1989) relative to B[*a*]P to calculate the interim SF for sulfur mustard. The subcommittee noted that Watson et al. (1989) also compared the potency of sulfur mustard with that of the direct-acting carcinogen *bis*-(chloromethyl)ether (BCME). Both compounds are alkylating agents, are powerful lung and eye irritants, cause necrotic skin lesions, and exhibit comparable modes of action in biological systems. The median relative potency of sulfur mustard compared with BCME for a variety of biological effects was 0.6 (Watson et al. 1989), which is within an order of magnitude of the potency derived for sulfur mustard when compared with B[*a*]P. Because the SF for BCME is 220 per mg/kg per day (EPA 1992), the SF for sulfur mustard calculated on the basis of its potency factor relative to that of BCME is 132 per mg/kg per day (220 per mg/kg per day × 0.6), compared with 9.5 per mg/kg per day derived in relation to B[*a*]P.

ORNL also considered calculating an SF on the basis of the U.S. Environmental Protection Agency's (EPA 1991) estimated inhalation unit risk (8.5×10^{-2} per $\mu g/m^3$) of sulfur mustard. Normalizing the inhalation unit risk for a 70-kg person inhaling 20 m^3 of air per day would yield an SF of 0.3 per μg/kg per day. ORNL decided not to use this method because the inhalation study (McNamara et al. 1975) used to estimate the inhalation unit risk resulted in rat skin tumors that appeared to be caused by dermal exposure rather than by systemic absorption and distribution to the skin, and inhalation-to-oral extrapolation was not considered appropriate. Furthermore, the McNamara et al. (1975) study contained a number of deficiencies, such as outdated testing protocols, brief exposures, and small numbers of animals, which made quantitative analysis difficult.

The subcommittee agrees with ORNL that calculating an SF for sulfur mustard using the relative potency approach was more appropriate than using estimates from inhalation unit risk. The subcommittee notes, however, that a recent study by Culp et al. (1998) reported a lower carcinogenic potency value for B[*a*]P. That chronic exposure study of B[*a*]P in feed was conducted under Good Laboratory Practice conditions in $B6C3F_1$ female mice (Culp et al. 1998). The incidence of forestomach tumors was found to be 1 of 48, 3 of 47, and 36 of 46 at concentrations

of 0, 5, and 25 ppm, respectively. The carcinogenic potency of B[*a*]P was estimated to be less than 1.2 per mg/kg per day, assuming equal potency between animals and humans for dose adjusted by body weight to the ¾ power. That is one-sixth of the current EPA potency value for B[*a*]. On the basis of that new potency, if sulfur mustard is 1.3 times more potent than B[*a*]P (Watson et al. 1989), the upper limit for carcinogenic potency for sulfur mustard is estimated to be less than 1.6 per mg/kg per day (1.2 mg/kg per day × 1.3).

The subcommittee also used another approach to estimate the upper limit for carcinogenic potency of sulfur mustard. This approach involved evaluating the carcinogenic potency of sulfur mustard relative to its maximum tolerated dose (MTD). In a study of 139 animal carcinogens tested in the National Toxicology Program, Gaylor and Gold (1995) found that carcinogenic potency can be estimated by dividing 0.74 by the MTD (expressed in terms of milligrams per kilogram per day). The subcommittee applied the MTD approach to BCME, as well as to sulfur mustard, to determine whether the approach is predictable for direct-alkylating agents. The MTD for BCME is estimated to be 0.0078 mg/kg per day on the basis of a 6-month inhalation study in male rats (Leong et al. 1988). That dose averaged over a 2-year lifetime is about 0.002 mg/kg per day. Assuming equivalent potency of BCME by oral and inhalation exposure, the MTD approach yields a carcinogenic potency of 370 per mg/kg per day (0.74 ÷ 0.002 mg/kg per day) for BCME, which is close to the slope factor of 220 per mg/kg per day reported by EPA (1992). For sulfur mustard, the MTD was estimated to be 0.2 mg/kg per day on the basis of a study by Sasser et al. (1989a), who reported significant body-weight depression in rats administered sulfur mustard at 0.3 mg/kg per day for 90 days but no toxic effects at 0.1 mg/kg per day. With an MTD of 0.2 mg/kg per day for 5 days per week, the average daily dose would be 0.14 mg/kg per day (0.2 mg/kg per day × (5/7)). Using the method of Gaylor and Gold (1995), an estimate of the carcinogenic potency of sulfur mustard is less than 5.3 per mg/kg per day (0.74 ÷ 0.14 mg/kg per day).

In the absence of a chronic bioassay for sulfur mustard, the two approaches described above for estimating an upper limit on the carcinogenic potency give remarkably similar results—1.6 and 5.3 per mg/kg per day for lifetime exposure. Those potency values are less than an order of magnitude lower than the 9.5 per mg/kg per day derived by ORNL (see Table 7-2). That would indicate that the potency estimate of 132 per mg/kg per day relative to BCME (described earlier) is too high

and that the potency estimate relative to B[*a*]P should be used. The subcommittee believes that the updated potency of B[*a*]P reported by Culp et al. (1998) should be used as the basis for calculating the SF for sulfur mustard and recommends an SF of 1.6 per mg/kg per day.

TABLE 7-2 Estimates of the Upper Limit for Carcinogenic Potency of Sulfur Mustard

Method of Estimation	Estimate (per mg/kg/d)	Reference
Potency relative to B[*a*]P potency from EPA's IRIS database	9.5	Watson et al. 1989
Potency relative to B[*a*]P potency from GLP study	1.6	Culp et al. 1998
Potency relative to the MTD	5.3	Gaylor and Gold 1995
Potency relative to BCME potency from EPA's IRIS database	132	Watson et al. 1989

Abbreviations: B[*a*]P, benzo[*a*]pyrene; BCME, *bis*-(chloromethyl)ether; GLP, good laboratory practice; IRIS, Integrated Risk Information System; MTD, maximum tolerated dose.

Cancer risk is estimated by multiplying the carcinogenic potency with the average lifetime daily dose. Thus, if the potential carcinogenic risk from ingestion of sulfur mustard is restricted to less than 1×10^{-5} (1 in 100,000 persons) for those individuals exposed for a lifetime, daily oral doses should be limited to 6×10^{-6} mg/kg per day ($10^{-5} \div 1.6$ per mg/kg per day). That value is similar to the RfD of 7×10^{-6} mg/kg per day for noncancer effects. The level of carcinogenic risk might even be lower, because exposure duration is likely to be less than 70 years. According to an analysis by Israeli and Nelson (1992), only about 5% of all households are expected to stay at the same residence for over 23 years in a 70-year lifetime. Additionally, past regulations concerning acceptable risk levels generally correspond to implied estimates of excess lifetime cancer risk of less than 10^{-4} (Rodricks et al. 1987). Those facts strongly suggest that the RfD recommended by the subcommittee should be adequately protective of health with respect to possible carcinogenic effects.

WEIGHT AND STRENGTH OF EVIDENCE

The strength of evidence for the SF of sulfur mustard is poor because no epidemiological or animal carcinogenicity studies have been conducted

on long-term oral exposure to sulfur mustard. Therefore, only indirect approaches can be used to calculate the SF. Two indirect approaches, one using relative potency calculations and the other estimating the carcinogenic potency of orally administered sulfur mustard, resulted in estimated potency values that were within a factor of 3 of each other, lending some credibility to the indirect comparative approach used by ORNL. However, the subcommittee used an updated estimate for B[*a*]P potency in its relative potency calculations.

CONCLUSIONS

The approach used by ORNL to calculate the interim RfD for sulfur mustard is consistent with the guidelines of the EPA. On the basis of available toxicity and related data on sulfur mustard, the subcommittee concludes that the Army's RfD for sulfur mustard of 7×10^{-6} mg/kg per day is scientifically valid, although the subcommittee believes that slightly different uncertainty factors should be used.

The approach selected by ORNL to calculate the SF for sulfur mustard was scientifically valid given the absence of epidemiological or animal carcinogenicity studies of sulfur mustard. Another approach using the MTD for estimating the potency of sulfur mustard yielded a similar result. Using an updated estimate for B[*a*]P potency, the subcommittee believes that the Army's interim SF of 9.5 per mg/kg per day should be lowered to 1.6 per mg/kg per day.

DATA GAPS AND RESEARCH RECOMMENDATIONS

The major gap in the available information on sulfur mustard is the lack of long-term oral toxicity and carcinogenicity studies from which to derive the RfD and SF directly. Because of that deficiency, the RfD for sulfur mustard can only be estimated by extrapolating from subchronic exposure studies of adverse effects in animals in to humans, and the SF can only be established by applying comparative carcinogenic potency methods. The absence of long-term data can be addressed by conducting a long-term oral exposure study in which such routes as diet are used to deliver sulfur mustard to animals at a slow rate.

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8

Evaluation of the Army's Interim Reference Dose for Lewisite

THE CHEMICAL-WARFARE agent lewisite is an organic, trivalent arsenic compound. It is classified as a vesicating agent because of its ability to cause blisters on exposed skin. Lewisite is present at several stockpile and nonstockpile munitions sites in the United States. At the request of the U.S. Army, Oak Ridge National Laboratory (ORNL) conducted a health risk assessment of lewisite. The assessment included a detailed analysis of lewisite's physical and chemical properties, environmental fate, toxicokinetics, mechanism of action, and animal and human toxicity data (see Appendix F, *Health Risk Assessment of Lewisite,* ORNL 1996). On the basis of that assessment, ORNL proposed a reference dose (RfD) of 1×10^{-4} mg/kg of body weight per day for noncancer health effects of lewisite. Because there was no evidence that lewisite is carcinogenic, a slope factor was not derived. The Army's Surgeon General accepted ORNL's proposed RfD as an interim exposure value until an independent evaluation of the proposed RfD was conducted by the National Research Council (NRC). This chapter contains the NRC's independent assessment of the scientific validity of the Army's interim RfD for lewisite.

DERIVATION OF THE ARMY'S INTERIM RFD

The Army's interim RfD for lewisite is 1×10^{-4} mg/kg per day. ORNL (1996) calculated that value on the basis of a two-generation reproduc

tive study (Sasser et al. 1989a) in which the highest oral dose of lewisite did not produce forestomach lesions (necrosis and hyperplasia) in rats. In that study, male and female rats were intragastrically intubated with lewisite for 5 days per week for 13 weeks. Female rats were also dosed for 7 days per week during the 3-week gestation period and 4 days per week during the 3-week lactation period. The highest no-observed-adverse-effect level (NOAEL) for lewisite was 0.6 mg/kg per day. Because of the discontinuous exposure regimen, ORNL adjusted the NOAEL ($NOAEL_{adj}$) to a time-weighted average. That adjustment was done by calculating the total dose administered during the different exposure protocols:

0.6 mg/kg per day × (5/7) = 0.43 mg/kg per day for 13 weeks.
0.6 mg/kg per day × (7/7) = 0.6 mg/kg per day for 3 weeks.
0.6 mg/kg per day × (4/7) = 0.34 mg/kg per day for 3 weeks.

Using those values, ORNL calculated the $NOAEL_{adj}$ to be 0.44 mg/kg per day as follows:

$$\frac{(0.43\ \text{mg/kg/day} \times 13\ \text{wk}) + (0.6\ \text{mg/kg/day} \times 3\ \text{wk}) + (0.34\ \text{mg/kg/day} \times 3\ \text{wk})}{19\ \text{wk}} = 0.44\ \text{mg/kg/day}$$

The RfD for lewisite was calculated to be 1×10^{-4} mg/kg per day by dividing the $NOAEL_{adj}$ by 3,000, the product of the uncertainty factors and the modifying factor selected by ORNL.

The subcommittee is aware that the interim RfD for lewisite was reviewed by the Material/Chemical Risk Assessment (MCRA) Working Group of the Environmental Risk Assessment Program, which represents multiagency (U.S. Environmental Protection Agency (EPA), Department of Defense, and Department of Energy) expertise in deriving and validating toxicity values. The MCRA Working Group concluded that the forestomach lesions appeared to be an artifact of administering doses of lewisite directly to the forestomach in a short period of time and that the overall data base for lewisite was poor. Its consensus was that the RfD for lewisite be considered unverifiable due to data deficiencies. For those reasons and because it also considered lewisite to degrade to inor

ganic arsenic in environmental media, the MCRA Working Group decided that the existing RfD for inorganic arsenic (3×10^{-4} mg/kg per day) would be an applicable surrogate, although the group recognized that the chemical structure of lewisite might imply toxic activity different from that of inorganic arsenic.

APPROPRIATENESS OF THE CRITICAL STUDY

ORNL estimated the NOAEL for lewisite by considering two studies—a two-generation reproductive study (Sasser et al. 1989a) and a 90-day toxicity study (Sasser et al. 1989b, 1996). In the two-generation reproductive study (Sasser et al. 1989a), Sprague-Dawley rats (20 males and 25 females per group) were intragastrically intubated with lewisite dissolved in sesame oil at doses of 0.10, 0.25, and 0.60 mg/kg per day. Males and females were dosed for 5 days per week for 13 weeks before mating. Female rats were also dosed for 7 days per week during the 3-week gestation period and 4 days per week during the 3-week lactation period. No significant adverse effects on reproductive performance or fertility were found at any dose through two consecutive generations, nor were any other toxic effects observed.

In the 90-day toxicity study (Sasser et al. 1989b, 1996), Sprague-Dawley rats (10 male and 10 females per group) were intragastrically intubated with lewisite dissolved in sesame oil at doses of 0.01, 0.1, 0.5, 1.0, and 2.0 mg/kg per day for 5 days per week for 13 weeks. The most significant adverse effects observed were necrosis and hyperplasia of the forestomach. Those forestomach lesions were found only in rats treated with lewisite at 1.0 or 2.0 mg/kg per day; the incidence was 1 of 20 and 12 of 20, respectively. Thus, the highest NOAEL for this study was 0.5 mg/kg per day. ORNL noted that no forestomach lesions were found at a slightly higher dose of 0.6 mg/kg per day in the two-generation reproductive study (Sasser et al. 1989a) and concluded that this higher value would be an appropriate estimate of the NOAEL. As described earlier, that NOAEL was adjusted to a time-weighted average of 0.44 mg/kg per day, and ORNL used the adjusted value to calculate the RfD.

ORNL also considered a teratogenicity study (Hackett et al. 1987) in which rabbits were administered lewisite by intragastric intubation on days 6–19 of gestation at doses of 0.07, 0.2, and 0.6 mg/kg per day. Increased mortality of 13% (in fact, 15% but reported incorrectly by

Hackett et al. (1987) and by ORNL), 46%, and 69%, respectively, were reported. In addition, gastric lesions (mucosal inflammation, edema, necrosis, and mucosal sloughing) were found at all doses, and teratogenic effects (fetal stunting and supernumerary ribs) were observed at the highest dose. On the basis of gastric lesions and mortality, the lowest-observed-adverse-effect level (LOAEL) for this study was 0.07 mg/kg per day.

ORNL considered this study to be compromised statistically primarily because of the small number of survivors in each treatment group. The subcommittee disagrees with that conclusion because the statistical weakness identified appears to compromise only the detection of teratogenic effects and not necessarily the effects of mortality or marked gastric lesions that were observed in the adult female rabbits at all administered doses. Furthermore, comparisons between the results of this study and a similar teratogenicity study in rats conducted by the same investigators (Hackett et al. 1987) suggest that rabbits might be more susceptible to lewisite than rats. On the basis of these considerations, the subcommittee concludes that even though the teratogenicity study in rabbits has shortcomings, the data indicating a LOAEL of 0.07 mg/kg per day for mortality and gastric lesions in the maternal rabbits should not be dismissed.

The subcommittee also considered other possible critical studies. In a study by Leitch et al. (1941), groups of 10 rats each were fed drinking water containing lewisite at 10 or 16 ppm for 98 or 133 days, respectively. No adverse effects were observed. However, the subcommittee noted that the study had a number of deficiencies, including an undefined effect concentration and lack of data on the actual concentration of lewisite consumed in drinking water. Earlier, Daniels (1990) came to the same conclusion. In addition, the test animals' water consumption, which is critical for determining an actual or estimated dose of lewisite, was not reported. Furthermore, the consumed concentration of lewisite might have varied from the target concentration because of degradation. Lewisite is hydrolyzed to 2-chlorovinylarsine oxide in the presence of water (IOM 1993); therefore, the actual exposure in the Leitch et al. (1941) study was not to lewisite but to its hydrolysis product. The subcommittee also reviewed the Institute of Medicine's (IOM 1993) evaluation of health effects of lewisite and found no other relevant studies with respect to derivation of the RfD.

On the basis of its evaluation of the available studies on lewisite, the

subcommittee recommends that the teratogenic study in rabbits (Hackett et al. 1987) be used as the critical study from which to derive the RfD for lewisite instead of the two-generation reproductive study in rats by Sasser et al. (1989a). Although the study was not ideal and the administration period was only 14 days, the rabbit appears to be more susceptible than the rat to lewisite, and the range of doses and number of animals in each dose group are considered credible. This recommendation contradicts the consensus judgment of the MCRA Working Group, who concluded that the available data on lewisite were inadequate for deriving an RfD for lewisite, and the RfD for inorganic arsenic should be used as a surrogate RfD for lewisite. The working group's recommendation was based on the assumption that lewisite degrades to inorganic arsenic in environmental media, an assumption the subcommittee regards as not necessarily certain under all circumstances. The subcommittee believes that use of the Hackett et al. (1987) rabbit study allows for the possibility that the chemical structure of lewisite represents a toxic activity that exceeds that of inorganic arsenic; the study is reasonable despite the limitations of the data; and the study does not presume lewisite will degrade to inorganic arsenic.

APPROPRIATENESS OF CRITICAL END POINT

The $NOAEL_{adj}$ of 0.44 mg/kg per day used by ORNL for derivation of the interim RfD for lewisite was based on the highest dose at which necrosis and hyperplasia of the forestomach were not observed in rats (Sasser et al. 1989a). The subcommittee recommends, however, the use of the reproductive rabbit study (Hackett et al. 1987) in which a LOAEL of 0.07 mg/kg per day was identified on the basis of maternal mortality and gastric lesions. Although the possibility exists that those effects resulted from administration of lewisite directly to the stomach over a short period, resulting in an RfD that could be overprotective of noncancer health effects, there are two reasons for applying such a conservative value at this time. First, the available dose-response data are too sparse to establish conclusively that the dose-administration process primarily is responsible for the observed effects. Second, the available data on lewisite are inadequate for determining its carcinogenic potential. Because the chemical structure of lewisite includes arsenic and vinyl groups, the possibility exists for lewisite to be degraded in the environ

ment or to be metabolized into inorganic arsenic and for vinyl chloride to be formed. Inorganic arsenic and vinyl chloride are considered human carcinogens (EPA 1997a,b; NRC 1996).

APPROPRIATENESS OF UNCERTAINTY FACTORS

Because the subcommittee recommends the use of the teratogenicity study in rabbits (Hackett et al. 1987) as the basis for deriving the RfD for lewisite, it assigned values to the uncertainty factors and the modifying factor with respect to that study below.

EXTRAPOLATION FROM ANIMAL TO HUMAN

The subcommittee believes that lewisite is a highly corrosive agent, and when it is introduced directly into the stomach in a short period, its behavior is likely to be similar across species. For that reason, the subcommittee considers the typical default value of 10 for the uncertainty factor for extrapolation of data from animals to humans (UF_A) to be too high and recommends a lower value of 3. The lower value is meant to indicate some similarity in action across species without excluding the possibility of some greater human susceptibility.

PROTECTING SUSCEPTIBLE SUBPOPULATIONS

The subcommittee considers the appropriate value for the uncertainty factor for protecting susceptible subpopulations (UF_H) to be 3 because the rabbits were pregnant females and can be considered more susceptible than healthy nonpregnant animals but not the most susceptible subpopulation.

EXTRAPOLATION FROM LOAEL TO NOAEL

Because a LOAEL was used to derive the RfD, the subcommittee assigns a value of 10 to the uncertainty factor for extrapolation from a LOAEL to a NOAEL (UF_L).

EXTRAPOLATION FROM SUBCHRONIC TO CHRONIC EXPOSURES

The subcommittee considers it appropriate to use a value of 10 for the uncertainty factor for extrapolation from subchronic to chronic exposures (UF_S) because the LOAEL was estimated from a 14-day exposure study in rabbits.

DATA-BASE ADEQUACY

The subcommittee believes the uncertainty factor for data-base adequacy (UF_D) should be assigned a value of 10 because no long-term exposure studies involving lewisite are available, only a few studies that address the acute or subchronic toxicity of lewisite are available, and little or no information about the metabolism of lewisite or its degradation products is available.

MODIFYING FACTOR FOR ADDITIONAL UNCERTAINTY

The subcommittee believes that the values assigned to the uncertainty factors above are supported by the data on lewisite and, therefore, supports a modifying factor (MF) of 1.

SUMMARY

Table 8-1 presents the uncertainty factors recommended by the subcommittee for deriving an RfD based on the rabbit teratogenic study (Hackett et al. 1987). The product of those factors is 9,000. On the basis of that value, the RfD for lewisite is 8×10^{-6} mg/kg per day (0.07 mg/kg per day ÷ 9,000), which reasonably can be rounded to 1×10^{-5} mg/kg per day.

WEIGHT AND STRENGTH OF EVIDENCE

Because of a poor data base on lewisite, the strength of evidence for deriving the RfD for lewisite is weak.

TABLE 8-1 Uncertainty Factors Used to Calculate the RfD for Lewisite on the Basis of the Hackett et al. (1987) Study

Uncertainty Factor	Description	NRC
UF_A	For animal-to-human extrapolation	3
UF_H	To protect susceptible subpopulations	3
UF_L	For LOAEL-to-NOAEL extrapolation	10
UF_S	For Subchronic-to-chronic extrapolation	10
UF_D	For data-base adequacy	10
MF	Modifying factor for additional uncertainty	1
TOTAL UF		9,000

Abbreviations: LOAEL, lowest-observed-adverse-effect level; MF, modifying factor; NOAEL, no-observed-adverse-effect level; NRC, National Research Council; RfD, reference dose; UF, uncertainty factor

CONCLUSIONS

The approach used by ORNL to calculate the RfD for lewisite is consistent with the guidelines of the EPA. The subcommittee does not agree, however, with ORNL's proposed RfD of 1×10^{-4} mg/kg per day, which is based on studies in the rat, and recommends deriving the RfD on the basis of a rabbit study. The RfD for lewisite recommended by the subcommittee is 1×10^{-5} mg/kg per day, which is an order of magnitude more conservative than the Army's interim RfD.

DATA GAPS AND RESEARCH RECOMMENDATIONS

The major gaps in the available information on lewisite are the lack of information on the implications of administering lewisite directly to the stomach over a short time and the absence of chronic oral toxicity data from which to derive an RfD. Because of those deficiencies, the RfD for lewisite is estimated by extrapolating from a less-than-ideal animal study to humans. Confidence in the RfD can be increased if subchronic studies in rabbits and rats are conducted that compare the effects of chronic oral exposure to low concentrations of lewisite with the effects of short-term intragastric administration of small volumes. These crucial studies will provide not only the data needed to better understand the implications

of dosing techniques but also more pertinent information on whether the rabbit is a more appropriate animal model than the rat for deriving an RfD for lewisite.

The subcommittee believes the potential environmental and metabolic breakdown products of lewisite are not well identified. There is a possibility that inorganic arsenic and perhaps even vinyl chloride, two known carcinogens, may be break down products. Accordingly, the subcommittee recommends that the environmental degradation and metabolic products of lewisite be determined, and, if those breakdown products are found to be produced, that the carcinogenic potential of those substances, as well as lewisite, be considered in future assessments.

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Glossary

acetylcholinesterase	True cholinesterase (ChE). Acetylcholinesterase hydrolyzes acetylcholine within the central nervous system and peripheral neuro-effector functions.
benchmark dose	A dose with a specified low level of excess health risk, generally in the range of 1% to 10%, that can be estimated from data with little or no extrapolation outside the experimental dose range.
chronic exposure	For laboratory animals, an exposure (usually at low concentrations) of long duration, such as months or years. For human populations, an exposure that lasts at least 7 years and could last as long as a lifetime.
cholinesterase	An enzyme capable of catalyzing the hydrolysis of acetylcholine.

dose	The amount of a substance that enters or interacts with organisms. An administered dose is the amount of substance administered to an animal or human, usually measured in milligrams per kilogram of body weight; milligrams per square meter of body-surface area; or parts per million of the diet, drinking water, or ambient air. An effective dose is the amount of the substance reaching the target organ.
epithelial acanthosis	An increase in the thickness of the stratum spinosum of the epithelial tissue.
LD_{50}	Lethal dose to 50% of test animals.
lowest-observed-adverse-effect level (LOAEL)	The lowest exposure level at which there are statistically or biologically significant increases in frequency or severity of adverse effects between the exposure population and its appropriate control group.
maximum tolerated dose (MTD)	The highest dose that can be administered to animals for two years without causing mortality from causes other than cancer.
microgram (µg)	One millionth of a gram.
milligram (mg)	One thousandth of a gram.
miosis	A decrease in pupil size.
modifying factor	A factor that is used to account for uncertainties not accounted for by uncertainty factors.
nonstockpile chemical materiel (NSCM)	NSCM includes a host of lethal wastes from past disposal efforts, unserviceable munitions, chemically contaminated

	containers, chemical production facilities, newly located chemical munitions, known sites containing significant quantities of buried chemical weapons and wastes, and binary weapons and components. NSCM is not part of the stockpile of lethal chemical agents and munitions kept by the military for retaliatory purposes.
no-observed-adverse-effect level (NOAEL)	An exposure level at which there are no statistically or biologically significant increases in the frequency or severity of adverse effects between the exposed population and its appropriate control; some effects may be produced at this level, but they are not considered as adverse, nor precursors to adverse effects.
potency	The degree to which an agent can cause strong or toxic effects.
reference dose	As estimate (with uncertainty spanning perhaps on order of magnitude or greater) of a daily dose to the human population (including susceptible subpopulations) that is likely to be without an appreciable risk of deleterious health effects during a lifetime.
slope factor	The slope of the dose-response curve in the low-dose region. When low-dose linearity cannot be assumed, the slope factor is the slope of the straight line from 0 dose (and 0 excess risk) to the dose at 1% excess risk. An upper bound on this slope is usually used instead of the slope itself.
subchronic exposure	For laboratory animals, multiple or continuous exposures occurring usually over 3 months. For human populations, exposures that last from 1 to 7 years.

threshold	The lowest dose of a substance at which a specified measurable effect is observed and below which it is not observed.
toxicity	The study of adverse effects of chemicals on living organisms.
uncertainty factors	Factors used to divide a no-observed-adverse-effect level (LOAEL) to obtain a safe exposure level.

Appendix A

Health Risk Assessment for The Nerve Agent GA

HEALTH RISK ASSESSMENT FOR THE NERVE AGENT GA

DRAFT REPORT

September 1996

(editorial corrections made April 1997)

Prepared for

U.S. Department of the Army

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Prepared by

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Submitted to

Material/Chemical Risk Assessment Working Group

Advisory and Coordinating Committee

Environmental Risk Assessment Program

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PREFACE

This report assesses the potential non-cancer and cancer effects of chemical agent GA (CAS No. 77-81-6).

This document supports the activities of the Material/Chemical Risk Assessment Working Group of the Environmental Risk Assessment Program, a cooperative endeavor of the Department of Defense, Department of Energy, and Environmental Protection Agency. This working group is developing toxicity values for selected chemicals of concern at federal facilities. Toxicity values will be submitted for consideration by the EPA's IRIS Consensus Process for inclusion on IRIS (EPA's Integrated Risk Information System). The Material/Chemical Risk Assessment Working Group consists of Drs. Jim Cogliano (chair) and Harlal Choudhury (U.S. EPA), Dr. Bruce Briggs (Geo-Centers); Lt. Cmdr. Warren Jederberg and Dr. Robert L. Carpenter (U.S. Naval Medical Research Institute); Dr. Elizabeth Maull and Mr. John Hinz (U.S. Air Force Occupational and Environmental Health Directorate); Drs. Glenn Leach and Winnie Palmer (U.S. Army Center for Health Promotion and Preventive Medicine); Drs. Robert Young and Po-Yung Lu (Oak Ridge National Laboratory).

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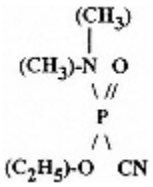
1. INTRODUCTION

Military nerve agents are organophosphate compounds containing either a fluorine, sulfur, or cyanide substituent group (Dacre, 1984). GA contains a cyanide substituent group (VX contains a sulfur group and GB a fluorine group). The chemical synonyms, Chemical Abstract Service (CAS) and Army identification numbers (DA, 1974, 1992; Dacre, 1984), and chemical formula for GA are as follows:

Dimethylphosphoramidocyanidic acid, ethyl ester;
Dimethylaminoethoxy-cyanophosphine oxide;
Dimethylamidoethoxyphosphoryl cyanide;
Ethyl *N, N*-dimethylphosphoramidocyanidate;
Ethyl *N, N*-dimethylaminocyanophosphate
Ethyl dimethylphosphoramidocyanidate;
Ethyl dimethylamidocyanophosphate;
Ethylphosphorodimethylamidocyanidate;
Tabun;
CAS No. 77-81-6;
Edgewood Arsenal No. 1205



1.1. PHYSICAL/CHEMICAL PROPERTIES

Agent GA is a colorless to brown-colored liquid with a molecular weight of 162.1 (DA, 1974; MacNaughton and Brewer, 1994); it has a vapor density of 5.6 (air = 1) and a liquid density of 1.08 g/mL at 25° C (DA, 1974). The vapor pressure of GA is 0.07 mm Hg at 25°C; its solubility in distilled water is 9.8 g per 100 g at 25°C and 7.2 g per 100 g at 20°C (DA, 1974).

1.2. ENVIRONMENTAL FATE

1.2.1 Air

The vapor pressure for GA is 0.07 mm Hg at 25°C indicating a moderate potential for volatilization. A vapor concentration of 610 mg/m^3 has been reported for a temperature of 25°C (DA, 1974) (although not adequately described in the reference, this presumably is the saturation concentration above a pure liquid).

1.2.2 Water

GA has a water solubility of 50–100 mg/L (MacNaughton and Brewer, 1994)); therefore, it is a potential water contaminant. However, because it is subject to hydrolysis, it is not expected to be very persistent in aqueous systems. The half-life of GA is less than 10 min at pH levels greater than 9, 2–3 hr at pH 8–9, and about 6 hr at a pH of 4 (MacNaughton and Brewer, 1994).

The Henry's Law Constant for GA has been estimated to be 1.3×10^{-6} atm m^3/mol (MacNaughton and Brewer, 1994), indicating that GA may volatilize slowly from water.

1.2.3 Soil

Although a soil half-life of 1 to 1.5 days has been reported for GA (DA, 1974), information was not provided on the temperature, pH, or moisture content and other environmental conditions for which this estimate was made.

The volatility potential (slope of the vapor pressure vs. concentration in soil organics) of GA is 2.4×10^{-7} mm Hg/mg/kg and its air-soil partition coefficient (for a soil density of 1.4 g/cm^3) of 1×10^{-4} mg/m^3 (MacNaughton and Brewer, 1994), indicate that GA will evaporate from soil into the air. Results of a field trial with GA showed 10% evaporation in 0.27 hours and 90% evaporation in 4.66 hours (Morrill et al., 1985).

Binding of GA to soil organics is likely to be limited considering the relatively low log K_{ow} of 0.11 and low K_{oc} values of 25 (MacNaughton and Brewer, 1994); therefore, a potential exists for leaching and groundwater contamination. MacNaughton and Brewer (1994) calculated a leaching index of 2 for GA, (i.e., the number of leachings required to reduce the GA soil concentration to one-tenth of the original amount, assuming that for each leaching one kilogram of soil is in equilibrium with one liter of water). However, the amount of GA reaching ground water is likely to be limited by hydrolysis.

2. MECHANISM OF ACTION

Nerve agents are inhibitors of acetylcholinesterase (AChE), an enzyme responsible for deactivating the neurotransmitter acetylcholine at some neuronal synapses and myoneural junctions. By a mechanism of phosphorylation, nerve agents act as substrates for the enzyme thereby preventing deactivation of acetylcholine. The organophosphate-inhibited enzyme can be reactivated by dephosphorylation, but this occurs at a rate that is slower than the rate of reactivation of acetylcholine (deactivated by acetylcholinesterase). Consequently, there is a depletion of acetylcholinesterase and a buildup of acetylcholine. In addition, the nerve agent-enzyme complex can also undergo an "aging" process (thought to be due to a loss of an alkyl or alkoxy group), whereby it becomes resistant to dephosphorylation (see review by Munro et al., 1994). Differences in rates of aging and reactivation may be important in evaluating toxicity data especially when extrapolating from animal studies to humans. *In vitro* tests conducted by Grob and Harvey (1958) indicate that both GA and GB combine with cholinesterase almost irreversibly during the first hour of their reaction. Sidell and Groff (1974) reported that the GB-ChE complex ages very rapidly *in vivo,* with 45–70% completion by 5 hours after infusion. In contrast, the complex formed between ChE and the nerve agent VX does not age significantly, and the rate of spontaneous reactivation can be as fast as 1%/hr in humans (Sidell and Groff, 1974).

2.1 Effects of Organophosphate Agents on the Nervous System

The anticholinesterase effects of the organophosphate nerve agents can be characterized as being muscarinic, nicotinic, or central nervous system (CNS)-related. Muscarinic effects occur in the parasympathetic system (bronchi, heart, pupils of the eyes; and salivary, lacrimal and sweat glands) and result in signs of pulmonary edema, bradycardia, miosis, tearing, and sweating. Nicotinic effects occur in somatic (skeletal/motor) and sympathetic systems, and result in muscle fasciculation, muscle weakness, tachycardia, and diarrhea. Effects on the CNS by organophosphates are manifested as giddiness, anxiety, emotional lability, ataxia, confusion, and depression (O'Brien, 1960).

Although the inhibition of cholinesterase within neuro-effector junctions or the effector itself is thought to be responsible for the major toxic effects of organophosphate agents, these compounds can apparently affect nerve-impulse transmission by more direct processes as well. Direct effects may occur on excitable tissues, receptors, and ionic channels. According to Somani et al. (1992), the direct action of nerve agents on nicotinic and muscarinic ACh receptors may occur when concentrations in the blood rise above micromolar levels, whereas at lower levels the action is mainly the result of inhibition of AChE. Albuquerque et al. (1985) have shown that agent GA, as well as agents GB and GD are capable of changing receptor sites in a manner similar to that exhibited by acetylcholine, which promotes the conductance of electrophysiological signals associated with stimulation of neuromuscular function. VX "may directly affect a small population of muscarinic ACh receptors that have a high affinity for [^{3}H]-*cis*-methyldioxalane binding" (Somani et al., 1992). VX may also counteract the effects of ACh by acting as an open channel blocker at the neuromuscular junction, thereby interrupting neuromuscular function (Rickett et al., 1987).

Exposure to some organophosphate cholinesterase inhibitors results in a delayed neuropathy characterized by degeneration of axons and myelin. This effect is not associated with the inhibition of acetylcholinesterase, but rather with the inhibition of an enzyme described as neuropathy target esterase (NTE); however, the exact mechanism of toxicity is not yet fully understood (Munro et al., 1994). For some organophosphate compounds, delayed neuropathy can be induced in experimental animals at relatively low exposure levels, whereas for others the effect is only seen following exposure to supralethal doses when the animal is protected from the acute toxic effects caused by cholinesterase inhibition.

Although there is the potential for nerve agents to have direct toxic effects on the nervous system, there is no evidence that such effects occur in humans at doses lower than those causing cholinesterase inhibition. For the purpose of evaluating potential health effects, inhibition of blood cholinesterase is generally considered the most useful biological endpoint.

2.2 Effect on Blood Cholinesterases

In addition to being found in the nervous system, acetylcholinesterase also occurs in the blood where it is bound to the surface of red blood cells (termed RBC-ChE or RBC-AChE). RBC-AChE activity, as well as the activity of a second type of cholinesterase found in blood plasma (butyrylcholinesterase, or plasma cholinesterase) have been used to monitor exposure to organophosphate compounds (pesticides and nerve agents). Both RBC-AChE and plasma-ChE activity have been used as bioindicators of potential toxic effects. There is some evidence that RBC-AChE is as sensitive as brain ChE to the effects of nerve agents. Grob and Harvey (1958) reported that the *in vitro* concentrations producing 50% depression of brain-ChE and RBC-AChE activity were the same in the case of GA (1.5×10^{-8} mol/L),

and only slightly different (3×10^{-9} mol/L and 3.3×10^{-9} mol/L) in the case of GB. However, *in vivo* animal studies indicate a poor correlation between brain and RBC-AChE in cases of acute exposures (Jimmerson et al., 1989), and this is reflected in the fact that blood cholinesterase activity may not always be correlated with exposure or with signs and symptoms of toxicity (Holmstedt, 1959). Acute exposures to high concentrations may cause immediate toxic effects before significant changes occur in blood ChE activity, and repeated exposures over a period of several days or more may result in a sudden appearance of symptoms due to cumulative effects (Grob and Harvey, 1958). Conversely, blood ChE activity can become very low without overt signs or symptoms during chronic exposures to low concentrations of organophosphates. This may be due to a slower rate of recovery of RBC-AChE compared to tissue ChE, or to noncholinesterase-dependent recovery pathways for neural tissue (Grob and Harvey, 1958). Sumerford et al. (1953) reported that orchard workers exposed to organophosphate insecticides had RBC-AChE values as low as 13% of preexposure values without any other signs or symptoms of toxicity. Animal studies have demonstrated that chronic exposures to low concentrations of organophosphate insecticides and nerve agents can result in increased tolerance levels (Barnes, 1954; Rider et al., 1952; Dulaney et al., 1985). Similarly, Sumerford et al. (1953) reported increased levels of tolerance to organophosphate insecticides in people living near orchards treated with organophosphate insecticides. Such adaptation may result from increased rates of formation of blood ChE, or from increased rates of detoxification. Additional information on the development of tolerance to organophosphate cholinesterase inhibitors can be found in a review paper by Hoskins and Ho (1992).

The blood cholinesterases may, to some degree, provide a protective effect by binding with some fraction of the anticholinesterase compound (Wills, 1972). However, not all nerve agents bind equally well with all cholinesterases. In tests conducted on dogs, Holmstedt (1951) found that GA affected RBC and plasma cholinesterase to a nearly equal degree. In contrast, agent VX preferentially inhibits RBC-AChE (70% compared with about 20% inhibition of plasma ChE) (Sidell and Groff, 1974). Rodents (but not humans) have other enzymes in the blood, termed aliesterases, which can bind to organophosphates, thereby reducing the amount available for binding with acetylcholinesterase (Fonnum and Sterri, 1981). Agent GB binds with aliesterases; however, according to Fonnum and Sterri (1981), VX has a quaternary ammonium group which prevents it from being a substrate for aliesterases. The strong specificity of agent VX to AChE may account, in part, for the fact that it is much more acutely toxic than agents GA and GB (see Appendix A).

2.2.1 Intra- and Interspecies Variation in Blood Cholinesterase Activity

Although blood cholinesterase activity is used as a measure of exposure to organophosphate compounds, baseline activity levels can vary between individuals and between species. According to Wills (1972), both plasma- and RBC-AChE activity are generally lower in women than in men. Sidell and Kaminskis (1975) reported that, for a test population of 22 human subjects, the highest coefficient of variation of RBC-AChE was 4.1% per single subject; the average range of variation was ± 2.1% for men and ± 3.1% for women. In individuals studied for one year, the RBC-AChE activity varied by 11% in men and 16% in women. Yager et al. (1976) reported a 10.0% intra-individual coefficient of variation for RBC-AChE activity and 14.4% for plasma-ChE activity. Callaway et al. (1951) estimated that with only one pre-exposure measurement, the smallest measurable decrease was 15% of the baseline value for RBC-AChE activity and 20% of the baseline for plasma-ChE activity.

A small subpopulation of men and women have a genetic defect causing their blood cholinesterase activity to be abnormally low (Evans et al., 1952; Harris and Whittaker, 1962). For homozygous individuals, the activity can be as low as 8–21% of the normal mean (Bonderman and Bonderman, 1971).

Morgan (1989) suggests that these individuals may be unusually sensitive to organophosphate anticholinesterase compounds.

Data compiled by Ellin (1981) reveal that the RBC-AChE activity for humans is slightly higher than that for monkeys and much higher than that for rats and other laboratory animals (Table 1). These differences in RBC-AChE activity may affect a species' sensitivity to a particular organophosphate compound. At the same time, the relative amount of plasma cholinesterase and other compounds in the blood that can bind to the organophosphate agents must also be considered. For example, rodents, but not humans, have high levels of aliesterases (AE) in the blood, and these compounds may provide rats and mice with a higher level of resistance to some anticholinesterase compounds (McNamara and Leitnaker, 1971).

Table 1. RBC-AChE activity in different species

Species	**RBC-AChE activity (µmol/mL/min)**	**Optimum substrate**[a] concentration (M)
Human	12.6	2×10^{-3}
Monkey	7.1	2×10^{-3}
Pig	4.7	1×10^{-3}
Goat	4.0	2×10^{-3}
Sheep	2.9	2×10^{-3}
Mouse	2.4	2×10^{-3}
Dog	2.0	2×10^{-2}
Guinea pig	2.7	2×10^{-3}
Rabbit	1.7	5×10^{-3}
Rat	1.7	5×10^{-3}
Cat	1.5	5×10^{-3}

Source: Ellin, 1981

[a] Acetylthiocholine iodide concentration for maximum RBC-AChE activity.

2.2.2 Potency of Nerve Agents as Cholinesterase Inhibitors

The potency of the anticholinesterase activity of nerve agents and other organophosphates is expressed by the bimolecular rate constant (k_i) for the reaction of the phosphate compound with the enzyme and by the molar concentration causing 50% inhibition of the enzyme when tested *in vitro* (I_{50}). I_{50} data for several organophosphate nerve agents have been tabulated by Dacre (1984). The relationship between I_{50} and k_i as a function of time (t) is expressed by the following equation (Eto, 1974):

$$I_{50} = \frac{0.693}{t \times k_i} \qquad (1)$$

The pI_{50} (negative log of the molar concentration causing 50% inhibition) for GA was reported to be 8.4 by Holmstedt (1959), 8.6 by Dacre (1984), and calculated as 7.8 from an I_{50} of 1.5×10^{-8} mol/L reported by Grob and Harvey (1958). Grob and Harvey (1958) reported that the potency of GA in inhibiting RBC-AChE was only one-fifth of that for GB ($I_{50} = 0.3 \times 10^{-8}$ mol/L).

Relative potency of nerve agents can also be expressed in terms of the *in vivo* dose necessary to produce the same level of cholinesterase inhibition by a specific exposure route. As would be expected, the effectiveness of the agents in inhibiting cholinesterase is closely correlated with their acute toxicity (see Appendix A).

3. TOXICOLOGY

3.1 Introduction

Health and environmental impacts of nerve agents and related compounds (organophosphate insecticides) have been reviewed by O'Brien (1960), Matsumura (1976), Dacre (1984), Carnes and Watson (1989), Watson et al. (1989), and Munro et al. (1994). A brief general discussion of the toxicology of nerve agents and related organophosphate pesticides is given below.

Nerve agents are toxic by all routes of exposure. Initial symptoms of acute poisoning are fatigue, headache, mild vertigo, weakness, and loss of concentration. Moderate exposures result in miosis and excessive sweating, tearing, and salivation. Acidosis and hyperglycemia may also occur in addition to muscular weakness, muscular twitching, lacrimation, urination, and defecation. Acute poisoning can result in prostration, clonic convulsions (rapid repetitive movements), and tonic convulsions (limbs stretched and rigid) (Matsumura, 1976). Exposures sufficiently high to cause convulsions have resulted in brain lesions and cardiomyopathy in laboratory animals (Singer et al., 1987).

In addition to the immediate toxicity of the nerve agents, there is concern that acute exposures may lead to chronic neurological effects similar to those reported for some related organophosphate insecticides. Included among these possible effects are organophosphate-induced delayed neuropathy (OPIDN), EEG changes, and long-term psychological disturbances (Munro et al., 1994). OPIDN, which appears 5–30 days after exposure, manifests itself as muscle weakness, tingling, and twitching followed

by paralysis (see review by Munro et al., 1994). Histopathological changes, which consist of degeneration of axons and myelin of the nervous system, can be correlated, not with inhibition of acetylcholinesterase, but rather with inhibition of NTE; however, the exact mechanism of toxicity is not yet fully understood (Munro et al., 1994). There is no evidence that GA causes OPIDN in humans. The limited animal data indicate that GA may cause some inhibition of NTE and slight signs of OPIDN in some species, but OPIDN has only been observed in antidote-protected chickens dosed with 120 times the LD_{50} for GA (see section 3.5). The likelihood that GA would induce OPIDN in humans at dose levels below those causing acute toxicity or cholinesterase inhibition is very small.

Acute exposures to nerve agents are known to result in EEG changes and psychological effects (Grob and Harvey, 1958; Sidell, 1992). Some studies have indicated that changes in EEG patterns may persist for long periods of time after exposure (Metcalf and Holmes, 1969; Burchfiel et al., 1976; Duffy et al., 1979; Duffy and Burchfiel, 1980); however, the reported changes have been considered to be clinically insignificant and not correlated with behavioral or physiological changes (DHHS, 1988).

Although nerve agents can induce neuropsychological changes in acutely exposed individuals, there is no evidence of effects persisting for months or years as has been reported for some organophosphate insecticides (Savage et al., 1988). Although some studies have identified neurologic and psychological changes in workers occupationally exposed to organophosphate insecticides, it is unclear to what degree these effects may have been caused by intermittent acute exposures (Gershon and Shaw, 1961; Mick, 1974; Rodnitzky, 1974; Wagner, 1983; Tabershaw and Cooper, 1966). However, the available data on the organophosphate insecticides suggest that long-term toxicological effects do not occur in the absence of significant changes in blood cholinesterase activities, and a similar conclusion is likely to apply to the nerve agents.

3.2 Acute Toxicity

GA lethality data for animals and estimates of human LD_{50} values are given in Table 2. Oral LD_{50} values for humans have been estimated to be 357–714 µg/kg (Somani et al., 1992). A subcutaneous injection of 0.43 µmol/kg (0.0698 mg/kg) in dogs resulted in a depression in erythrocyte cholinesterase activity to about 30% of its baseline value; however, no overt toxic effects were observed (Holmstedt, 1951). In cats, a slow drip intravenous (i.v.) infusion of 0.05 mg/kg or more resulted in increased bronchial constriction and labored respiration (Holmstedt, 1951). In rabbits, an i.v. dose of about 0.04 mg/kg (25% of the lethal dose) caused a steady decrease in cardiac output (Holmstedt, 1951).

3.3 Subchronic Toxicity

The National Center for Toxicological Research evaluated the subchronic toxicity of agent GA on male and female CD rats (Bucci et al., 1992). The test animals (12/sex/dose group) were injected intraperitoneally with GA at dose levels equivalent to 0, 28.13, 56.25, or 112.5 µg/kg/day. The injections were given once per day, 5 days per week for 13 weeks. Animals were observed daily for clinical signs of toxicity and weighed weekly. Necropsy examination was performed on all animals. Terminal body and organ weights were recorded. Microscopic evaluation was performed on all high-dose and control animals as well as on all gross lesions and on animals dying or sacrificed before the end of the test period. Blood samples were taken from 6 rats/sex/dose during weeks -1, 1, 3, 7 and at necropsy. Hematological analyses consisted of blood cell counts, hemoglobin, hematocrit, and mean corpuscular volume, hemoglobin and hemoglobin concentration. Clinical chemistry included measurements of alanine

aminotransferase, aspartate aminotransferase, blood urea nitrogen, creatinine, creatinine kinase, and RBC and plasma cholinesterase. In addition, at necropsy, brain samples were tested for NTE.

Table 2. LD50 values for agent GA

Exposure route	Species[a]	LD_{50} (μg/kg)	References
Intravenous	human	14[b]	Robinson, 1967
	monkey	~50	DA, 1974
	pig	—	—
	rat	70	DA, 1974
Oral	human	357–714[c]	Somani et al., 1992
	monkey	—	—
	rat	3700	RTECS, 1995
Subcutaneous	human	—	—
	monkey	70	RTECS, 1995
	rat	162	RTECS, 1995
	rat	~300	DA, 1974
Percutaneous	human	14,000–21,000	DA, 1974
	human	2,857–14,286[c]	Somani et al., 1992
	monkey	9,300	RTECS, 1995
	pig	—	—
	rat	18,000	RTECS, 1995
	rat	12,600	Crook et al., 1983
Intramuscular	human	—	—
	monkey	34	RTECS, 1995
	rat	800	RTECS, 1995
Intraperitoneal	human	—	—
	monkey	—	—
	rat	~800	DA, 1974
		490	RTECS, 1995

[a] Values for humans estimated from animal data
[b] LD_{Lo}
[c] Estimated for 70 kg individuals

The results of the clinical chemistry tests indicated no adverse effects on liver, kidney, or muscle. Hematological parameters for the dosed animals were generally within the normal range, and brain NTE activity was not affected by GA administration. There were no GA-related neoplastic or non-neoplastic lesions.

Cholinesterase levels in the dosed rats were compared to control values for the same sampling times (Tables 3, 4). There was considerable variability in the RBC-AChE data. Mean baseline values for both male and female rats were elevated substantially (5029 versus 1848 IU/L in females and 4045 versus 1552 IU/L in males) when compared to control levels recorded in previous nerve agent studies conducted at the same laboratory. The elevated pre-exposure RBC-AChE readings in the current study were attributed to faulty reagents. Mean RBC-AChE activity levels in dosed and concurrent control animals also showed irregular fluctuations over time, with unusually high readings occurring in females at week 3 and in males at week 7. It was reported that the percent reduction in RBC-AChE at week 3

was about 37% in dosed females and 18% in males. Statistical analysis of the RBC-AChE data indicated significant reductions in RBC-AChE activity (relative to controls) in only a few cases; i.e., in high-dose females at week 3; in mid-dose females at week 7; and in males of all dose groups at week 1.

Table 3. RBC-AChE levels in 90-day subchronic rat study using agent GAa

		Week of treatment								
Dose (µg/kg/d)	**Sex**	**-1**	**1**	**%[b]**	**3**	**%[b]**	**7**	**%[b]**	**13**	**%[b]**
0	F	4604 (1451)	2949 (370)	64	5472 (801)	119	4483 (441)	97	4457 (261)	97
28.13	F	5272 (589)	3181 (331)	60	4360 (179)	83	3723 (397)	71	4386 (414)	83
56.25	F	5168 (623)	2975 (180)	58	4315 (514)	83	3169 (381)[c]	61	4205 (545)	81
112.50	F	5073 (428)	2421 (184)	48	3428 (174)[c]	68	3404 (364)	67	4229 (721)	83
0	M	3939 (434)	3588 (297)	91	4427 (422)	112	5582 (455)	142	4789 (290)	122
28.13	M	4115 (356)	2549 (311)[c]	62	4106 (557)	99	5025 (98)	>100	4058 (465)	99
56.25	M	4369 (499)	2409 (788)[c]	55	3630 (417)	83	5200 (340)	>100	4601 (553)	>100
112.50	M	3760 (541)	2367 (534)[c]	63	3612 (582)	96	5145 (281)	>100	4126 (491)	>100

Source: Bucci et al., 1992
[a] Results given in IU/L, mean and (SEM)
[b] Percent of baseline.
[c] $p \leq 0.05$, different from control value (analysis by Bucci et al., 1992)

The RBC-AChE data were re-analyzed by ORNL (using standard deviations) with ANOVA, and Dunnett's Comparison (see Appendix B). The ORNL analysis indicated that RBC-AChE levels in males at week 1 were not significantly lower than control values, but were significantly lower than pre-exposure values ($p < 0.05$) for the two lowest dose groups, but not the high-dose group. Similar re-analysis of the female RBC-AChE data indicated that the high-dose group had significant reductions from pre-exposure values at weeks 1, 3, and 7 (Appendix B).

Changes in plasma-ChE activity in dosed and control animals are shown in Table 4. Over the course of the study, plasma-ChE activity levels in dosed and control animals appear to be more stable than RBC-AChE activity. It was reported that plasma-ChE activity was decreased by about 55% in dosed females at week 7, and by 37.5% in dosed males at week 3. Mean plasma-ChE activity in the female controls exhibited a slow increase over the 13-week test period (from 1743 IU/L at week -1 to 2891 IU/L at week 13). A similar response was seen in the two lowest dose groups of females. In males, mean plasma-ChE activity in controls was lower than pre-exposure levels (401 IU/L at week -1) at all weeks except week 3 (413 IU/L). In the dosed groups of males, mean plasma-ChE levels were lower than pre-exposure values at all sampling times. Statistical analysis of the plasma-ChE activity indicated that mean values were significantly lower than controls in the mid- and high-dose females at weeks -1, 1, 3, and 7 but not at week 13, and in the high-dose males at weeks 3 and 7.

The plasma-ChE data were re-analyzed by ORNL (using standard deviations) with ANOVA, and

Dunnett's Comparison (see Appendix C). The ORNL analysis indicated that plasma-ChE levels in males were significantly lower ($p < 0.05$) than pre-exposure values at week 1, 3, 7 and 13 in the mid-dose group; and at weeks 1, 3, and 7 in the high-dose group. The two highest dose groups were also significantly lower ($p < 0.05$) than control values at weeks 3 and 7 (and at week 1 for the high-dose group). In females, plasma-ChE levels were not significantly lower than preexposure values, but were significantly lower ($p < 0.05$) than controls at weeks 1, 3 and 7 for all dose groups (Appendix C).

Table 4 Plasma-ChE levels in 90-day subchronic rat study using agent GAa

		Week of treatment								
Dose (μg/kg/d)	**Sex**	**-1**	**1**	**%[b]**	**3**	**%[b]**	**7**	**%[b]**	**13**	**%[b]**
0	F	1743(273)	2033 (284)	116	2204 (303)	126	2544 (334)	146	2891 (425)	166
28.13	F	1369(111)	1416 (125)	103	1540 (138)	112	1794 (188)	131	2311 (245)	169
56.25	F	1233(135)[c]	1288 (164)[c]	104	1424 (186)[c]	115	1548 (194)[c]	125	1928 (297)	156
112.50	F	1449(122)[c]	1202 (113)[c]	82	1136 (133)[c]	78	1148 (121)[c]	79	1755 (190)	121
0	M	401(25)	397(25)	99	413(37)	103	383(23)	96	344(26)	86
28.13	M	426(28)	394(25)	92	361(22)	85	362(17)	85	375(21)	88
56.25	M	415(13)	351(13)	85	319(12)	77	323(14)	78	330(18)	79
112.50	M	405(31)	311(16)	77	258(11)[c]	64	268(13)[c]	66	360(32)	89

Source: Bucci et al., 1992
[a] Results given in IU/L, mean and (SEM)
[b] Percent of baseline.
[c] $p \leq 0.05$, different from control value (analysis by Bucci et al., 1992).

Dulaney et al. (1985) evaluated the effects of GA on the growth rates of rats given daily subcutaneous doses of 100 μg/kg for 85 days. The dosed animals exhibited reduced growth rates (42% of controls in the first 15 days, 82% of controls in the next 22 days, and 95% of controls from the 38th day to the end of the study). AChE activity was determined in the striatum and the remainder of the brain 24 hr after the last exposure. Mean brain striatal AChE activity was only 13% of the control value; in the remaining parts of the brain the AChE activity was 22% of the control. In cumulative mortality studies, rats (8–11/dose group) were dosed with 75 or 100 μg/kg/day for 25 days. In the low-dose group one of 8 animals died on day 10; in the high-dose group, one animal died on day 15 and another on day 20. Dosing was continued for an additional 60 days without any further mortality. Because of the observed mortality, the subcutaneous dose of 75 μg/kg/day can be considered a lowest-observed-adverse-effect level (LOAEL) under the conditions of the study.

3.4 Chronic Toxicity

Data on the chronic toxicity of GA were not found in the available literature.

3.5 Nervous System Toxicity

There has been concern that organophosphate compounds like GA may have direct toxic effects on the nervous system. Some organophosphate compounds cause a neurotoxic effect (organophosphate-induced delayed neuropathy or OPIDN) that is not associated with AChE inhibition but rather with inhibition of NTE. Agent GA has not been shown to produce OPIDN in humans. Some animal data suggest that it might have the potential to do so, but only following extremely high doses (Munro et al., 1994). *In vitro* and *in vivo* studies have demonstrated that supralethal doses of GA can cause NTE inhibition in antidote-protected chickens (Lotti and Johnson, 1978; Vranken et al., 1982); however, small daily doses given over a prolonged time period do not appear to induce this effect. In studies conducted by Bucci et al. (1992), intraperitoneal injections of GA into CD rats at dose levels up to 112.5 μg/kg/day, 5 days per week for 13 weeks did not result in a significant change in brain NTE. Furthermore, there was no clinical evidence of neuropathology, even though blood cholinesterase activity decreased significantly in the dosed animals. Although a number of studies have been conducted on chickens, in only a few cases have signs of OPIDN been observed. Henderson et al. (1989, 1992) reported no signs of OPIDN in chickens receiving one 0.125 mg/kg dose by intramuscular injection or repeated injections of 0.07 mg/kg, 5 days/week for 90 days. OPIDN was also not observed in antidote-protected chickens receiving one 12 or 15 mg/kg dose or two 12 mg/kg doses (120–150 times the LD_{50}) by intramuscular injection (Willems et al., 1984; Johnson et al., 1988); however, mild signs of neuropathy occurred in one of two surviving chickens receiving two 6 mg/kg doses (Willems et al., 1984). Overall, the data suggest that it is unlikely that OPIDN would occur in humans at less-than-lethal doses (see Munro et al., 1994).

3.6 Developmental and Reproductive Effects

There are no data evaluating the potential developmental and reproductive toxicity of GA in humans. Limited animal data indicate that such effects are unlikely. In studies in which CD rats were injected intraperitoneally with 0, 75, 150, or 300 μg GA/kg/day on gestation days 6–15, no fetal malformations or developmental effects (with the exception of increased pre-implantation losses relative to controls) were observed (Bucci et al., 1993). Because dosing began on gestation day 6, which was near the end or after the time of implantation, the observed pre-implantation losses were not considered to be agent-related (Bucci et al., 1993). Blood cholinesterase levels were not monitored during the study. Signs of maternal toxicity (salivation and lacrimation) were seen at all dose levels, and the highest dose produced tremors in some animals. Mean maternal weight gain was also reduced in the high-dose animals when compared to that of controls. Maternal mortality rates were 1/31, 2/32, and 12/33 in the low-, mid-, and high-dose groups and all were considered to be agent-related. Therefore, the lowest dose of 75 μg/kg/day can be considered a LOAEL for maternal effects in rats under the conditions of the study.

In the tests in which agent GA (28.1, 56.3, and 112.5 μg/kg/day) was administered subcutaneously to New Zealand white rabbits on gestation days 6–19, no adverse effects on fetal implantations, fetal weight, and fetal malformations were observed (Bucci et al., 1993). However, maternal toxicity (indicated by salivation, diarrhea, and nasal discharge) was evident in the high-dose group which also experienced a mortality rate of 13.3% (4/30). Therefore, the highest dose of 112.5 μg/kg/day can be considered a LOAEL for maternal effects in rabbits.

3.7 Carcinogenicity

No information is available regarding the potential carcinogenicity of GA in humans. No long-

term animal carcinogenicity studies have been carried out on GA. Neoplastic lesions were not observed in male and female CD rats injected intraperitoneally with up to 28.13, 56.25, or 112.5 μg GA/kg/day for 90 days (Bucci et al., 1992); however, this subchronic study was of insufficient duration to fully evaluate tumor incidence rates. No other animal data are available to assess the potential carcinogenicity of GA.

3.8 Genotoxicity

No information is available regarding the genotoxicity of GA in humans; however, genotoxicity and mutagenicity data are available from microbial assays, and *in vitro* and *in vivo* tests on laboratory animals (Wilson et al., 1994). GA was found to be weakly mutagenic in eight of 11 Ames Salmonella assays using the revertant strains TA98, TA100, TA1535, and TA1538. GA also induced dose-related increases in mutation rates when tested on mouse L5178Y lymphoma cells without metabolic activation; the increase observed at a test concentration of 100 μg/mL was nearly three-times that of the control. An increase in sister chromatid exchanges (SCE) was observed in Chinese hamster ovary cells exposed *in vitro* to GA concentrations of 25–200 μg/mL. Dose-responses were linear and highly statistically significant; however, the number of SCEs did not exceed twice the control value at any of the concentrations tested. C57B1/6 mice treated *in vivo* with a maximally tolerated intraperitoneal dose of 700 μg GA/kg did not exhibit a significant increase in SCE in splenic lymphocytes. Exposure of rat hepatocytes to GA concentrations as high as 200 μg/mL resulted in inhibition of unscheduled DNA synthesis. From the results of these studies (i.e., three positive responses in five assays), Wilson et al. (1994) concluded that GA was a weakly acting mutagen.

4. ORAL REFERENCE DOSE FOR GA

4.1 Cholinesterase Inhibition as an RfD Endpoint

The endpoint for defining a maximum acceptable exposure level for nerve agents such as GA is considered to be the level at which no significant depression in blood cholinesterase activity occurs. In humans, 15% inhibition of RBC-AChE is generally considered to be the minimum change that can be observed with any statistical reliability (Callaway et al., 1951). Existing human response data (Marquis, 1988) indicate that human RBC-AChE inhibition of as much as 20% is not associated with adverse clinical signs or symptoms and should be considered only as evidence of organophosphate exposure. This contention is supported by the U.S. EPA (1995a) which reports scientific agreement that statistically significant inhibition of cholinesterase in multiple organs and tissues accompanied by clinical effects constitutes a hazard; however, in the absence of clinical effects, such inhibition may not be of biological significance. It is generally agreed that inhibition of RBC and/or plasma cholinesterase contributes to the overall hazard identification of cholinesterase inhibiting agents by serving as biomarkers (U.S. EPA, 1995a). Animal data have shown that exposure to low doses of nerve agents for extended periods of time

can result in low blood ChE activity levels without signs of toxicity. Bucci et al. (1992) found no evidence of toxicity in rats dosed intraperitoneally with GA (up to 112 μg/kg), even though RBC-AChE activity was reduced about 37% in females (relative to controls). In oral toxicity studies conducted on GB, Bucci et al. (1992) found that gavage doses of 0.3 mg/kg/day to rats caused nearly a 50% reduction in RBC-AChE activity without signs of toxicity. Goldman et al. (1988) reported no signs of toxicity, but 78–80% reduction in RBC-AChE activity, in Sprague-Dawley rats dosed subcutaneously with 1.0 μg VX/kg/day over 30 days. Rice et al. (1971) reported that whole blood cholinesterase of sheep dosed with 15 μg VX/day was reduced to 4–5% of the normalized baseline values (during the last 3 weeks of the dosing period) without any signs of toxicity. Rice et al. (1971) also found that sheep showing signs of toxicity (not described) at higher dose levels recovered fully after the exposures ended. Further complicating the evaluation is the extreme variability in ChE levels of individual animals and different sexes and ages of the same species (Halbrook et al., 1992). Possible changes in blood ChE that may occur with increasing age of the animals requires comparisons with concurrent controls, because the absence of a significant difference from pre-exposure value may be due to age-related increases in ChE in the dosed animals.

Blood ChE activity has been used by EPA as the critical endpoint in the establishment of oral RfDs for organophosphate insecticides (U.S. EPA 1995a,b). In the case of malathion (U.S. EPA, 1995a), the no-observed-effect level (NOEL) was identified as the highest oral dose level at which no significant change in RBC-AChE or plasma-ChE activity was recorded in 5 human volunteers who received the compound orally for 47 days (Moeller and Rider, 1962). The next highest dose was associated with a depression of about 25% in both RBC-AChE and plasma ChE, but no clinical signs of toxicity. The EPA approach, also used for other organophosphate pesticides (U.S. EPA, 1995b), is, therefore, to identify the lowest-effect level (LEL) as the dose at which statistically significant decreases in ChE levels (RBC-AChE, plasma-ChE, or brain-ChE) occur, and then to base an RfD on the dose level where the change in ChE is not statistically significant. This approach is also used in this report so that the RfDs developed for the nerve agents will not be disproportionally different from those for organophosphate insecticides; however, it should be emphasized that these values may be overly conservative. Furthermore, in evaluating the experimental data for the nerve agents, added weight was given to those cases where significant changes in ChE occurred relative to both control and pre-exposure values and where there was evidence of a dose-response relationship.

4.2 Derivation of the Oral RfD for GA

For the derivation of a chronic oral RfD, chronic or subchronic human oral exposure data are preferred; however, the only available human data for GA pertain to acute exposures. Although such data can be used to establish short-term exposure limits, acute toxicity endpoints are generally not used for developing subchronic or chronic reference values since they do not provide information on the possibility of cumulative effects following prolonged exposures. There are no subchronic or chronic animal studies on GA using the oral exposure route. Subchronic exposure data are available for GA from one rat study in which the animals were injected intraperitoneally once per day for 90 days (Bucci et al., 1992) and from another rat study in which rats were injected subcutaneously for 85 days (Dulaney et al., 1985). Non-oral exposure data are not normally used by EPA for deriving an oral RfD because of difficulties in estimating equivalent dose levels for oral exposures. EPA has used non-oral exposure data to derive an oral RfD for silver, but only because adequate data were available to estimate equivalent oral doses from experimental intravenous data by using information on the absorption of the element through the gastrointestinal tract (U.S. EPA, 1991a). The Bucci et al. (1992) study is used here to derive an oral RfD for GA because it included a series of doses and a more comprehensive evaluation of the potential toxicity

than did the Dulaney et al. (1985) study; however, an oral dosing study would have been preferred. Ideally, information on the relative absorption of GA through both the oral and i.p. routes should be used to estimate an equivalent oral dose from i.p. data. Because such data are not available, a very provisional estimate of the equivalent oral dose was derived from a comparison of oral and i.p. LD_{50} values for the rat. The reported rat oral LD_{50} value of 3700 μg/kg is 4.6 and 7.6 times larger than the reported i.p. LD_{50}s of 800 and 490 μg/kg, respectively. The assumption is made that a similar relationship would occur under a subchronic exposure protocol.

The use of a rat study for developing an RfD for GA is complicated by the fact that rodents have a much lower RBC-AChE activity level compared to humans (Ellin, 1981). By itself, this could cause rats to be relatively more sensitive than humans to anticholinesterase compounds; however, the lower RBC-AChE activity may be offset by the presence of aliesterases in the blood of rats. Aliesterases, which are not found in human blood plasma, are known to bind to and, therefore, reduce the toxicity of GB, and a similar mechanism may operate in the case of GA. Other species differences, such as in the rates of aging of the GA-ChE complex, in the rates of synthesis of plasma-ChE in the liver, and in the levels of AChE in the nervous system (see Ivanov et al., 1993) may also result in difference between species in sensitivity to GA. Data are insufficient to more fully evaluate these possibilities. There is little human acute toxicity data that can be compared with the available rat data; however, acute toxicity data for primates in general (see Table 2) suggests that humans are likely to be more sensitive than rats. Therefore, for the purpose of this assessment, the standard EPA method will be followed which assumes that humans can be as much as ten times more sensitive to a chemical than laboratory animals.

In the Bucci et al. (1992) study, 12 CD rats/sex/dose group were injected intraperitoneally with GA at dose levels equivalent to 0, 28.13, 56.25, or 112.5 μg/kg/day. The injections were given once per day, 5 days per week for 13 weeks. Details of the study are given in section 3.3. The only significant changes observed in the dosed animals were decreases in blood ChE activity levels. In the case of RBC-AChE levels, considerable fluctuations occurred between and within test and control groups (0 μg/kg/day) (see Table 3). The variability in the RBC-AChE data in the male and female control groups makes these data sets less reliable for identifying a LOAEL and a no-observed-adverse-effect level (NOAEL) for ChE inhibition (Appendix B).

Of the parameters measured in the Bucci et al. study, changes in plasma-ChE values in male rats provided the least variable indication of a LOAEL and NOAEL for GA (see Appendix C); in the two highest dose groups plasma-ChE was significantly lower ($p < 0.05$) than both pre-exposure values and control levels at weeks 3 and 7, and significantly ($p < 0.05$) lower than pre-exposure values at week 1 (also significantly lower than controls in the high-dose group at week 1). There is also evidence (based on mean plasma ChE values) of a dose-response relationship for all sampling times (i.e., plasma-ChE was lower at the higher doses). Maximum depression of plasma-ChE occurred at 3–7 weeks, a condition also seen in a study of rats dosed with the nerve agent VX (Goldman et al., 1988). Therefore, because of the significantly lower levels of plasma-ChE in male rats (relative to both controls and pre-exposure vales), the mid-dose of 56.25 μg/kg/day is considered a LOAEL for plasma-ChE inhibition, and because of the lack of consistent change in plasma and RBC-AChE (relative to controls or preexposure values), the dose of 28.13 μg/kg/day is considered a NOAEL.

The equivalent oral NOAEL is estimated by comparing oral and intraperitoneal LD_{50} values for the rat and assuming that about the same ratio would apply for longer term exposures. A rat oral LD_{50} of 3700 μg/kg, and i.p. LD_{50} values of 490 and 800 μg/kg (average 645 μg/kg) have been reported. Therefore the equivalent oral NOAEL is:

$$oral\ NOAEL = 28.13 \times \left[\frac{3700}{645}\right] = 161\ \mu g/kg/day \qquad (2)$$

The estimated oral NOAEL of 161 μg/kg/day can be used to estimate a human oral reference dose (RfD) by first adjusting the NOAEL for a 7 days/week exposure period by using a factor of 5/7; i.e., 5/7 × 161 μg/kg = 115 μg/kg/day, and then applying the result to the following EPA formula:

$$RfD = \frac{115\ \mu g/kg/day}{UF_1 \times UF_2 \times UF_3 \times UF_4 \times UF_5 \times MF} \qquad (3)$$

where:

UF_1	=	10 (sensitive subpopulations)
UF_2	=	10 (animal to human extrapolation)
UF_3	=	3 (extrapolation from subchronic to chronic exposures).
UF_4	=	1 (LOAEL to NOAEL extrapolation)
UF_5	=	3 (data base incomplete)
MF	=	3 (modifying factor).

A total uncertainty factor of 1000 was applied, accounting for protection of sensitive subpopulations (10), subchronic-to-chronic extrapolation (3), animal-to-human extrapolation (10), and lack of a complete data base (3). In addition, a Modifying Factor of 3 was used because the RfD was based on a non-oral study.

An uncertainty factor of 10 for sensitive subpopulations is considered necessary because some individuals have a genetic defect causing their blood cholinesterase activity to be abnormally low (Evans et al., 1952; Harris and Whittaker, 1962). For homozygous individuals, the activity can be as low as 8–821% of the normal mean (Bonderman and Bonderman, 1971). These individuals may be unusually sensitive to organophosphate anticholinesterase compounds (Morgan, 1989).

The standard uncertainty factor of 10 is used for animal-to-human extrapolation because there is no evidence to suggest that humans are less sensitive to GA than animals.

An uncertainty factor of 3 is used to extrapolate from a subchronic to chronic exposure. In the derivation of oral RfDs for other organophosphate compounds, EPA has used NOAELs for cholinesterase inhibition following short-term exposures without adjustment for a more prolonged exposure period because of the unlikelihood that the endpoint would change over time (i.e., a subchronic-to-chronic UF of 1 was used). In addition, animal data for other organophosphate cholinesterase inhibitors such as agent VX indicate that maximum ChE inhibition usually occurs 30–60 days after exposure begins and then levels off or even shows signs of recovery. However, an uncertainty factor of 3 is used here because chronic studies are not available to verify that additional effects would not occur following chronic exposures.

The data base for GA consists of two subchronic toxicity studies in rats, teratology studies in two species (rats and rabbits), and delayed neuropathy studies in chickens. These studies generally support the use of cholinesterase inhibition as the critical endpoint for deriving an oral RfD. Deficiencies in the data base include the lack of a multi-generation reproductive toxicity study, a standard toxicity study in a second species, and toxicity studies by the oral exposure route. Because studies on other organophosphate cholinesterase inhibitors, including a multi-generational study on agent VX, indicate that reproductive effects are unlikely, a full Uncertainty Factor of 10 is not considered necessary for data base deficiencies.

The principal study involved a non-oral exposure route (intraperitoneal) and required route-to-route extrapolation using acute toxicity data. Because of uncertainties associated with the use of this nonstandard methodology, a Modifying Factor of 3 was applied to the RfD.

Therefore:

$$\text{RfD} = \frac{115\ \mu\text{g/kg/day}}{10 \times 10 \times 3 \times 1 \times 3 \times 3} \quad (4)$$

$$\text{RfD} = 0.04\ \mu\text{g GA/kg body weight/day} \quad (5)$$

4.3 Overall Confidence in the Oral RfD

Study: Medium
Data Base: Low
RfD: Low

The data base for GA consists of intraperitoneal and subcutaneous subchronic studies in rats, teratology studies in rats and rabbits, and delayed neuropathy studies in rats and chickens. Deficiencies in the data base include the lack of a multi-generation reproductive toxicity study, a standard toxicity study in a second species, and adequate toxicity studies by the oral exposure route. Although well-designed and well-conducted, the principal study involved a non-oral (intraperitoneal) exposure route. Consequently, overall confidence in the RfD is low.

4.4 Comparison of the Oral RfD with Human Toxicity Data

There are no human data available for GA for the oral exposure route. The intravenous LD_{50} was estimated to be 0.014 mg/kg (Robinson, 1967). Inhalation data indicate that severe effects would occur

at a concentration of 50 mg-min/m^3 (Reutter, unpublished data). The LCt_{50} is 135 mg min/m^3 for time periods of 0.5–2.0 min (DA, 1974). DHHS (1988) has set an inhalation maximum control limit of 0.000003 mg/m^3 for the general public (72 hr time-weighted average).

5. CARCINOGENICITY ASSESSMENT

The potential carcinogenicity of GA cannot be determined. Data are inadequate for performing a quantitative assessment of agent GA.

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APPENDIX A COMPARISON OF RFDS, CHE INHIBITION AND TOXICITY DATA FOR GA, GB, GD AND VX

Endpoint	GA (μg/kg/day)	GB (μg/kg/day)	GD (μg/kg/day)	VX (μg/kg/day)	Ref.
RfD	0.04	0.02	0.004	0.0006	This report
Estimated no-effect level for RBC-AChE inhibition	-	1.0	-	0.24	**GB**[d] **VX**[a]
27–33% inhibition of RBC-AChE in humans/oral dose	-	2.3 (3 days)	-	0.2–2.0	**GB** - Grob and Harvey, 1958; **VX**-this report
RBC-AChE inhibition in humans/ i.v. dose	-	-	1.5–2.0 (30%)	1.0 (50%)	DA, 1974; Sidell and Groff, 1974
50–60% RBC-AChE inhibition in humans/oral dose	-	10	-	2.4	**GB** - Grob and Harvey, 1958; **VX** - Sidell and Groff, 1974
50% brain ChE inhibition *in vitro*	1.5×10^{-8} (c)	0.3×10^{-8} (c)	-	-	Grob and Harvey, 1958
Acute toxic effects in humans/oral dose	-	20–30	-	2–4.5	**GB** - Thienes and Haley 1972; Grob and Harvey, 1958; **VX** - Sidell and Groff, 1974
human oral LD_{50} (estimated)	25–50[b]	5–20[b]	5–20	3–10[b]	Somani et al., 1992
rat oral LD_{50}	3700	800–1060 600	400	77–128	DA, 1974 Grob & Harvey, 1958
monkey i.v. LD_{50}	50	20	-	6–11	DA, 1974
rat i.v. LD_{50}	70	45–63	50	6.9–10.1	Dacre, 1984
rat i.p. LD_{50}	490, 800	250 218	-	37–55	DA, 1974 RTECS, 1995

[a] Based on ratio of oral to i.v. doses (2.4 and 1.0 μg/kg, respectively) required for 50% Rbc-ChE inhibition and the estimated i.v. no effect dose of 0.1 μg/kg.
[b] Values were estimated from animal data.
[c] Molar concentration
[d] Estimated from RBC-ChE_{50} values for GB and VX.

APPENDIX B STATISTICAL ANALYSIS OF GA-INDUCED RBC-ACHE INHIBITION IN RATS (BUCCI ET AL., 1992)

GA - RBC-AChE Inhibition in Female Rats (ANOVA and Dunnett's)

	Week				
I.P. Dose[a] (μg/kg/day)	-1	1	3	7	13
0	←/↑[b]	ns/	ns/	ns/	ns/
28.13	_/ns	S/ns	ns/ns	S/ns	ns/ns
56.25	_/ns	S/ns	ns/ns	S/ns	ns/ns
112.5	_/ns	S/ns	S/ns	S/ns	ns/ns

[a] Six animals/dose group
[b] Comparison to pre-exposure value (week -1)/comparison to control (0 μg/kg/day) value.
ns. Not statistically significant.
S. Statistically significant at $p < 0.05$.

GA - RBC-AChE Inhibition in Male Rats (ANOVA and Dunnett's)

	Week				
I.P. Dose[a] (μg/kg/day)	-1	1	3	7	13
0	←/↑[b]	ns/	ns/	S/	ns/
28.13	_/ns	S/ns	ns/ns	ns/ns	ns/ns
56.25	_/ns	S/ns	ns/ns	ns/ns	ns/ns
112.5	_/ns	ns/ns	ns/ns	ns/ns	ns/ns

[a] Six animals/dose group
[b] Comparison to pre-exposure value (week -1)/comparison to control (0 μg/kg/day) value.
ns. Not statistically significant.
S. Statistically significant at $p < 0.05$.

APPENDIX C STATISTICAL ANALYSIS OF GA-INDUCED PLASMA-CHE INHIBITION IN RATS (BUCCI ET AL., 1992)

GA - Plasma-ChE Inhibition in Female Rats (ANOVA and Dunnett's)

	Week				
I.P. Dose[a] (μg/kg/day)	-1	1	3	7	13
0	←/↑[b]	ns/	ns/	ns/	ns/
28.13	_/ns	ns/S	ns/S	ns/S	S/ns
56.25	_/ns	ns/S	ns/S	ns/S	ns/ns
112.5	_/ns	ns/S	ns/S	ns/S	ns/ns

[a] Six animals/dose group
[b] Comparison to pre-exposure value (week-1)/comparison to control (0 μg/kg/day) value.
ns. Not statistically significant.
S. Statistically significant at p <0.05.

GA - Plasma-ChE Inhibition in Male Rats (ANOVA and Dunnett's)

	Week				
I.P. Dose[a] (μg/kg/day)	-1	1	3	7	13
0	←/↑[b]	ns/	ns/	ns/	ns/
28.13	_/ns	ns/ns	ns/ns	ns/ns	ns/ns
56.25	_/ns	S/ns	S/S	S/S	S/ns
112.5	_/ns	S/S	S/S	S/S	ns/ns

[a] Six animals/dose group
[b] Comparison to pre-exposure value (week -1)/comparison to control (0 μg/kg/day) value.
ns. Not statistically significant.
S. Statistically significant at p <0.05.

Appendix B

Health Risk Assessment for The Nerve Agent GB

HEALTH RISK ASSESSMENT FOR THE NERVE AGENT GB

DRAFT REPORT

September 1996

(editorial corrections made April 1997)

Prepared for

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Prepared by

Life Sciences Division

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Oak Ridge, Tennessee 37831

Submitted to

Material/Chemical Risk Assessment Working Group

Advisory and Coordinating Committee Environmental Risk Assessment Program

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PREFACE

This report assesses the potential non-cancer and cancer effects of chemical agent GB (CAS No. 107-44-8).

This document supports the activities of the Material/Chemical Risk Assessment Working Group of the Environmental Risk Assessment Program, a cooperative endeavor of the Department of Defense, Department of Energy, and Environmental Protection Agency. This working group is developing toxicity values for selected chemicals of concern at federal facilities. Toxicity values will be submitted for consideration by the EPA's IRIS Consensus Process for inclusion on IRIS (EPA's Integrated Risk Information System). The Material/Chemical Risk Assessment Working Group consists of Drs. Jim Cogliano (chair) and Harlal Choudhury (U.S. EPA), Dr. Bruce Briggs (Geo-Centers); Lt. Cmdr. Warren Jederberg and Dr. Robert L. Carpenter (U.S. Naval Medical Research Institute); Dr. Elizabeth Maull and Mr. John Hinz (U.S. Air Force Occupational and Environmental Health Directorate); Drs. Glenn Leach and Winnie Palmer (U.S. Army Center for Health Promotion and Preventive Medicine); Drs. Robert Young and Po-Yung Lu (Oak Ridge National Laboratory).

This document was written by Dr. Dennis M. Opresko, Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN. Internal peer review was provided by Dr. Robert Young, Dr. Annetta Watson, and Mr. Robert Ross. External review of the toxicity data was provided by Dr. Thomas J. Bucci, Integrated Services, White Hall, AR and Dr. I.K Ho of the U. of Mississippi Medical Center, Jackson MS. External review of the derivation of the RfDs was provided by Drs. Michael Dourson and Susan Velazquez of Toxicology Excellence for Risk Assessment, Cincinnati, OH, and Dr. William Hartley of Tulane Medical Center, New Orleans LA. Additional reviews were provided by Mr. Joe King, Dr. Jack Heller, Ms. Veronique Hauschild, Ms. Bonnie Gaborek, Mr. Maurice Weeks, Maj. Robert Gum, and Mr Kenneth Williams of the U.S Army.

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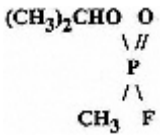
1. INTRODUCTION

Military nerve agents are organophosphate compounds containing either a fluorine, sulfur, or cyanide substituent group (Dacre, 1984). GB contains a fluorine substituent group (GA contains a cyanide substituent group and VX a sulfur group). The chemical synonyms, Chemical Abstract Service (CAS) and Army identification numbers (DA, 1974, 1992; Dacre, 1984), and chemical formula for GB are as follows:

Phosphonofluoridic acid, methyl-, 1-methylethyl ester
Phosphonofluoridic acid, methyl-, isopropyl ester
Isopropoxymethylphosphoryl fluoride;
Isopropyl methylfluorophosphate;
Isopropyl methanefluorophosphonate;
O-Isopropyl methylphosphonofluoridate;
O-Isopropyl methylisopropoxyfluorophosphine oxide;
Isopropyl-methyl-phosphoryl fluoride:
Isopropoxymethylphosphonyl fluoride;
Methylphosphonofluoridic acid isopropyl ester;
Methylfluorophosphonic acid, isopropyl ester;
Methylphosphonofluoridic acid 1-methylethyl ester;
Methylisopropoxyfluorophosphine oxide;
Sarin
CAS No. 107-44-8;
Edgewood Arsenal No. 1208



1.1 PHYSICAL/CHEMICAL PROPERTIES

Agent GB is a colorless liquid with a molecular weight of 140.1 (DA, 1974, MacNaughton and Brewer, 1994); it has a vapor density of 4.8 (air = 1) and a liquid density of 1.09 g/mL at 25°C (DA, 1974). The vapor pressure of GB is 2.9 mm Hg at 25°C. It is miscible with water and readily soluble in organic solvents (DA, 1974).

1.2. ENVIRONMENTAL FATE

1.2.1. Air

GB is very volatile with a vapor pressure of 2.9 mm Hg at 2519MMacNaughton and Brewer, 1994). A vapor concentration of 22 g/m^3 has been reported for a temperature of 25°C (DA, 1974) (although not adequately described in the reference, this presumably is the saturation concentration above a pure liquid). No information was found on the atmospheric half-life of GB.

1.2.2 Water

GB is completely miscible with water (DA, 1974). Its rate of hydrolysis is dependent on temperature, pH, and other water quality parameters (Epstein, 1974; Morrill et al., 1985; Clark, 1989). At 20°C, the half-life ranges from 461 hr at pH 6.5 to 46 hr at pH 7.5. At 25°C, the half-life is 237 hr at pH 6.5 and 24 hr at pH 7.5. GA is much more persistent at low temperature; at 0°C, its half-life is 8,300 hours at pH of 6.5. The rate of hydrolysis under natural conditions is accelerated by the presence of ions (dissolved solids) in solution. Metal cations such as copper and manganese in seawater increase the rate of hydrolysis (Epstein, 1974).

Based on an estimated Henry's Law Constant of 5.4×10^{-7} atm m^3/mol (MacNaughton and Brewer, 1994), evaporation of GB from water is expected to be slow.

1.2.3 Soil

According to Morrill et al. (1985), evaporation is the primary mechanism for the loss of GB from soil, and this is supported by the estimated volatility potential (slope of the vapor pressure vs. concentration in soil organics) of 4.9×10^{-8} mm Hg/mg/kg and by the air-soil partition coefficient of 135×10^{-5} mg/m^3 (for a soil density of 1.4 g/cm^3) as reported by MacNaughton and Brewer (1994). In a field test conducted in Finland detectable concentrations of GB ($\geq$1 pg/dm^3) were found in the air for up to 9 days following application of 10 mg of GB over a 10 × 10 meter area of moss (temperature 2.5–8 °C, humidity 60–100%, wind speed 1–10 m/s) (Sanches et al., 1993).

Studies conducted with soil samples from Dugway Proving Ground and Edgewood Arsenal showed that 90% of GB added to soil and maintained in closed containers at room temperature (20–25°C) was lost in the first 5 days (Small, 1984).

Binding of GB to soil organics is likely to be limited considering the relatively low log K_{ow} of 0.72 and low K_{oc} value of 59 (MacNaughton and Brewer, 1994); therefore, there is a potential for leaching and groundwater contamination. MacNaughton and Brewer (1994) calculated a leaching index of 3.7 for GB, (i.e., the number of leachings required to reduce the GB soil concentration to one-tenth of the original amount, assuming that for each leaching one kilogram of soil is in equilibrium with one liter of water). However, the amount reaching ground water is likely to be limited by hydrolysis.

2. MECHANISM OF ACTION

Nerve agents are inhibitors of acetylcholinesterase (AChE), an enzyme responsible for deactivating the neurotransmitter acetylcholine at some neuronal synapses and myoneural junctions. By a mechanism of phosphorylation, nerve agents act as substrates for the enzyme, thereby preventing deactivation of acetylcholine. The organophosphate-inhibited enzyme can be reactivated by dephosphorylation, but this occurs at a rate that is slower than the rate of reactivation of acetylcholine. Consequently, there is a depletion of acetylcholinesterase and a buildup of acetylcholine. In addition, the nerve agent-enzyme complex can also undergo an "aging" process (thought to be due to a loss of an alkyl or alkoxy group), whereby it becomes resistant to dephosphorylation (see review by Munro et al., 1994). Differences in rates of aging and reactivation may be important in evaluating toxicity data especially when extrapolating from animal studies to humans. *In vitro* tests conducted by Grob and Harvey (1958) indicate that both GA and GB combine with cholinesterase almost irreversibly during the first hour of their reaction. Sidell and Groff (1974) reported that the GB-ChE complex ages very rapidly *in vivo,* with 45–70% completion by 5 hours after infusion. In contrast, the complex formed between ChE and the nerve agent VX does not age significantly, and the rate of spontaneous reactivation can be as fast as 1%/hr in humans (Sidell and Groff, 1974).

2.1 Effects of Organophosphate Agents on the Nervous System

The anticholinesterase effects of the organophosphate nerve agents can be characterized as being muscarinic, nicotinic, or central nervous system (CNS)-related. Muscarinic effects occur in the parasympathetic system (bronchi, heart, pupils of the eyes; and salivary, lacrimal and sweat glands) and result in signs of pulmonary edema, bradycardia, miosis, tearing, and sweating. Nicotinic effects occur in somatic (skeletal/motor) and sympathetic systems, and result in muscle fasciculation, muscle weakness, tachycardia, and diarrhea. Effects on the CNS by organophosphates are manifested as giddiness, anxiety, emotional lability, ataxia, confusion, and depression (O'Brien, 1960).

Although the inhibition of cholinesterase within neuro-effector junctions or the effector itself is thought to be responsible for the major toxic effects of organophosphate agents, these compounds can apparently affect nerve-impulse transmission by more direct processes as well. Direct effects may occur on excitable tissues, receptors, and ionic channels. According to Somani et al. (1992), the direct action of nerve agents on nicotinic and muscarinic ACh receptors may occur when concentrations in the blood rise above micromolar levels, whereas at lower levels the action is mainly the result of inhibition of AChE. Albuquerque et al. (1985) have shown that agent GA, as well as agents GB and GD are capable of changing receptor sites in a manner similar to that exhibited by acetylcholine, which promotes the conductance of electrophysiological signals associated with stimulation of neuromuscular function. VX "may directly affect a small population of muscarinic ACh receptors that have a high affinity for [^{3}H]-*cis*-methyldioxalane binding" (Somani et al., 1992). VX may also counteract the effects of ACh by acting as an open channel blocker at the neuromuscular junction, thereby interrupting neuromuscular function (Rickett et al., 1987).

Exposure to some organophosphate cholinesterase inhibitors results in a delayed neuropathy characterized by degeneration of axons and myelin. This effect is not associated with the inhibition of acetylcholinesterase, but rather with the inhibition of an enzyme described as neuropathy target esterase (NTE); however, the exact mechanism of toxicity is not yet fully understood (Munro et al., 1994). For some organophosphate compounds, delayed neuropathy can be induced in experimental animals at relatively low exposure levels, whereas for others the effect is only seen following exposure to supralethal

doses when the animal is protected by antidotes from the acute toxic effects caused by cholinesterase inhibition.

Although there is the potential for nerve agents to have direct toxic effects on the nervous system, there is no evidence that such effects occur in humans at doses lower than those causing cholinesterase inhibition. For the purpose of evaluating potential health effects, inhibition of blood cholinesterase is generally considered the most useful biological endpoint.

2.2 Effect on Blood Cholinesterases

In addition to being found in the nervous system, acetylcholinesterase also occurs in the blood where it is bound to the surface of red blood cells (termed RBC-ChE). RBC-ChE activity, as well as the activity of a second type of cholinesterase found in blood plasma (butyrylcholinesterase, or plasma cholinesterase) have been used to monitor exposure to organophosphate compounds (pesticides and nerve agents). Both RBC-AChE and plasma-ChE have been used as bioindicators of potential toxic effects. There is some evidence that RBC-AChE is as sensitive as brain ChE to the effects of nerve agents. Grob and Harvey (1958) reported that the *in vitro* concentrations producing 50% depression of brain-ChE and RBC-AChE activity were the same in the case of GA (1.5×10^{-8} mol/L), and only slightly different (3×10^{-9} mol/L and 3.3×10 mol/L) in the case of GB. However, *in vivo* animal studies indicate a poor correlation between brain and RBC-AChE in cases of acute exposures (Jimmerson et al., 1989), and this is reflected in the fact that blood cholinesterase activity may not always be correlated with exposure or with signs and symptoms of toxicity. Acute exposures to high concentrations may cause immediate toxic effects before significant changes occur in blood ChE activity, and repeated exposures over a period of several days may result in a sudden appearance of signs and symptoms due to cumulative effects (Grob and Harvey, 1958). Conversely, blood ChE activity can become very low without overt signs or symptoms during chronic exposures to low concentrations of organophosphates. This may be due to a slower rate of recovery of RBC-ChE compared to tissue ChE, or to a noncholinesterase-dependent recovery pathway for neural tissue (Grob and Harvey, 1958). Sumerford et al. (1953) reported that orchard workers exposed to organophosphate insecticides had RBC-AChE values as low as 13% of average preexposure levels without any other signs or symptoms of toxicity. Animal studies have demonstrated that chronic exposures to low concentrations of organophosphate insecticides can also result in increased tolerance levels (Barnes, 1954; Rider et al., 1952; Dulaney et al., 1985). Similarly, Sumerford et al. (1953) reported increased levels of tolerance to organophosphate insecticides in people living near orchards subject to insecticide applications. Such adaptation may result from increased rates of formation of blood ChE, or from increased rates of detoxification. Additional information on the development of tolerance to organophosphate cholinesterase inhibitors can be found in a review paper by Hoskins and Ho (1992).

The blood cholinesterases and other esterases may, to some degree, provide a protective effect by binding with some fraction of the anticholinesterase compound (Wills, 1972). However, not all nerve agents bind equally well with all cholinesterases. Agent GB inhibits both RBC-ChE (80–100%) as well as plasma-ChE (30–50%) (Grob and Harvey, 1958). In contrast, agent VX preferentially inhibits RBC-ChE (70% compared with about 20% inhibition of plasma ChE) (Sidell and Groff, 1974). Rodents (but not humans) have other enzymes in the blood, termed aliesterases, which can bind to organophosphates, thereby reducing the amount available for binding with acetylcholinesterase (Fonnum and Sterri, 1981). Agent GB binds with aliesterases; however, according to Fonnum and Sterri (1981), VX has a quaternary ammonium group which prevents it from being a substrate for aliesterases. The strong specificity of agent VX to AChE may account, in part, for the fact that it is much more acutely toxic than agents GA and GB (see Appendix A).

2.2.1 Intra- and Interspecies Variation in Blood Cholinesterase Activity

Although blood cholinesterase activity is used as a measure of exposure to organophosphate compounds, baseline activity levels can vary between individuals and between species. According to Wills (1972), both plasma- and RBC-ChE activity are generally lower in women than in men. Sidell and Kaminskis (1975) reported that, for a test population of 22 human subjects, the highest coefficient of variation of RBC-ChE was 4.1% per single subject; the average range of variation was ± 2.1% for men and ± 3.1% for women. In individuals studied for one year, the RBC-ChE activity varied by 11% in men and 16% in women. Yager et al. (1976) reported a 10.0% intra-individual coefficient of variation for RBC-ChE and 14.4% for plasma-ChE. Callaway et al. (1951) estimated that with only one pre-exposure measurement, the smallest measurable decrease was 15% of the baseline value for RBC-ChE activity and 20% of the baseline for plasma-ChE.

A small subpopulation of men and women have a genetic defect causing their blood cholinesterase activity to be abnormally low (Evans et al., 1952; Harris and Whittaker, 1962). For homozygous individuals, the activity can be as low as 8–21% of the normal mean (Bonderman and Bonderman, 1971). Morgan (1989) suggests that these individuals may be unusually sensitive to organophosphate anticholinesterase compounds.

Data compiled by Ellin (1981) reveal that the RBC-ChE activity for humans is slightly higher than that for monkeys and much higher than that for rats and other laboratory animals (Table 1).

Table 1. RBC-ChE activity in different species

Species	**RBC-ChE activity (μmol/mL/min)**	**Optimum substrate**[a] concentration (M)
Human	12.6	2×10^{-3}
Monkey	7.1	2×10^{-3}
Pig	4.7	1×10^{-3}
Goat	4.0	2×10^{-3}
Sheep	2.9	2×10^{-3}
Mouse	2.4	2×10^{-3}
Dog	2.0	2×10^{-2}
Guinea pig	2.7	2×10^{-3}
Rabbit	1.7	5×10^{-3}
Rat	1.7	5×10^{-3}
Cat	1.5	5×10^{-3}

Source: Ellin, 1981

[a] Acetylthiocholine iodide concentration for maximum RBC-ChE activity.

These differences in RBC-ChE activity may affect a species' sensitivity to a particular organophosphate compound. At the same time, the relative amount of plasma cholinesterase and other compounds in the blood that can bind to the organophosphate agents must also be considered. For example, rodents, but

not humans, have high levels of aliesterases (AE) in the blood, and these compounds may provide rats and mice with a higher level of resistance to some anticholinesterase compounds (McNamara and Leitnaker, 1971).

2.2.2 Potency of Nerve Agents as Cholinesterase Inhibitors

The potency of the anticholinesterase activity of nerve agents and other organophosphates is expressed by the bimolecular rate constant (k_i) for the reaction of the phosphate compound with the enzyme and by the molar concentration causing 50% inhibition of the enzyme (I_{50}). The relationship between I_{50} and k_i as a function of time (t) is expressed by the following equation (Eto, 1974):

$$I_{50} = \frac{0.693}{t \times k_i} \quad (1)$$

I_{50} data for several organophosphate nerve agents have been tabulated by Dacre (1984). The pI_{50} (negative log of the molar concentration causing 50% inhibition) for GB was reported to be 8.8 by Tammelin (1958) and 8.9 by Dacre (1984), and calculated as 8.5 from an I_{50} of 3.7×10^{-9} mol/L reported by Grob and Harvey (1958).

The potency of nerve agents can also be expressed in terms of the dose necessary to produce 50% inhibition of cholinesterase (ChE_{50}). In humans, RBC-ChE_{50} values for GB are 0.003 mg/kg and 0.01 mg/kg, respectively, for i.v. and oral doses (Grob and Harvey, 1958). The relative effectiveness of nerve agents in inhibiting cholinesterase is closely correlated with their acute toxicity (see Appendix A).

3. TOXICOLOGY

3.1 Introduction

Health and environmental impacts of nerve agents and related compounds (i.e., organophosphate insecticides) have been reviewed by O'Brien (1960), Matsumura (1976), Dacre (1984), Carnes and Watson (1989), Watson et al. (1989), and Munro et al. (1994). A brief general discussion of the toxicology of nerve agents and related organophosphate pesticides is given below.

Nerve agents are acutely toxic by all routes of exposure. Initial symptoms of acute poisoning are fatigue, headache, mild vertigo, weakness, and loss of concentration. Moderate exposures result in miosis and excessive sweating, tearing, and salivation. Acidosis and hyperglycemia may also occur in addition to muscular weakness, muscular twitching, lacrimation, urination, and defecation. Acute poisoning can result in prostration, clonic convulsions (rapid repetitive movements) and tonic convulsions (limbs stretched and rigid) (Matsumura, 1976). Exposures sufficiently high to cause convulsions have resulted in brain lesions and cardiomyopathy in laboratory animals (Singer et al., 1987).

In addition to the immediate toxicity of the nerve agents, there is concern that exposures may lead to chronic neurological effects similar to those reported for some organophosphate insecticides. Included among these possible effects are organophosphate-induced delayed neuropathy (OPIDN), EEG changes, and long-term psychological disturbances (Munro et al., 1994). OPIDN, which appears 5–30 days after

exposure, manifests itself as muscle weakness, tingling, and twitching followed by paralysis (Munro et al., 1994). Histopathological changes, which consist of degeneration of axons and myelin of the nervous system, can be correlated, not with inhibition of AChE, but rather with inhibition of NTE. There is no evidence that agent GB causes OPIDN in humans. There are some data indicating that GB can induce this effect in chickens but only at supralethal dose levels when the animals are protected from immediate toxic effects by pretreatment with antidotes (see section 3.5). Neuropathological changes suggestive of OPIDN have also been observed in one study on mice exposed to GB; but the exposure levels were relatively high, and such effects were not reported in other studies on rodents (see section 3.5). The overall data indicate that GB is not likely to cause OPIDN in humans at exposure levels below those causing acute toxicity or cholinesterase inhibition.

Acute exposures to nerve agents are known to cause EEG changes (Grob and Harvey, 1958; Sidell, 1992) which may persist for long periods of time after exposure (Metcalf and Holmes, 1969; Burchfiel et al., 1976; Duffy et al., 1979; Duffy and Burchfiel, 1980); however, the reported changes have been considered to be clinically insignificant and not correlated with behavioral or physiological changes (DHHS, 1988). Acute exposures can also induce neuropsychological changes; however, there is no evidence of these effects persisting for months or years as has been reported for some organophosphate insecticides (Savage et al., 1988; Gershon and Shaw, 1961; Mick, 1974; Rodnitzky, 1974; Wagner, 1983; Tabershaw and Cooper, 1966). The available data for the organophosphate insecticides suggest that chronic neuropsychological effects (excluding OPIDN) do not occur in the absence of significant changes in blood cholinesterase. The same conclusion may apply to the organophosphate nerve agents.

3.2 Acute Toxicity

In tests on humans, Grob and Harvey (1958) found that a single oral dose of 0.022 mg GB/kg produced mild toxic effects including anorexia, nausea, heartburn, tightness in the stomach and chest, increased fatigue, nervous tension, anxiety, and other CNS responses including insomnia and excessive dreaming. An additional dose of 0.008 mg/kg within 8 hr resulted in moderate toxic effects including stomach cramps, vomiting, diarrhea, increased salivation and lacrimation, slightly decreased heart rate, and abnormal breathing. According to Thienes and Haley (1972), a single dose of 0.002 mg GB/kg caused excessive dreaming and talking during sleep and a dose of 0.020 mg/kg caused insomnia, excessive dreaming, withdrawal, and depression. At high exposures, brain damage may occur as a result of oxygen deprivation in brain tissue during GB-induced convulsions (Sidell, 1992).

Grob and Harvey (1958) reported that the first appearance of toxicity in humans occurred when RBC-ChE activity was depressed 88% (to 12% of the baseline value) following a single oral dose of GB. The single dose oral ChE_{50} value was reported to be 0.01 mg GB/kg, and the lethal oral dose was estimated to be 0.14 mg/kg. In comparison, the single dose intra-arterial ChE_{50} was reported to be 0.003 mg/kg, and the lethal intramuscular dose was estimated to be 0.03 mg/kg. Following i.v. administration, toxic effects occurred when RBC-ChE activity was depressed 40–50% (60-50% of baseline) indicating a more immediate effect on the nervous system than that caused by oral dosing (Grob and Harvey, 1958). LD_{50} data for selected species are shown in Table 2. Values for humans are estimates derived from animal data.

Table 2. Lethality data for agent GB

Exposure route	Species[a]	LD_{50} (μg/kg)	References
oral	human	71—285[b];	Somani et al., 1992;
	human	140[c]	Grob and Harvey, 1958
	monkey	—	—
	rat	550;	RTECS, 1995
	rat	600;	Grob and Harvey, 1958
	rat	870–1060	DA, 1974
dermal	human	28,000	RTECS, 1995
	human	24,000;	DA, 1974
	human	1,429–7,143[b]	Somani et al., 1992
	monkey	—	—
	pig	115,900	DA, 1974
	rat	2500	DA, 1974
	mouse	1080	RTECS, 1995
intravenous	human	14	DA, 1974
	monkey	20	DA, 1974
	pig	15	DA, 1974
	rat	39	RTECS, 1995
		45	DA, 1974
subcutaneous	human	—	—
	monkey	—	—
	rat	103–108	RTECS, 1995
intramuscular	human	30[c]	Grob and Harvey, 1958
	monkey	22	RTECS, 1995
	rat	170	Grob and Harvey, 1958
		108	RTECS, 1995
		112	DA, 1974
intraperitoneal	human	—	—
	monkey	—	—
	rat	218	DA, 1974
		250	RTECS, 1995

[a] Values for humans estimated from animal data
[b] Based on 70 kg body weight
[c] Lethal level

Grob and Harvey (1958) also administered to human volunteers multiple oral doses of GB over a period of 3 days (3–24 hr apart; average 7.5 hr). In two individuals, doses of 0.0005 or 0.005 mg/kg, totaling 0.007 mg/kg over the 3-day period, reduced RBC-ChE 33% and 27%, respectively, but neither produced toxic effects. Multiple doses of 0.008-0.016 mg/kg, totaling 0.088 mg/kg over the 3-day period, produced mild symptoms of toxicity. Similar incremental doses, totaling 0.102 mg/kg over 3 days, produced moderate symptoms of toxicity and >90% reduction in RBC-ChE activity. Grob and Harvey (1958) reported that exposure to GB had a cumulative effect that resulted in increased sensitivity to the chemical.

Bucci et al. (1991) and Bucci and Parker (1992) conducted range-finding studies with GB Type I (GB containing tributylamine as a stabilizer) and GB Type II (GB containing diisopropylcarbodiimide as a stabilizer). The chemicals were administered by gavage once per day, 5 days per week for 3 weeks. These studies indicated that for both GB mixtures, the maximum tolerated dose was 0.3 mg/kg/day and a dose of 0.5 mg/kg/day was lethal to the test animals.

3.3 Subchronic Toxicity

In a subchronic study conducted by the National Center for Toxicological Research (NCTR), male and female CD rats were administered GB Type I (GB containing tributylamine as a stabilizer) or GB Type II (GB containing diisopropylcarbodiimide as a stabilizer) by gavage at dose levels equivalent to 0, 0.075, 0.15, or 0.3 mg GB/kg/day (Bucci et al., 1991; Bucci and Parker, 1992). The doses were given once per day, 5 days per week for 13 weeks. All animals were observed daily for clinical signs of toxicity and weighed weekly. Necropsy examination was performed on all animals. Terminal body and organ weights were recorded. Microscopic evaluation was performed on all high-dose and control animals, and on those tissues of lower dose animals that were abnormal at necropsy Hematological analyses and clinical chemistry (including RBC and plasma cholinesterase) were evaluated in the same 6 male and 6 female rats in each dose group one week before the exposures began and also at weeks 1, 3, 7, and 13. In addition, at necropsy a hemisection of each brain was prepared and tested for NTE activity. In both studies there were several statistically significant changes in clinical chemistry (i.e., aspartate aminotransferse in mid-dose males exposed to GB Type II) and hematology (decrease at week 7 in white blood cells in high-dose males exposed to GB Type II and increase at week 13 in erythrocytes in mid-dose females exposed to GB Type II); however, these effects were not sufficiently consistent to suggest organ dysfunction. Brain NTE was not altered significantly in any rats dosed with GB Type II; however, it was significantly decreased ($p < 0.05$) in female rats dosed with 0.3 mg/kg/day GB Type I. The latter, however, did not exhibit any histological signs of delayed neuropathy. GB Type II was not associated with any neoplastic or non-neoplastic lesions. Two high-dose females and one low-dose female dosed with GB Type I had brain lesions consisting of necrosis and vacuolization of individual hippocampal pyramidal cells. It was reported by Bucci et al. (1991) that this type of lesion is consistent with hippocampal hypoxia resulting from the respiratory convulsant effects of GB; however, Bucci et al. (1991) also noted that post-mortem autolysis could have mimicked cerebral necrosis in two of the animals which were found dead. The third animal exhibited signs consistent with nerve agent toxicity (e.g., rapid breathing, salivation, lacrimation, hemorrhage in the urinary wall, and possible right forelimb paralysis), and the observed neural lesions were attributed to GB. This animal was in the test group receiving 0.075 mg/kg/day. As noted above, none of the rats dosed with up to 0.3 mg/kg/day of GB Type II exhibited brain lesions. GB Type I contains the stabilizer tributylamine and GB Type II contains diisopropylcarbodiimide. Subchronic and chronic toxicity data for these stabilizers are lacking and in terms of acute toxicity, the stabilizers have only a fraction of the toxicity of the nerve agents (e.g., the oral LD_{50} for tributylamine is 114 mg/kg in rats; RTECS, 1995). Although there is one report indicating that tributylamine is a CNS stimulant (Windholz et al., 1983), there is no evidence to suggest that it contributed to the neurotoxic effect seen in the GB Type I study.

In the NCTR studies, RBC cholinesterase levels in the dosed animals were compared to control values for the same sampling times (Tables 3–5). In both studies there were significant decreases in plasma and RBC-ChE levels at certain time periods. The results for the GB Type II study were more internally consistent than those for GB Type I; i.e., the control values did not vary as greatly and the test groups in general exhibited more clearly defined dose-related changes in enzyme activity. For GB Type II, plasma ChE activity in high- and mid-dose males at week 1; in high- and mid-dose females at weeks 1 and 7; and in high-dose females at week 3 was significantly lower than the corresponding control values for the same time periods (Table 5).

Table 3. RBC-ChE levels in 90-day subchronic study of GB Type I in CD ratsa

		Week of treatment								
Dose (μg/kg/d)	**Sex**	**-1**	**1**	**%[b]**	**3**	**%[b]**	**7**	**%[b]**	**13**	**%[b]**
0	F	2343(118)	1885 (196)	80	1765 (143)	75	3177 (118)	136	1533 (375)	65
75	F	2420(168)	1964 (121)	81	1584 (109)	65	2101(94)[c]	87	1265 (383)	52
150	F	2656(94)	1814 (188)	68	1867 (143)	70	2217 (158)[c]	83	1318 (288)	50
300	F	2518(127)	1564(99)	62	1557(97)	62	2061 (126)[c]	82	1673 (441)	66
0	M	2085(267)	1771 (205)	85	1954(97)	94	2346 (124)	113	1547 (285)	74
75	M	2219(114)	1886 (282)	85	1834(53)	83	1749(82)	79	1405 (166)	63
150	M	2444(177)	1835 (151)	75	1907 (104)	78	1917 (135)	78	1522 (173)	62
300	M	2632(218)	1921 (126)	73	1990(96)	76	2006(94)	76	1478(98)	56

Source: Bucci et al., 1991
[a] Values given as mean IU/L and (SEM)
[b] Percent of baseline
[c] p <0.05, different from control value (0 μg/kg)

Table 4. RBC-ChE levels in 90-day subchronic study of GB Type II in CD ratsa

		Week of treatment								
Dose (μg/kg/d)	**Sex**	**-1**	**1**	**%[b]**	**3**	**%[b]**	**7**	**%[b]**	**13**	**%[b]**
0	F	1728(174)	1777 (223)	103	1497 (182)	87	1828 (280)	106	2313 (285)	130
75	F	1603(149)	1405(67)	88	1545 (221)	96	1353 (135)	84	1753 (249)	109
150	F	1678(132)	1060(54)[c]	63	1085(62)	65	985(83)[c]	59	1553 (161)[c]	93
300	F	1653(75)	968(41)[c]	59	895(41)	54	865(87)[c]	52	1510(79)[c]	91
0	M	1118(20)	1032(40)	92	1270(42)	114	1002(76)	90	1097(54)	98
75	M	1180(78)	728(33)	62	910(47)[c]	77	748(96)[c]	63	992(72)	84
150	M	1097(46)	665(51)	61	782(73)[c]	71	602(36)[c]	55	835(78)[c]	76
300	M	1156(59)	612(25)	53	764(91)[c]	66	606(30)[c]	52	910(97)[c]	79

Source: Bucci and Parker, 1992
[a] Mean IU/L and (SEM)
[b] Percent of baseline (week -1).
[c] p <0.05, different from control value (0 μg/kg)

Inhibition of RBC-AChE was dose-related for females in the two highest dose groups and for males in all dose groups. Maximum RBC-ChE depression (48%) occurred in week 7 in both high-dose males and females. Male rats exposed to the lowest dose of GB Type II exhibited a 38% decrease in RBC-ChE activity in week 1; females exhibited a 12% decrease. By week 13, RBC-ChE activity levels

in females returned to near pre-exposure levels (>90%); however, levels in males were still depressed 16–24%.

The AChE data were re-analyzed by ORNL (using standard deviations) with ANOVA and Dunnett's Comparison (see Appendix B). This analysis indicated that RBC-AChE levels in males were significantly lower ($p < 0.05$) than baseline values in all dose groups at weeks 1, 3, and 7, and for the two highest dose groups at week 13. The values for all dose groups were also significantly lower than controls at week 1, 3, and 7. Similar results were seen in females except that the RBC-AChE levels were not significantly different from controls or baseline values in the low-dose group (Appendix B).

Table 5. Plasma ChE levels in 90-day subchronic study of GB Type II in CD ratsa

		Week of treatment								
Dose (μg/kg/d)	**Sex**	**-1**	**1**	**%[b]**	**3**	**%[b]**	**7**	**%[b]**	**13**	**%[b]**
0	F	1267(189)	1935 (304)	153	1461 (246)	115	1761 (353)	139	2100 (350)	166
75	F	1381(288)	1460 (206)	106	1483 (350)	107	1475 (188)	107	1574 (280)	114
150	F	1136(105)	705(104)[c]	62	736(53)	65	790(136)[c]	70	1280 (149)	113
300	F	1316(55)	611(73)[c]	46	475(58)[c]	36	481(95)[c]	36	1311 (146)	100
0	M	437(33)	578(59)	132	353(29)	81	254(15)	58	313(18)	72
75	M	461(25)	407(53)	88	296(22)	64	174(12)	38	312(25)	68
150	M	443(40)	239(26)[c]	54	229(9)	52	122(7)	28	246(13)	56
300	M	375(19)	249(52)[c]	66	187(15)	50	109(9)	29	257(4)	69

Source: Bucci and Parker, 1992

[a] Mean IU/L and (SEM)

[b] Percent of baseline (week -1).

[c] $p < 0.05$, different from control value (0 μg/kg)

In a subchronic inhalation study conducted on Fischer 344 rats, no signs of toxicity were observed in animals exposed to 0.0001 or 0.001 mg GB/m^3, 6 hr/day, 5 days/week, for up to 24 weeks (Weimer et al., 1979). Compared to the dose levels used in the subchronic oral studies described above, the exposures used in the Weimer et al. (1979) study were relatively low. For example, assuming an inhalation rate of 0.29 m^3/day and an average body weight of 0.35 kg for rats and 100% pulmonary absorption, the highest concentration in the Weimer et al. study would be equivalent to an internal dose of only 0.15 μg/kg/day.

3.4 Chronic Toxicity

There is limited information concerning the effects of GB following prolonged exposure to low concentrations. In a retrospective study of workers occupationally exposed to GB for one year or longer prior to the testing, increased brain beta activity, increased delta and theta slowing, decreased alpha activity, and increased amounts of REM (rapid-eye-movement) sleep were observed (Duffy et al., 1979;

Burchfiel and Duffy, 1982). DHHS (1988) considered these changes to be of questionable importance due to the absence of clinically significant neuropsychological effects.

There are no animal studies involving chronic oral exposures to GB. In chronic inhalation studies conducted by Weimer et al. (1979), I.C.R. Swiss and strain A mice, Sprague-Dawley/Wistar and Fischer 344 rats, and purebred beagle dogs were exposed to 0, 0.0001, or 0.001 mg GB/m^3, 6 hr/day, 5 days/week, for up to 52 weeks. Four male and 8 female beagles were exposed to each test concentration. In the rodent studies, 50 animals of each sex of each strain were exposed to each test concentration. The control groups were identical to the test groups except that an additional 100 F344 rats and A strain mice were used. Animals were sacrificed according to the schedule listed in Table 6. RBC-AChE activity levels were monitored throughout the study for all the test species. No dose-related, statistically significant changes in RBC-AChE occurred in any species at any sampling time. Using an inhalation rate of 0.29 m^3/day for rats, and assuming 100% pulmonary absorption, the 6 hr/day, 5 day/week exposure would correspond to an average daily dose of 0.00015 mg/kg. This dose is considerably below the gavage doses of 0.075 mg/kg/day that produced cholinesterase depression in the subchronic studies described in Section 3.3.

Table 6. Sacrifice schedule for GB chronic study

Species	**Number of animals sacrificed**								
	Months of exposure	**6-month post exposure**							
	1		**2**	**3**	**4**	**5**	**6**	**9**	**12**
Colony rats	10	10	10	10	10	10	10	10	20
Fischer 344 rats	—	—	20	—	—	20	20	20	20
Colony mice	10	10	10	10	10	10	10	10	20
A strain mice	—	—	20	—	—	20	20	20	20
Beagle dogs	2	2	2	—	—	2	2	2	—

Source: Weimer et al., 1979

Five of 20 dogs exhibited abnormal EKGs at the time of sacrifice; elevated P waves were suggestive of right atrial hypertrophy; however, there was no evidence of enlargement or physical abnormalities of the heart. The absence of pre-exposure data precludes identifying this effect as due to GB exposure. A higher incidence of tracheitis occurred in colony rats (a Sprague Dawley/Wistar population) and in Fischer rats exposed to GB in comparison to control animals (Table 7). The most severe cases occurred in the high-exposure group. The investigators could not determine whether the occurrence of tracheitis was agent-related. No other overt signs of GB-related toxicity were observed at either exposure level. Atrophy of the seminiferous tubules, starting at 12 weeks of exposure, was also seen in the Fischer rats. The investigators noted that this inbred strain of rat is susceptible to numerous genetically based defects which may appear under experimental conditions of stress. The tests were repeated using the same experimental protocol for 12 and 24 weeks. None of the rats in this second assay exhibited testicular atrophy.

Table 7. Incidence of tracheitis in colony rats

	Dose group		
Exposure period (wk)	**Control**	**0.0001 mg/m^3**	**0.001 mg/m^3**
4	0/10	5/10	0/10
8	0/10	4/10	9/10
12	0/10	5/8	5/7
16	0/9	0/10	1/10
20	0/10	0/5	2/6
24	1/10	1/5	1/10
36	0/9	2/5	2/7
52	2/20	1/20	6/20
6-month post-exposure	0/20	7/19	9/28
Totals	3/108	25/98	34/108

Source: Weimer et al., 1979

3.5 Nervous System Toxicity

As noted in Section 3.1, the neurotoxic effects following acute exposures to nerve agent such as GB can range from minor symptoms such as fatigue, headache, mild vertigo, weakness, and loss of concentration to convulsions, respiratory arrest, and death. In laboratory animals exposed to GB, single subcutaneous injections sufficiently high to cause convulsions resulted in brain lesions (Singer et al., 1987; see also McLeod, 1985). Brain lesions in the absence of convulsions have also been reported in rats dosed by gavage with 0.3 mg GB Type I/kg/day for 90 days (Bucci et al., 1991).

Exposures to nerve agents have also been associated with subtle neurological effects manifested as altered EEGs (Metcalf and Holmes, 1969; Burchfiel et al., 1976; Duffy et al., 1979; Duffy and Burchfiel, 1980) and psychological changes (Sidell, 1992). The reported EEG abnormalities have not been correlated with behavioral or physiological parameters.

Organophosphate-induced delayed neuropathy (OPIDN) has not been observed in humans exposed to acutely toxic levels of GB (Munro et al., 1994), nor in cats receiving single supralethal doses or multiple low doses of GB for up to 10 days (Goldstein et al., 1987; Goldstein, 1989). In subchronic rat studies, Bucci et al. (1991) found that male and female rats receiving 0.3 mg GB Type I/kg/day for 90 days had reduced brain NTE activity levels (significant at the $p < 0.05$ level in females) but no histopathological signs of OPIDN. Decreases in brain NTE were not seen in a related study in which rats were dosed with the same amount of GB Type II (Bucci and Parker, 1992)). However, signs suggestive of OPIDN have been observed in female Swiss albino mice exposed to 5 mg GB/m^3 for 20 min daily for 10 days (Husain et al., 1993). Muscular weakness of the limbs and slight ataxia occurred on the 14th day after the start of the exposures. These changes were accompanied by significant ($p < 0.001$) inhibition of NTE activity in the brain (59.2%), spinal cord (47.4%), and platelets (55.4%). Histological examination

of the spinal cord revealed focal axonal degeneration which was reported to be moderate in two animals and light in four. The same exposure inhibited AChE in blood by 27.3% and in brain by 19.2% but was not associated with any anti-AChE symptoms. Some studies have also demonstrated that GB can induce OPIDN in chickens, but only at supralethal doses. Davies et al. (1960), Davies and Holland (1972) and Gordon et al. (1983) reported that signs of OPIDN appeared in chickens dosed with 20 and 30 times the LD_{50} if the animals were protected from acute toxic effects by the use of antidotes such as atropine and oxime compounds. However, Bucci et al. (1992a) reported that White Leghorn chickens (atropine-protected) given single oral doses of 70.2, 140.4, or 280.7 μg/kg GB Type II exhibited no clinical signs of OPIDN such as ataxia when evaluated 8 to 43 days after the treatment. At sacrifice, samples of nervous tissue were examined microscopically, but showed no evidence of OPIDN pathology. In a related study conducted by Bucci et al. (1992a), hens were administered the same total doses of GB but in one-third increments given one week apart. There were no signs of neuropathology when the animals were sacrificed 43 days later. In a third study, Bucci et al. (1992a) evaluated the effects of GB on NTE in hens given single doses of GB Type II ranging from 70.2 to 750 μg/kg. Bucci et al. (1992a) reported that under the conditions of the test GB Type II did not cause a significant dose-related change in NTE in the brain or spinal cord.

3.6 Developmental and Reproductive Effects

No data are available to evaluate the potential reproductive and developmental effects of GB in humans; however, studies in laboratory animals indicate that such effects are not likely even at dose levels that are maternally toxic. LaBorde and Bates (1986) conducted developmental toxicity studies on agent GB Type I and GB Type II using CD rats and New Zealand rabbits. In the rat studies, the test animals were dosed with 0, 100, 240, or 380 μg/kg of GB orally on days 6–15 of gestation. Females were weighed on gestational days 0, 6–16 and prior to sacrifice on gestational day 20. The test animals were observed for clinical signs of toxicity. At sacrifice, gravid uteri were weighed and examined for number and status of implants (alive, resorbed or dead). Individual fetal body weight and internal or external malformations were recorded. Maternal toxicity (evidenced by excessive salivation, ataxia, lacrimation) and mortality (8/29 for GB Type I and 13/29 for GB Type II) occurred in the high-dose group. There were no significant differences among treatment groups in the incidence of resorptions or in the average body weight of live fetuses per litter. The only fetal morphological anomaly was fetal hydroureter which occurred at a rate of 5.2, 1.9, 5.3 and 2.1% with GB Type I and 4, 5, 3.2, and 0.5% with GB Type II in the 0, 100, 240, and 300 μg/kg dose groups, respectively. The observed effect was not dose-related and was, therefore, considered to be a spontaneous variant. Skeletal and cartilage variants occurred between dose group but these were not statistically significant. In similar studies conducted on New Zealand rabbits using the same experimental protocol, oral doses of 0, 5, 10, or 15 μg/kg/day on gestational days 6–19, resulted in no fetal toxicity or teratogenicity (Laborde and Bates, 1986). The only observed fetal anomaly was retinal folding which occurred at a rate of 6.8, 3.9, 4.3, and 7.4% for GB Type I and 17, 18, 25, and 19% for GB Type II in the 0, 5, 10, and 15 μg/kg dose groups, respectively. The frequency of the anomaly was not dose-related and the variant was, therefore, considered to be a spontaneously occurring malformation. Maternal toxicity, evidenced by excessive salivation, ataxia, and lacrimation, occurred at the highest dose.

The developmental toxicity of GB was also evaluated by Denk (1975). In this study Sprague-Dawley rats were exposed to GB vapors (0.1 and 1 $\mu g/m^3$) for 6 hr/day, 5 days/wk, for varying time periods. In one series of tests, male rats were exposed for 1 week to 1 yr, and then mated to unexposed females. Nineteen days after mating, the females were sacrificed and examined for number of corporalutea, deciduomata, number of fetal deaths, and number of live fetuses. In a second series of tests mated pairs of rats were exposed to GB for 1, 2, or 3 weeks or until the pups were whelped. The incidence of

intrauterine deaths was recorded and all fetuses were examined for abnormalities. In a third series of tests, males and females were exposed to GB for 10 months and then mated. The F_1 generation was mated, as was the F_2 generation. The number and sex of offspring, number of preweaning deaths, number weaned, and pup weights at various ages were recorded. Denk (1975) reported that GB, at the doses and by the route used, had no adverse effects with respect to dominant lethal mutations, reproductive performance, fetal toxicity, and teratogenesis.

3.7 Carcinogenicity

There are no human data to suggest that GB is carcinogenic. As part of chronic inhalation studies conducted by Weimer et al. (1979) (see section 3.4), the tissues of animals exposed to GB for up to one year were examined for microscopic lesions including tumors. The test species included I.C.R. Swiss mice, strain A mice, Sprague-Dawley/Wistar rats, Fischer 344 rats, and purebred beagle dogs. The exposures were to 0.0001 or 0.001 mg GB/m^3, 6 hr/day, 5 days/week. Weimer et al. (1979) reported that agent-related tumors did not occur in any of the exposed species. Pulmonary tumors did occur in strain A mice; after 52 weeks of exposure, pulmonary adenomas were present in 3/19 animals exposed to 0.0001 mg GB/$m^{3'}$ in 3/20 animals exposed to 0.001 mg GB/m^3, and in 0/20 controls; and for animals maintained for 6 months post-exposure, the incidence rates for pulmonary adenocarcinomas were 5/19, 6/18, and 9/29, respectively. However, these lesions were not considered to be agent-related. Strain A mice have a high natural propensity to form pulmonary tumors; the incidence of spontaneous pulmonary tumors being about 53% in animals 12 months of age and 90% in animals 18 months of age (Heston, 1942). Overall, the studies of Weimer et al. (1979) indicate that agent GB is not carcinogenic.

3.8 Genotoxicity

No information is available regarding the genotoxicity of GB in humans. In bioassays using bacteria and mammalian cell cultures, GB was not genotoxic or mutagenic when tested with or without metabolic activation (Goldman et al., 1987). GB did not induce biologically significant increases in mutations when tested in the Ames Salmonella assay using five revertant strains (TA135, TA100, TA98, TA1537, and TA1538) (Goldman et al., 1987). GB Type I and GB Type II did not induce a significant increase in forward mutations when tested on mouse L5178Y lymphoma cells at concentrations of 50, 100, or 200 μg/mL (Goldman et al., 1987). An increase in sister chromatid exchanges (SCE) was not observed in Chinese hamster ovary cells exposed *in vitro* to 200 µg/mL of GB (Goldman et al., 1987). Mice treated *in vivo* with a maximally tolerated intraperitoneal dose of 360 µg GB/kg did not exhibit a significant increase in SCE in splenic lymphocytes (Goldman et al., 1987). Exposure of rat hepatocytes to GB concentrations as high as 2.4×10^{-3} M resulted in a decrease in DNA repair synthesis, leading Goldman et al. (1987) to conclude that GB probably did not damage DNA directly but that it might inhibit DNA synthesis after non-agent-induced DNA damage had occurred.

4. ORAL REFERENCE DOSE FOR GB

4.1 Cholinesterase Inhibition as an RfD Endpoint

The endpoint for identifying a no-observed-adverse-effect level (NOAEL) for nerve agents such as GB is the level at which there is no significant depression in blood cholinesterase activity. In humans, 15% inhibition of RBC-AChE is generally considered to be the minimal change that can be observed with any statistical reliability (Callaway et al., 1951). Existing response data for other organophosphates indicate that RBC-ChE inhibition of as much as 20% is not associated with adverse clinical signs or symptoms in humans and should be considered only as evidence of exposure (Marquis, 1988). This contention is supported by the U.S. EPA (1995a) which reports scientific agreement that statistically significant inhibition of cholinesterase in multiple organs and tissues accompanied by clinical effects constitutes a hazard; however, in the absence of clinical effects, such inhibition may not be of biological significance. It is generally agreed that inhibition of RBC and/or plasma cholinesterase contributes to the overall hazard identification of cholinesterase inhibiting agents by serving as biomarkers (U.S. EPA, 1995a). Animal data have shown that exposure to low doses of nerve agents for extended periods of time can result in low blood ChE activity levels without signs of toxicity. Bucci et al. (1992b) found no evidence of toxicity in rats dosed i.p. with GA (up to 112 μg/kg), even though RBC-AChE activity was reduced about 37% in females (relative to controls). In oral toxicity studies conducted on GB, Bucci and Parker (1992) found that gavage doses of 0.3 mg/kg/day to rats caused nearly a 50% reduction in RBC-AChE activity without signs of toxicity. Goldman et al. (1988) reported no signs of toxicity, but 78–80% reduction in RBC-AChE activity, in Sprague-Dawley rats dosed subcutaneously with 1.0 μg VX/kg/day over 30 days. Rice et al. (1971) reported that whole blood cholinesterase of sheep dosed orally with 15 μg VX/day was reduced to 5% of the normalized baseline values (during the last 3 weeks of the dosing period) without any signs of toxicity. Rice et al. (1971) also found that sheep showing signs of toxicity at higher dose levels recovered fully after the exposures ended. Further complicating the evaluation is the extreme variability in ChE levels of individual animals and different sexes and ages of the same species (Halbrook et al., 1992). Possible changes in blood ChE that may occur with increasing age of the animals requires comparisons with concurrent controls, because the absence of a significant difference from preexposure value may be due to age-related increases in ChE in the dosed animals.

Blood ChE activity has been used by EPA as the critical endpoint in the establishment of oral RfDs for organophosphate insecticides (U.S. EPA 1995b-d). In the case of malathion (U.S. EPA, 1995a), the no-observed-effect level (NOEL) was identified as the highest oral dose level at which no significant change in RBC-AChE or plasma-ChE activity was recorded in 5 human volunteers who received the compound orally for 47 days (Moeller and Rider, 1962). The next highest dose was associated with a depression of about 25% in both RBC-AChE and plasma ChE, but no clinical signs of toxicity. The EPA approach, also used for other organophosphate pesticides (U.S. EPA, 1995b), is, therefore, to identify the lowest-effect level (LEL) as the dose at which statistically significant decreases in ChE levels (RBC-AChE, plasma-ChE, or brain-ChE) occur, and then to base an RfD on the dose level where the change in ChE is not statistically significant. This approach is also used in this report so that the RfDs developed for the nerve agents will not be disproportionally different from those for organophosphate insecticides; however, it should be emphasized that these values may be overly conservative. Furthermore, in evaluating the experimental data for the nerve agents, added weight was given to those cases where significant changes in ChE occurred relative to both control and pre-exposure values and where there was evidence of a dose-response relationship.

4.2 Derivation of the Oral RfD

In the derivation of an oral RfD, human oral exposure data are preferred (as in the case of malathion); however, the only available human data for GB pertain to acute exposures. Although such data can be used to establish short-term exposure limits; acute toxicity endpoints are generally not used for developing subchronic or chronic reference values.

The only subchronic or chronic exposure studies for GB that were found in the available literature consist of a 90-day study in which rats were given GB Type I (Bucci et al., 1991) or GB Type II (Bucci and Parker, 1992) by gavage, and a 1-year study in which rats, mice, and dogs were exposed to GB by inhalation (Weimer et al., 1979). For the development of an oral RfD, a study involving the same exposure pathway is preferred even though the exposure period may be less than chronic.

The use of the subchronic rat study for developing an oral RfD for GB is complicated by the fact that rodents have a much lower RBC-AChE activity level compared to humans (Ellin, 1981, see Table 1). By itself, this could cause rats to be relatively more sensitive than humans to anticholinesterase compounds; however, the lower RBC-ChE activity may be offset by the presence of aliesterases in rat blood. Aliesterases, which are not present in humans (Cohen et al., 1971), are known to bind to and thereby reduce the toxicity of GB (Fonnum and Sterri, 1981). Other species differences, such as the rates of aging of the GB-ChE complex, the rates of synthesis of plasma cholinesterase in the liver, and the levels of AChE in various parts of the nervous system (see Ivanov et al., 1993) may also result in differences in species' sensitivities. There is insufficient data to determine the relative susceptibilities of humans and rodents to GB; therefore, for the purpose of this assessment, the EPA method will be followed which assumes that humans may be as much as ten times more sensitive to a chemical than laboratory animals.

The subchronic rat study conducted by Bucci and Parker (1992) with GB Type II is used here to derive an oral RfD for GB. This study is described in detail in Section 3.3. Briefly summarized, the results of this study showed statistically significant ($p < 0.05$) decreases in plasma and RBC-ChE activity levels in male and female CD rats dosed by gavage once per day, 5 days per week for 13 weeks. The RBC-ChE levels are shown in Table 4. Significant reductions in RBC-AChE relative to controls and to baseline values were seen in male rats in all dose groups (Appendix B). Although no other toxic effects were observed in the rats dosed with GB Type II, brain lesions (hippocampal necrosis) occurred in rats dosed with GB Type I (in 1 of 12 females dosed with 0.075 mg/kg/day, and in 2 of 12 females dosed with 0.3 mg/kg/day, but in none of the females dosed with 0.150 mg/kg/day, and in the of the males dosed with 0.075, 0.15, or 0.3 mg/kg/day) (Bucci et al., 1991). The absence of effects at the mid-dose, and the possibility that post-mortem autolysis contributed to the findings (see section 3.3) makes it difficult to select a LOAEL or NOAEL for this endpoint.

The lowest tested dose (0.075 mg/kg/day) is considered a lowest-observed-adverse-effect level (LOAEL) because of the statistically significant reduction in RBC-AChE seen in male rats dosed with GB Type II. This dose is adjusted to a 7 days/week exposure period by using a factor of 5/7; i.e., $5/7 \times 0.075$ mg/kg/day = 0.054 mg/kg/day. The RfD can then be calculated according to the following formula:

$$RfD = \frac{0.054 \text{ mg/kg/day}}{UF_1 \times UF_2 \times UF_3 \times UF_4 \times UF_5 \times MF} \quad (2)$$

where:

UF_1	=	10 (sensitive subpopulations)
UF_2	=	10 (animal to human extrapolation)
UF_3	=	3 (subchronic-to-chronic extrapolation)
UF_4	=	3 (LOAEL-to-NOAEL extrapolation)
UF_5	=	3 (data base incomplete)
MF	=	1 (modifying factor - not needed)

An uncertainty factor of 10 for sensitive subpopulations is considered necessary because some individuals have a genetic defect causing their blood cholinesterase activity to be abnormally low (Evans et al., 1952; Harris and Whitaker, 1962). For homozygous individuals, the activity can be as low as 8–21% of the normal mean (Bonderman, and Bonderman, 1971). These individuals may be unusually sensitive to organophosphate anticholinesterase compounds (Morgan, 1989).

An uncertainty factor of 10 is used for animal-to-human extrapolation because there is ample evidence that humans are more sensitive to GB than laboratory rodents. In humans, the single dose oral RBC-AChE $_{50}$ (dose required to lower red blood cell cholinesterase by 50%) is 0.01 mg/kg (Grob and Harvey, 1958), and an average daily dose of 0.034 mg/kg for three days resulted in moderate signs of toxicity. In comparison, rats receiving 0.3 mg GB Type II/kg/day for 90 days exhibited decreases in blood cholinesterase levels but no signs of toxicity (Bucci and Parker, 1992).

An uncertainty factor of 3 is used to extrapolate from a subchronic to chronic exposure. In the derivation of oral RfDs for other organophosphate compounds, EPA has used NOAELs for cholinesterase inhibition following short-term exposures without adjustment for a more prolonged exposure period because of the unlikelihood that the endpoint would change over time (i.e., a subchronic-to-chronic UF of 1 was used). In addition, animal data indicate that maximum ChE inhibition may occur 30–60 days or more after exposure begins after which it levels off or even shows signs of recovery. In the Bucci and Parker study plasma and RBC-ChE activity levels at week 13 were no longer significantly different from both baseline and control values, particularly for the lowest dose level (see Appendix B); therefore, increased ChE inhibition is not expected to occur at longer exposure periods. However, an uncertainty factor of 3 is used here because studies are not available to verify that adverse effects would not occur following chronic exposures.

A LOAEL-to-NOAEL uncertainty factor of 3 is used instead of 10 because the endpoint, cholinesterase inhibition, was not associated with signs of toxicity.

The data base for GB consists of two well designed and well conducted subchronic toxicity studies in rats, developmental studies in two species (rats and rabbits), delayed neuropathy studies in chickens and rats, and chronic inhalation studies in mice, rats and dogs. In addition, there is substantial human data for acute and short-term exposures. These studies support the use of cholinesterase inhibition as the critical endpoint for deriving an oral RfD. The data base for GB is, however, lacking a multi-generational reproductive toxicity study. Because studies on other organophosphate cholinesterase inhibitors, including a multi-generational study on agent VX, indicate that reproductive effects are unlikely, a full Uncertainty Factor of 10 is not considered necessary.

Therefore,

$$\text{RfD} = \frac{0.054 \text{ mg/kg/day}}{10 \times 10 \times 3 \times 3 \times 3 \times 1} \quad (3)$$

$$\text{RfD} = 0.00002 \text{ mg GB/kg/day} \quad (4)$$

$$\text{RfD} = 0.02 \text{ µg GB/kg/day} \quad (5)$$

4.3 Overall Confidence in the Oral RfD

Study: High
Data Base: Medium
RfD: Medium

The principal study was well-designed and well-conducted, used a relevant exposure pathway, and examined the appropriate toxicological endpoints. The data base for GB also contains a second oral subchronic study in rats, chronic inhalation studies in rats, mice and dogs, teratology studies in rats and rabbits, and delayed neuropathy studies in mice and chickens. Deficiencies in the data base consist primarily of a lack of a multi-generational reproductive toxicity study, and a standard toxicity study in a second species. Consequently, the overall confidence in the RfD is medium.

4.4 Comparison of the RfD with Human Toxicity Data

The RfD is compared to the available human toxicity data in Table 8. One study in humans indicated that an oral dose of 2.3 µg/kg/day for three days resulted in 27 and 33% RBC-AChE inhibition but no toxic effects. This dose is about 115 times greater than the derived RfD. For an adverse effect level (i.e., mild toxic effect at 29 µg/kg/day; Grob and Harvey, 1958), the "margin of safety" would be about 10 times greater than that for the 27–33% RBC-AChE inhibition.

5. CARCINOGENICITY ASSESSMENT

The potential carcinogenicity of GB cannot be determined; however, limited data from animal inhalation studies suggest that agent GB is not carcinogenic (see Section 3.7). The results of mutagenicity assays on bacteria, *in vitro* tests on mammalian cell cultures, and *in vivo* studies on mice (see Section 3.8) indicate that GB is not genotoxic or mutagenic. These data provide supporting evidence that GB is not likely to be carcinogenic.

Table 8. Comparison of RfD with Human Toxicity Data for GB

Dose (μg/kg)	Exposure Route	Endpoint	References
0.02	oral	RfD - no inhibition of RBC-AChE	This report
2	oral	excessive dreaming	Thienes and Haley, 1972
2.3	oral; average daily dose for three days	RBC-AChE reduced 27 and 33%, but no toxic effects	Grob and Harvey, 1958
10	oral	50% inhibition of AChE	Grob and Harvey, 1958
20	oral	insomnia, withdrawal, depression	Thienes and Haley, 1972
22	oral	mild toxic effects, anorexia, nausea, hearburn	Grob and Harvey, 1958
29	oral; average daily dose for three days	mild toxic effects	Grob and Harvey, 1958
30	oral	moderate toxic effects	Grob and Harvey, 1958
34	oral; average daily dose for three days	moderate toxic effects; > 90% reduction in RBC-AChE activity	Grob and Harvey, 1958
140	oral	Estimated lethal oral dose	Grob and Harvey, 1958

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APPENDIX A COMPARISON OF RFDS, CHE INHIBITION AND TOXICITY DATA FOR GA, GB, GD, AND VX

Endpoint	GA (μg/kg/day)	GB (μg/kg/day)	GD (μg/kg/day)	VX (μg/kg/day)	Ref.
RfD	0.04	0.02	0.004	0.0006	This report
Estimated no-effect level for RBC-AChE inhibition	-	1.0	-	0.24	**GB**[d] **VX**[a]
27–33% inhibition of RBC-AChE in humans/oral dose	-	2.3 (3 days)	-	0.2-2.0	**GB** - Grob and Harvey, 1958; **VX**-this report
RBC-AChE inhibition in humans/ i.v. dose	-	-	1.5-2.0	1.0 (50%)	DA, 1974; Sidell and Groff, 1974
50–60% RBC-AChE inhibition in humans/oral dose	-	10	-	2.4	**GB** - Grob and Harvey, 1958; **VX**-Sidell and Groff, 1974
50% brain ChE inhibition *in vitro*	1.5×10^{-8} (c)	0.3×10^{-8} (c)	-	-	Grob and Harvey, 1958
Acute toxic effects in humans/oral dose	-	20–30	-	2–4.5	**GB** - Thienes and Haley 1972; Grob and Harvey, 1958; **VX**-Sidell and Groff, 1974
human oral LD_{50} (estimated)	25–50[b]	5–20[b]	5–20	3–10[b]	Somani et al., 1992
rat oral LD_{50}	3700	870–1060 600	400	77–128	DA, 1974 Grob & Harvey, 1958
monkey i.v. LD_{50}	50	20	-	6–11	DA, 1974
rat i.v. LD_{50}	70	45–63	50	6.9–10.1	Dacre, 1984
rat i.p. LD_{50}	490, 800	250 218	-	37–55	DA, 1974 RTECS, 1995

[a] Based on ration of oral to i.v. doses (2.4 and 1.0 μg/kg, respectively) required for 50% RbC-ChE inhibition and the estimated i.v. no effect dose of 0.1 μg/kg.
[b] Values were estimated from animal data.
[c] Molar concentration
[d] Estimated from RBC-ChE_{50} values for GB and VX.

APPENDIX B STATISTICAL ANALYSIS OF GB-INDUCED CHE INHIBITION IN RATS

GB Type II - RBC Cholinesterase Inhibition in Female Ratsa (ANOVA and Dunnett's Comparison)

Oral Dose (μg/kg/day)	Week				
	-1	1	3	7	13
0	←/↑[b]	ns/	ns/	ns/	ns/
75	_/ns	ns/ns	ns/ns	ns/ns	ns/ns
150	_/ns	S/S	S/ns	S/S	ns/S
300	_/ns	S/S	S/S	S/S	ns/S

Source: Re-evaluation of data from Bucci and Parker, 1992.
[a] Six animals/dose group
[b] Comparison to pre-exposure value (week -1); ↑ comparison to control (0 μg/kg/day) value.
ns. Not statistically significant.
S. Statistically significant at $p < 0.05$.

GB Type II - RBC Cholinesterase Inhibition in Male Ratsa (ANOVA and Dunnett's Comparison)

Oral Dose (μg/kg/day)	Week				
	-1	1	3	7	13
0	←/↑[b]	ns/	ns/	ns/	ns/
75	_/ns	ns/ns	ns/ns	ns/ns	ns/ns
150	_/ns	S/S	S/ns	S/S	ns/S
300	_/ns	S/S	S/S	S/S	ns/S

Source: Re-evaluation of data from Bucci and Parker, 1992.
[a] Six animals/dose group
[b] Comparison to pre-exposure value (week -1)/comparison to control (0 μg/kg/day) value.
ns. Not statistically significant.
S. Statistically significant at $p < 0.05$.

GB Type II - RBC Cholinesterase Inhibition in Female Ratsa (ANOVA and Dunnett's Comparison)

Oral Dose (μg/kg/day)	Week				
	-1	1	3	7	13
0	←/↑[b]	ns/	ns/	ns/	ns/
75	_/ns	ns/ns	ns/ns	ns/ns	ns/ns
150	_/ns	S/S	S/ns	S/S	ns/S
300	_/ns	S/S	S/S	S/S	ns/S

Source: Re-evaluation of data from Bucci and Parker, 1992.
[a] Six animals/dose group
[b] Comparison to pre-exposure value (week -1)/comparison to control (0 μg/kg/day) value.
ns. Not statistically significant.
S. Statistically significant at $p < 0.05$.

GB Type II - RBC Cholinesterase Inhibition in Male Ratsa (ANOVA and Dunnett's Comparison)

Oral Dose (μg/kg/day)	Week				
	-1	1	3	7	13
0	0	ns/	ns/	ns/	ns/
75	_/ns	S/S	S/S	S/S	ns/ns
150	_/ns	S/S	S/S	S/S	S/ns
300	_/ns	S/S	S/S	S/S	S/ns

Source: Re-evaluation of data from Bucci and Parker, 1992.
[a] Six animals/dose group
[b] Comparison to pre-exposure value (week -1)/comparison to control (0 μg/kg/day) value.
ns. Not statistically significant.
S. Statistically significant at $p < 0.05$.

Appendix C

Health Risk Assessment for The Nerve Agent GD (Soman)

HEALTH RISK ASSESSMENT FOR NERVE AGENT GD (SOMAN)

DRAFT REPORT

September 1996

(editorial corrections made April 1997)

Prepared for

Environmental Risk Assessment Program

Strategic Environmental Research Development Program

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Submitted to

Material Chemical Working Group

Advisory and Coordinating Committee

Environmental Risk Assessment Program Strategic Environmental Research Development Program

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This document is an internal review draft for review purposes only and does not constitute U.S. Government policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

PREFACE

This report assesses the potential non-cancer and cancer effects of chemical agent GD (CAS No. 96-64-0).

This document supports the activities of the Material/Chemical Risk Assessment Working Group of the Environmental Risk Assessment Program, a cooperative endeavor of the Department of Defense, Department of Energy, and Environmental Protection Agency. This working group is developing toxicity values for selected chemicals of concern at federal facilities. Toxicity values will be submitted for consideration by the EPA's IRIS Consensus Process for inclusion on IRIS (EPA's Integrated Risk Information System). The Material/Chemical Risk Assessment Working Group consists of Drs. Jim Cogliano (chair) and Harlal Choudhury (U.S. EPA), Dr. Bruce Briggs (Geo-Centers); Lt. Cmdr. Warren Jederberg and Dr. Robert L. Carpenter (U.S. Naval Medical Research Institute); Dr. Elizabeth Maull and Mr. John Hinz (U.S. Air Force Occupational and Environmental Health Directorate); Drs. Glenn Leach and Winnie Palmer (U.S. Army Center for Health Promotion and Preventive Medicine); Drs. Robert Young and Po-Yung Lu (Oak Ridge National Laboratory).

This document was written by Dr. Robert A. Young, Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN. Internal peer review was provided by Dr. Robert Young, Dr. Annetta Watson, and Mr. Robert Ross.

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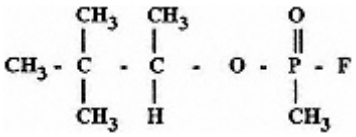
1. INTRODUCTION

Military nerve agents are organophosphate compounds containing either a fluorine, sulfur, or cyanide substituent group (Dacre, 1984). GD contains a fluoride substituent group (GA contains a cyanide substituent group and VX a sulfur group). The chemical synonyms, Chemical Abstract Service (CAS) and Army identification numbers (DA, 1974; Dacre, 1984), and chemical formula for GD are as follows:

Agent GD:	Methylphosphonofluoridic acid, 1,2,2-trimethylpropyl ester; Pinacoloxymethylphosphoryl fluoride; Pinacolyl methylphosphonofluorididate; Soman CAS No. 96-64-0;



1.1 PHYSICAL/CHEMICAL PROPERTIES

Agent GD is a colorless liquid with a molecular weight of 182.2 (DA, 1974); it has a vapor density of 6.3 (air = 1) and a liquid density of 1.02 g/mL at 25°C (DA, 1974). The vapor pressure of GD is 0.4 mm Hg at 25°C. In distilled water it has a solubility of 21 g/L at 20°C (DA, 1974).

1.2 ENVIRONMENTAL FATE

1.2.1 Air

Data specifically regarding the fate of GD in the atmosphere were not located. However, because of its volatility, GD is expected to disperse realtively quickly.

1.2.2 Water

Agent GD may hydrolyze to relatively nontoxic hydroflouric and pinacolyl methylphosphonic acids (MacNaughton and Brewer, 1994; Rosenblatt et al., 1995). The hydrolysis rate is a function of temperature and pH; the rate is minimum between pH 4 and 6. The $t_{1/2}$ for GD is approximately 100 hours with $20 \times t_{1/2}$ being required to attain a 1×10^6 reduction in GD concentration.

1.2.3 Soil

GD is likely to undergo hydrolysis in most soils. As noted above, the rate of hydrolysis will be dependent upon temperature and pH. According to Morrill et al. (1985), evaporation is the primary mechanism for the loss of the GA and GB nerve agents from soil. Although the G agents are liquids under ordinary environmental conditions, their relatively high volatility and vapor pressure permits them to be disseminated in vapor form. Because of this volatility, GD is not expected to persist in soils.

2. MECHANISM OF ACTION

Nerve agents are inhibitors of acetylcholinesterase (AChE), an enzyme responsible for deactivating the neurotransmitter acetylcholine (AChE) at some neuronal synapses and myoneural junctions. By a mechanism of phosphorylation, nerve agents act as substrates for the enzyme thereby preventing deactivation of acetylcholine. The organophosphate-inhibited enzyme can be reactivated by dephosphorylation, but this occurs at a rate that is slower than the rate of reactivation of acetylcholine (deactivated by acetylcholinesterase). Consequently, there is a depletion of acetylcholinesterase and a buildup of acetylcholine. In addition, the nerve agent-enzyme complex can also undergo an "aging" process (thought to be due to a loss of an alkyl or alkoxy group), whereby it becomes resistant to dephosphorylation (see review by Munro et al., 1994). Differences in rates of aging and reactivation may be important in evaluating toxicity data especially when extrapolating from animal studies to humans. In vitro tests conducted by Grob and Harvey (1958) indicate that both GA and GB combine with cholinesterase almost irreversibly during the first hour of their reaction. Sidell and Groff (1974) reported that the GB-ChE complex ages very rapidly in vivo, with 45–70% completion by 5 hours after infusion. In contrast, the complex formed between ChE and the nerve agent VX does not age significantly, and the rate of spontaneous reactivation can be as fast as 1%/hr in humans (Sidell and Groff, 1974).

2.1 Effects of Organophosphate Agents on the Nervous System

The anticholinesterase effects of the organophosphate nerve agents can be characterized as being muscarinic, nicotinic, or central nervous system (CNS)-related. Muscarinic effects occur in the parasympathetic system (bronchi, heart, pupils of the eyes; and salivary, lacrimal and sweat glands) and result in signs of pulmonary edema, bradycardia, miosis, tearing, and sweating. Nicotinic effects occur in somatic (skeletal/motor) and sympathetic systems, and result in muscle fasciculation, muscle weakness, tachycardia, and diarrhea. Effects on the CNS by organophosphates are manifested as giddiness, anxiety, emotional lability, ataxia, confusion, and depression (O'Brien, 1960).

Although the inhibition of cholinesterase within neuro-effector junctions or the effector itself is thought to be responsible for the major toxic effects of organophosphate agents, these compounds can apparently affect nerve-impulse transmission by more direct processes as well. Direct effects may occur on excitable tissues, receptors, and ionic channels. According to Somani et al. (1992), the direct action of nerve agents on nicotinic and muscarinic ACh receptors may occur when concentrations in the blood rise above micromolar levels, whereas at lower levels the action is mainly the result of inhibition of AChE. Albuquerque et al. (1985) have shown that agent GA, as well as agents GB and GD are capable of changing receptor sites in a manner similar to that exhibited by acetylcholine, which promotes the conductance of electrophysiological signals associated with stimulation of neuromuscular function. VX "may directly affect a small population of muscarinic ACh receptors that have a high affinity for [^{3}H]-*cis*-methyldioxalane binding" (Somani et al., 1992). VX may also counteract the effects of ACh by acting as an open channel blocker at the neuromuscular junction, thereby interrupting neuromuscular function (Rickett et al., 1987).

Exposure to some organophosphate cholinesterase inhibitors results in a delayed neuropathy characterized by degeneration of axons and myelin. This effect is not associated with the inhibition of acetylcholinesterase, but rather with the inhibition of an enzyme described as neuropathy target esterase (NTE); however, the exact mechanism of toxicity is not yet fully understood (Munro et al., 1994). For some organophosphate compounds, delayed neuropathy can be induced in experimental animals at relatively low exposure levels, whereas for others the effect is only seen following exposure to supralethal doses when the animal is protected from the acute toxic effects caused by cholinesterase inhibition.

Although there is the potential for nerve agents to have direct toxic effects on the nervous system or to cause delayed neuropathy, there is no evidence that such effects occur in humans at doses lower than those causing cholinesterase inhibition. However, it should be noted that there is very little animal or human data evaluating the potential effects of long-term exposure to low doses. Nevertheless, for the purpose of evaluating potential health effects, inhibition of cholinesterase is generally considered the most useful biological endpoint.

2.2 Effect on Blood Cholinesterases

In addition to being found in the nervous system, acetylcholinesterase also occurs in the blood where it is bound to the surface of red blood cells (termed RBC-ChE or RBC-AChE). RBC-AChE activity, as well as the activity of a second type of cholinesterase found in blood plasma (butyrylcholinesterase, or plasma cholinesterase) have been used to monitor exposure to organophosphate compounds (pesticides and nerve agents). Because ACh is the primary neurotransmitter of the nervous system, changes in RBC-AChE activity are generally considered to be the more appropriate bioindicators of potential effects (Morgan, 1989). There is also evidence that RBC-AChE is as sensitive as brain ChE to the effects of nerve agents. Grob and Harvey (1958) reported that the *in vitro* concentrations producing 50% depression of brain-ChE and RBC-AChE activity were the same in the case of GA (1.5×10^{-8} mol/L), and only slightly different (3×10^{-9} mol/L and 3.3×10^{-9} mol/L) in the case of GB. However, *in vivo* animal studies indicate a poor correlation between brain and RBC-AChE in cases of acute exposures (Jimmerson et al., 1989), and this is reflected in the fact that blood cholinesterase activity may not always be correlated with exposure or with signs and symptoms of toxicity (Holmstedt, 1959). Acute exposures to high concentrations may cause immediate toxic effects before significant changes occur in blood ChE activity, and repeated exposures over a period of several days or more may result in a sudden appearance of symptoms due to cumulative effects (Grob and Harvey, 1958). Conversely, blood ChE activity can become very low without overt signs or symptoms during chronic exposures to low concentrations of organophosphates. This may be due to a slower rate of recovery of RBC-AChE compared to tissue ChE, or to noncholinesterase-dependent recovery pathways for neural tissue (Grob and Harvey, 1958). Sumerford et al. (1953) reported that orchard workers exposed to organophosphate insecticides had RBC- and plasma-ChE values as low as 15% of normal values without any other signs or symptoms of exposure. Animal studies have demonstrated that chronic exposures to low concentrations of organophosphate insecticides and nerve agents can result in increased tolerance levels (Barnes, 1954; Rider et al., 1952; Dulaney et al., 1985). Similarly, Sumerford et al. (1953) reported increased levels of tolerance to organophosphate insecticides in people living near orchards treated with organophosphate insecticides. Such adaptation may result from increased rates of formation of blood ChE, or from increased rates of detoxification. Additional information on the development of tolerance to organophosphate cholinesterase inhibitors can be found in a review paper by Hoskins and Ho (1992).

The blood cholinesterases may, to some degree, provide a protective effect by binding with some fraction of the anticholinesterase compound (Wills, 1972). However, not all nerve agents bind equally well with all cholinesterases. In tests conducted on dogs, Holmstedt (1959) found that GA affected RBC and plasma cholinesterase to a nearly equal degree. In contrast, agent VX preferentially inhibits RBC-AChE (70% compared with about 20% inhibition of plasma ChE) (Sidell and Groff, 1974). Rodents (but not humans) have other enzymes in the blood, termed aliesterases, which can bind organophosphates, thereby reducing the amount available for binding with acetylcholinesterase (Fonnum and Sterri, 1981). Agent GB binds with aliesterases; however, according to Fonnum and Sterri (1981), VX has a quartenary ammonium group which prevents it from being a substrate for aliesterases. The strong specificity of agent VX to AChE may account, in part, for the fact that it is much more acutely toxic than agents GA and GB (Munro et al., 1994).

2.2.1 Intra- and Interspecies Variation in Blood Cholinesterase Activity

Although blood cholinesterase activity is used as a measure of exposure to organophosphate compounds, baseline activity levels can vary between individuals and between species. According to Wills (1972), both plasma- and RBC-AChE activity are generally lower in women than in men. Sidell and Kaminskis (1975) reported that, for a test population of 22 human subjects, the highest coefficient of variation of RBC-AChE was 4.1% per single subject; the average range of variation was ± 2.1% for men and ± 3.1% for women. In individuals studied for one year, the RBC-AChE activity varied by 11% in men and 16% in women. Yager et al. (1976) reported a 10.0% intra-individual coefficient of variation for RBC-AChE and 14.4% for plasma-ChE. Callaway et al. (1951) estimated that with only one preexposure measurement, the smallest measurable decrease was 15% of the baseline value for RBC-AChE activity and 20% of the baseline for plasma-ChE.

A small subpopulation of men and women have a genetic defect causing their blood cholinesterase activity to be abnormally low (Evans et al., 1952; Harris and Whitaker, 1962). For homozygous individuals, the activity can be as low as 8–21% of the normal mean (Bonderman and Bonderman, 1971). Morgan (1989) suggests that these individuals may be unusually sensitive to organophosphate anticholinesterase compounds.

Data compiled by Ellin (1981) reveal that the RBC-AChE activity for humans is slightly higher than that for monkeys and much higher than that for rats and other laboratory animals (Table 1). These differences in RBC-AChE activity may affect a species' sensitivity to a particular organophosphate compound. At the same time, the relative amount of plasma cholinesterase and other compounds in the blood that can bind to the organophosphate agents must also be considered. For example, rodents, but not humans, have high levels of aliesterases (AE) in the blood, and these compounds may provide rats and mice with a higher level of resistance to some anticholinesterase compounds (McNamara and Leitnaker, 1971).

2.2.2 Potency of Nerve Agents as Cholinesterase Inhibitors

The potency of the anticholinesterase activity of nerve agents and other organophosphates is expressed by the bimolecular rate constant (k_i) for the reaction of the phosphate compound with the enzyme and by the molar concentration causing 50% inhibition of the enzyme when tested in vitro (I_{50}). I_{50} data for several organophosphate nerve agents have been tabulated by Dacre (1984). The relationship between I_{50} and k_i as a function of time (t) is expressed by the following equation (Eto, 1974):

$$I_{50} = \frac{0.693}{t \times k_i} \qquad \textbf{(1)}$$

Table 1. RBC-ChE Activity in Different Species

Species	RBC-ChE activity (μmol/mL/min)	Optimum substrate[a] concentration (M)
Human	12.6	2×10^{-3}
Monkey	7.1	2×10^{-3}
Pig	4.7	1×10^{-3}
Goat	4.0	2×10^{-3}
Sheep	2.9	2×10^{-3}
Mouse	2.4	2×10^{-3}
Dog	2.0	2×10^{-2}
Guinea pig	2.7	2×10^{-3}
Rabbit	1.7	5×10^{-3}
Rat	1.7	5×10^{-3}
Cat	1.5	5×10^{-3}

Source: Ellin, 1981
[a] Acetylthiocholine iodide concentration for maximum RBC-ChE activity.

The pI_{50} (negative log of the molar concentration causing 50% inhibition) for GD is 9.2 as reported by Dacre (1984).

Relative potency of nerve agents can also be expressed in terms of the in vivo dose necessary to produce the same level of cholinesterase inhibition by a specific exposure route. As would be expected, the effectiveness of the agents in inhibiting cholinesterase is closely correlated with their acute toxicity (see Appendix A).

3. TOXICOLOGY

3.1 Introduction

Health and environmental impacts of nerve agents and related compounds (organophosphate insecticides) have been reviewed by O'Brien (1960), Matsumura (1976), Dacre (1984), Carnes and Watson (1989), Watson et al. (1989), and Munro et al. (1994). A brief general discussion of the toxicology of nerve agents and related organophosphate pesticides is given below.

Nerve agents are acutely toxic by all routes of exposure. Initial symptoms of acute poisoning are fatigue, headache, mild vertigo, weakness, and loss of concentration. Moderate exposures result in miosis and excessive sweating, tearing, and salivation. Acidosis and hyperglycemia may also occur in addition to muscular weakness, muscular twitching, lacrimation, urination, and defecation. Acute poisoning can result in prostration, clonic convulsions (rapid repetitive movements) and tonic convulsions (limbs stretched and rigid) (Matsumura, 1976). Exposures sufficiently high to cause convulsions have resulted

in brain lesions and cardiomyopathy in laboratory animals (Singer et al., 1987).

In addition to the immediate toxicity of the nerve agents, there is concern that exposures may lead to chronic neurological effects similar to those reported for some organophosphate insecticides. Included among these possible effects are organophosphorus-induced delayed neuropathy (OPIDN), EEG changes, and long-term psychological disturbances (Munro et al., 1994). OPIDN, which appears 5–30 days after exposure, manifests itself as muscle weakness, tingling, and twitching followed by paralysis (Munro et al., 1994). Histopathological changes, which consist of degeneration of axons and myelin of the nervous system, can be correlated, not with inhibition of acetylcholinesterase, but rather with inhibition of NTE; however, the exact mechanism of toxicity is not yet fully understood (Munro et al., 1994). OPIDN has not been observed in humans exposed to the nerve agent GD, however, it has been demonstrated that GB can induce this effect in chickens (a species which is normally used to test for OPIDN) (Gordon et al. 1983). The GB IC_{50} for NTE inhibition was 0.1 μM, a concentration considerably higher than known lethal concentrations in chickens (Willems et al., 1984).

Acute exposures to nerve agents are known to cause EEG changes (Grob and Harvey, 1958; Sidell, 1992) which may persist for long periods of time after exposure (Metcalf and Holmes, 1969; Duffy et al., 1979; Duffy and Burchfiel, 1980); however, the reported changes have been considered to be clinically insignificant and not correlated with behavioral or physiological changes (DHHS, 1988). Acute exposures can also induce neuropsychological changes; however, there is no evidence of these effects persisting for months or years as has been reported for some organophosphate insecticides (Savage et al., 1988; Gershon and Shaw, 1961; Mick, 1974; Rodnitzky, 1974; Wagner, 1983; Tabershaw and Cooper, 1966). The available data for the organophosphate insecticides suggest that chronic neuropsychological effects (excluding OPIDN) do not occur in the absence of significant changes in blood cholinesterase. The same conclusion may apply to the organophosphate nerve agents.

3.2 Short-term Toxicity

Bucci et al. (1992a) conducted range finding studies with GD. The test material was administered by gavage to male and female CD rats once per day, 5 days per week for 2 weeks. These studies indicated that the maximum tolerated dose was 70 μg/kg/day and a dose of 300 μg/kg/day was lethal to 100% of the test animals.

In a study by Blick et al. (1994), rhesus monkeys were administered GD (soman) parenterally daily for 5 days. The effects of the treatment on performance of a well-learned, compensatory trackning task was assessed, and an ED_{50} of 0.97 μg/kg/day for decrement in this performance was obtained. This decrement in performance was concurrent with a 85–90% inhibition of serum ChE. The 0.97 μg/kg/day dose was about 40% of the single acute dose required to produce a similar performance decrement. It was also noted that the single acute dose (2.43 μg/kg) was associated with a 65–70% serum ChE inhibition.

Acute lethality data for GD are summarized in Table 2.

Table 2. Lethality Data for Agent GD

Exposure route	Species	LD_{50} (μg/kg)	References
oral	human	5–20 (est.)	Somani et al., 1992
percutaneous	human	50–300 (est.)	Somani et al., 1992
intravenous	mouse	42	Tripathi and Dewey, 1989
	dog	10	Abbrecht et al., 1989
subcutaneous	rabbit	20	Harris et al., 1984
	guinea pig	26–30	Sterri, 1981; Maxwell et al., 1987b; Anderson et al., 1989; Sparenborg et al., 1989
	rat	70–165	Somani et al., 1986; Petrali, 1989; Maxwell et al., 1987a, 1987b; Lennox et al., 1985
intramuscular	monkey	3.8–15.3	Petras, 1984; Baze, 1993; Wall et al., 1990; Switzer et al., 1980
	rabbit	15	Olson et al., 1989
	mouse	98	Jones et al., 1984

3.3 Subchronic Toxicity

In a subchronic study conducted by the National Center for Toxicological Research (Bucci et al., 1992a), male and female CD rats (12/sex/group) were administered GD by gavage at dose levels equivalent to 17.5, 35.0 and 70 μg GD/kg/day. The doses were given once per day, 5 days per week for 13 weeks. All animals were observed daily for clinical signs of toxicity and weighed weekly. Necropsy examination was performed on all animals. Terminal body and organ weights were recorded. Microscopic evaluation was performed on all high-dose and control animals, and on those tissue of lower dose animals that were abnormal at necropsy. Hematological analyses and clinical chemistry (including RBC and plasma cholinesterase) were evaluated in the same 6 male and 6 female rats in each dose group one week before the exposures began and also at weeks 1, 3, 7, and 13. In addition, at necropsy a hemisection of each brain was prepared and tested for NTE.

Relative to untreated controls, the group mean body weight gain was significantly decreased ($p<0.001$) in the high-dose (70 μg/kg/day) males (b.w. change 160.9 in controls vs. 83.7 g). A decrease in body weight gain also occurred in female rats (75 vs 59 g decrease for high-dose and controls, respectively) but the difference was not statistically significant. A definitive dose-response was not present in either males or females.

Although changes were observed in some clinical chemistry and hematologic parameters, the changes were spurious, not dose-related and were not biologically relevant. Brain NTE was not altered in rats dosed with GD. Histopathological examinations revealed no gross or microscopic findings that could be attributed to treatment with GD. Special attention was given regarding intercostal and cardiac muscle lesions, and neurological lesions which have been previously reported in rats treated with GD (McLeod, 1985; Singer et al., 1987). However, none of these lesions was observed in GD-treated rats.

RBC-AChE and plasma-ChE levels are shown in Tables 3 and 4. Considerable variability was noted among the control and treatment group baseline values. A dose-related decrease in plasma-ChE levels in both male and female rats was observed for weeks 1 and 7. Relative to untreated rats, significant ($p<0.05$) depression of plasma cholinesterase levels was observed in both males and females of the high-dose group during week 1 (25% and 33% for males and females, respectively) and week 7 (20% and 33% for males and females, respectively), and in males of the 35 μg/kg/day group during week 7 (28%). In females, the plasma-AChE levels exceeded pretreatment (baseline) values by week 13 but remained depressed (54%, 66%, and 50% in the low, mid, and high dose groups, respectively) in males at week 13 although not significantly so. Upon comparing treatment groups with controls, no significant changes in RBC-AChE levels were noted by the study authors. Data for week 3 were highly variable and appear to reflect inaccuracies or problems with the AChE assay procedure.

Table 3. RBC-ChE Levelsa in 90-Day Subchronic Study of GD in CD Ratsa

		Week of treatment								
Dose (μg/kg/d)	**Sex**	**-1**	**1**	**%[b]**	**3**	**%[b]**	**7**	**%[b]**	**13**	**%[b]**
0	F	1609(252)	2461 (136)	153	2497 (186)	155	1674 (127)	104	1341 (397)	83
17.5	F	1462(153)	2445 (191)	167	1790 (144)	122	1236 (201)	85	1313 (280)	89
35.0	F	1466(167)	2244 (179)	153	1817 (127)	124	1381(90)	94	1310 (280)	89
70.0	F	1025(152)	2544 (367)	250	1695(52)	165	1556(96)	152	1296 (168)	126
0	M	1955(108)	2340 (149)	119	2043 (212)	105	2323 (116)	119	1904 (106)	97
17.5	M	1764(158)	1862(95)	106	1211 (125)	69	1784(90)	101	1774 (161)	100
35.0	M	1977(78)	1820 (153)	92	1435 (320)	73	1681(28)	85	1562(68)	79
70.0	M	1859(142)	1964 (205)	105	973(48)	52	1753 (139)	94	1720 (148)	92

Source: Bucci et al., 1992a
[a] Values given as mean IU/L and (SEM)
[b] Percent of baseline

Table 4. Plasma-ChE Levelsa in 90-Day Subchronic Study of GD in CD Ratsa

		Week of treatment								
Dose (μg/kg/d)	**Sex**	**-1**	**1**	**%[b]**	**3**	**%[b]**	**7**	**%[b]**	**13**	**%[b]**
0	F	1401(148)	1542 (175)	110	437(52)	31	1976 (198)	141	2959 (358)	190
17.5	F	1571(322)	893(185)	57	1131 (422)	72	968(109)	61	2275 (320)	145
35.0	F	1560(207)	593(50)	38	337(80)	22	673(96)	43	2303 (179)	148
70.0	F	1344(190)	446(81)*	33	950(180)	71	439(40) c*	33	1951 (179)	145
0	M	543(51)	377(44)	69	685(290)	126	340(18)	63	431(39)	78
17.5	M	631(131)	245(29)	39	500(293)	79	204(19)	32	339(34)	54
35.0	M	632(47)	198(16)	31	276(63)	44	174(14)*	28	414(32)	66
70.0	M	610(98)	153(11)*	25	370(59)	61	122(7)*	20	308(16)	50

Source: Bucci et al., 1992a
[a] Mean IU/L and (SEM)
[b] Percent of baseline (week -1).
* p<0.05, different from control value (0 μg/kg/day)

The plasma- and RBC-AChE data from the Bucci et al. (1992a) study were re-analyzed by ORNL (using standard deviations) with ANOVA and Dunnett's and Scheffe's Comparisons. In the re-evaluation, RBC and plasma cholinesterase levels were compared to respective controls for the same sampling times as well as to the pre-exposure values within each group (Tables 5–8). This analysis also indicated an absence of definitive changes in RBC-AChE levels that could be attributed to GD treatment. During week 3 in females and week 7 in males, RBC-AChE levels of all GD treatment groups were significantly lower (p<0.05) than controls but the response did not exhibit a dose-relation in either group. In females during week 1, RBC-AChE levels in the control and all treatment groups were inexplicably elevated relative to baseline (week -1) values. For plasma-AChE, a dose-related significant decrease (p<0.05) relative to controls was detected during weeks 1 and 7 for both male and female rats. With the exception of high-dose females at week 3, a comparison of values to pre-exposure levels indicated that plasma-AChE levels of both the mid and high-dose groups were significantly (p<0.05) lower at weeks 1, 3, 7 and 13 for females and males. Under the conditions of this study, GD treatment appeared to affect plasma-AChE levels at a dose as low as 17.5 μg/kg as exemplified by the significant (p<0.05) decrease relative to controls and the reduction in plasma-ChE at week 1 to 39% of baseline in males and to 57% of baseline in females. For plasma-AChE, decreases in activity appeared to be dose-related. It is uncertain why similar findings were not observed for RBC-AChE.

Table 5. GD-Induced RBC-AChE Inhibition in Female CD Rats (Dunnett's Comparison)

	Week				
Dose (μg/kg/day)	-1	1	3	7	13
0	1609	2461[a]	2497a[a]	1674	1341
17.5	1462	2445[a]	1790[b]	1236[b]	1313
35.0	1466	2244[a]	1817[b]	1381	1310
70.0	1025[b]	2544[a]	1695[a,b]	1556	1296

Source: Bucci et al., 1992a
[a] Significantly different ($p<0.05$) relative to pre-exposure baseline value (week -1)
[b] Significantly different ($p<0.05$) relative to respective weekly control value.

Table 6. GD-Induced RBC-AChE Inhibition in Male CD Rats (Dunnett's Comparison)

	Week				
Dose (μg/kg/day)	-1	1	3	7	13
0	1955	2340	2043	2323	1904
17.5	1764	1862	1211[a,b]	1784[b]	1774
35.0	1977	1820[b]	1435[a]	1681[b]	1562
70.0	1859	1964	973[a,b]	1753[b]	1720

Source: Bucci et al., 1992a
[a] Significantly different ($p<0.05$) relative to pre-exposure baseline value (week -1)
[b] Significantly different ($p<0.05$) relative to respective weekly control value.

Table 7. GD-Induced Plasma-AChE Inhibition in Female CD Rats (Dunnett's Comparison)

	Week				
Dose (μg/kg/day)	-1	1	3	7	13
0	1401	1542	437[a]	1976	2659[a]
17.5	1571	893[b]	1131	968[b]	2275
35.0	1560	593[a,b]	337[a]	673[a,b]	2303[a]
70.0	1344	446[a,b]	950	439[a,b]	1951[a]

Source: Bucci et al., 1992a
[a] Significantly different ($p<0.05$) relative to pre-exposure baseline value (week -1) within treatment group.
[b] Significantly different ($p<0.05$) relative to respective weekly control value.

Table 8. GD-Induced Plasma-AChE Inhibition in Male CD Rats (Dunnett's Comparison)

	Week				
Dose (μg/kg/day)	-1	1	3	7	13
0	543	377	685	340	431
17.5	631	245[b]	500	204[b]	339
35.0	632	198[a,b]	276[a]	174[a,b]	414[a]
70.0	610	153[a,b]	370[a]	122[a,b]	308[a,b]

Source: Bucci et al., 1992a
[a] Significantly different ($p<0.05$) relative to pre-exposure baseline value (week -1) within treatment group.
[b] Significantly different ($p<0.05$) relative to respective weekly control value.

3.4 Chronic Toxicity

There was no information concerning the effects of GD following chronic exposure.

3.5 Nervous System Effects

As noted in section 3.1, the neurotoxic effects following acute exposures to nerve agents such as GD can range from minor symptoms such as fatigue, headache, mild vertigo, weakness, and loss of concentration to convulsions, respiratory arrest and death.

Evidence of delayed neurotoxicity was not observed in the previously described rat study by Bucci et al. (1992a). A delayed neurotoxicity study in SPF white leghorn chickens was also negative (Bucci et al., 1992b).

3.6 Developmental and Reproductive Effects

There are no studies evaluating the developmental or reproductive effects of GD in humans or laboratory animals.

3.7 Carcinogenicity and Genotoxicity

No information is available regarding the potential carcinogenicity of agent GD in humans.

In a 90-day gavage study in rats, Bucci et al. (1992) found no neoplastic changes attributable to GD treatment. The study was, however, of insufficient duration to be suitable for a cancer bioassay. No additional data are available regarding the potential carciogenicity of GD in animals.

Goldman et al. (1987) reported on the results of genotoxicity studies of agent GD. In tests on bacteria and mammalian cell cultures, GD was not genotoxic or mutagenic when tested with and without metabolic activation. There were no biologically significant increases in mutations when tested in the Ames Salmonella assay using five revertant strains (TA135, TA100, TA98, TA1537, and TA1538) both with and without metabolic activation. GD did not induce a significant increase in forward mutations when tested on mouse L5178Y lymphoma cells at concentrations of 50, 100, or 200 μg/mL, and no increase in sister chromatid exchanges (SCE) was observed when Chinese hamster ovary cells were exposed *in vitro* to 200 μg/mL of GD. Mice treated *in vivo* with a maximally tolerated intraperitoneal dose of 300 μg GD/kg did not exhibit a significant increase in SCE in splenic lymphocytes. Exposure of rat hepatocytes to GD concentrations as high as 600 μl/3ml culture medium (2.5×10^6 hepatocytes) did not result in DNA damage or unscheduled DNA synthesis.

4. ORAL REFERENCE DOES FOR GD

4.1 Cholinesterase Inhibition as an RfD Endpoint

The endpoint for defining a maximum acceptable exposure level for nerve agents such as GD is considered to be the level at which no significant depression in blood cholinesterase activity occurs. In humans, 15% inhibition of RBC-AChE is generally considered to be the minimum change that can be observed with any statistical reliability (Callaway et al., 1951). Existing human response data (Marquis, 1988) indicate that human RBC-AChE inhibition of as much as 20% is not associated with adverse clinical signs or symptoms and should be considered only as evidence of organophosphate exposure. This contention is supported by the U.S. EPA (1995a) which reports scientific agreement that statistically significant inhibition of cholinesterase in multiple organs and tissues accompanied by clinical effects constitutes a hazard; however, in the absence of clinical effects, such inhibition may not be of biological significance. It is generally agreed that inhibition of RBC and/or plasma cholinesterase contributes to the overall hazard identification of cholinesterase inhibiting agents by serving as biomarkers (U.S. EPA, 1995a). Animal data have shown that exposure to low doses of nerve agents for extended periods of time can result in low blood ChE activity levels without signs of toxicity. Bucci et al. (1992c) found no evidence of toxicity in rats dosed i.p. with GA (up to 112 μg/kg), even though RBC-AChE activity was reduced about 37% in females (relative to controls). In oral toxicity studies conducted on GB, Bucci et al. (1992a) found that gavage doses of 0.3 mg/kg/day to rats caused nearly a 50% reduction in RBC-AChE activity without signs of toxicity. Goldman et al. (1988) reported no signs of toxicity, but 78–80% reduction in RBC-AChE activity, in Sprague-Dawley rats dosed subcutaneously with 1.0 μg VX/kg/day over 30 days. Rice et al. (1971) reported that whole blood cholinesterase of sheep dosed with 15 μg VX/day was reduced to 4–5% of the normalized baseline values (during the last 3 weeks of the dosing period) without any signs of toxicity. Rice et al. (1971) also found that sheep showing signs of toxicity (not described) at higher dose levels recovered fully after the exposures ended. Further complicating the evaluation is the extreme variability in ChE levels of individual animals and different sexes and ages of the same species (Halbrook et al., 1992). Possible changes in blood ChE that may occur with increasing age of the animals requires comparisons with concurrent controls, because the absence of a significant difference from pre-exposure values may be due to age-related increases in ChE in the dosed animals.

Blood ChE activity has been used by EPA as the critical endpoint in the establishment of oral RfDs for organophosphate insecticides (U.S. EPA 1995b; 1995c). In the case of malathion (U.S. EPA, 1995b), the no-observable-effect-level was identified as the highest oral dose level at which no significant change in RBC-AChE or plasma ChE was recorded in 5 human volunteers who received the compound orally for 47 days (Moeller and Rider, 1962). The next highest dose was associated with a depression of about 25% in both RBC-AChE and plasma ChE, but no clinical signs of toxicity. The EPA approach, also used for other organophosphate pesticides, is, therefore, to identify as LOAELs statistically significant decreases in ChE levels (RBC-AChE, plasma-ChE, or brain-ChE), and to base RfDs on NOAELs where the change in ChE is not statistically significant. This approach is also used in this report so that the RfDs developed for the nerve agents will not be disproportionally different from those for the organophosphate insecticides. In evaluating the experimental data for the nerve agents, added weight was given to those cases where significant changes in ChE occurred relative to both control and pre-exposure values and where there was evidence of a dose-response relationship.

In the derivation of an oral RfD, human oral exposure data are preferred (as in the case of malathion); however, such data are not available for GD. The only subchronic or chronic exposure studies for GD that were found in the available literature consist of a 90-day study in which rats were given GD by gavage (Bucci et al., 1992a).

The use of the subchronic rat study for developing an oral RfD for GD is complicated by the fact that rodents have a much lower RBC-AChE activity level compared to humans (Ellin, 1981, see Table 1). By itself, this could cause rats to be relatively more sensitive than humans to anticholinesterase compounds; however, the lower RBC-ChE activity may be offset by the presence of aliesterase in rat blood. Aliesterase, which is not present in humans (Cohen et al., 1971), is known to bind to and thereby reduce the toxicity of cholinesterase inhibitors (Fonnum and Sterri, 1981). Other species differences, such as the rates of aging of the nerve agent-ChE complex, the rates of synthesis of plasma cholinesterase in the liver, and the levels of AChE in various parts of the nervous system (see Ivanov et al., 1993) may also result in differences in species' sensitivities. There are insufficient data to determine the relative susceptibilities of humans and rodents to GD; therefore, for the purpose of this assessment, the EPA method will be followed which assumes that humans may be as much as ten times more sensitive to a chemical than laboratory animals.

4.2 Derivation of the Oral RfD for GD

The subchronic rat study conducted by Bucci et al. (1992a) is used here to derive an oral RfD for GD. This study is described in detail in section 3.2. Briefly summarized, the results of this study showed statistically significant ($p < 0.05$) decreases in plasma-ChE activity levels in male and female CD rats dosed by gavage once per day, 5 days per week for 13 weeks. There were no definitive dose-related changes in RBC-ChE, and NTE levels were not significantly affected by the GD treatment.

The lowest tested dose (17.5 μg/kg/day = 0.0175 mg/kg/day) is considered a LOAEL because of the statistically significant reduction in plasma ChE (relative to controls) and also because the plasma-ChE activity during week 1 was reduced to 39% of baseline in males and 57% of baseline in females. This dose is adjusted to a 7 day/week exposure period by using a factor of 5/7; i.e., 5/7 × 0.0175 mg/kg/day = 0.0125 mg/kg/day. The RfD can then be calculated according to the following formula.

$$\text{Oral RfD} = \frac{0.0125 \text{ mg/kg/day}}{UF_1 \times UF_2 \times UF_3 \times UF_4 \times UF_5 \times MF} \qquad (2)$$

where

UF_1	=	10 (sensitive subpopulations)
UF_2	=	10 (animal to human extrapolation)
UF_3	=	3 (although plasma-AChE is not expected to be inhibited at longer exposures, however, an uncertainty factor was incorporated to account for effects possibly unrelated to plasma-AChE inhibition)
UF_4	=	3 (LOAEL to NOAEL extrapolation; altered plasma-AChE is not overtly toxic)
UF_5	=	3 (data base incomplete due to lack of chronic oral studies in two species, and studies assessing reproductive/ developmental effects)
MF	=	1 (no additional modifications needed).

An uncertainty factor of 10 for sensitive subpopulations is considered necessary because some individuals have a genetic defect causing their blood cholinesterase activity to be abnormally low (Evans et al., 1952; Harris and Whitaker, 1962). These individuals, therefore, may be unusually sensitive to organophosphate anticholinesterase compounds.

An uncertainty factor of 10 is used for animal-to-human extrapolation because there is no evidence suggesting that humans less sensitive to GD than are laboratory animals.

An uncertainty factor of 3 is used to extrapolate from a subchronic to chronic exposure. In the derivation of the oral RfDs for other organophosphate compounds, the EPA has used NOAELs for cholinesterase inhibition following short-term exposures without adjustment for a more prolonged exposure period because of the unlikelihood that the endpoint would change over time (i.e., a subchronic-to-chronic UF of 1 was used). In addition, animal data indicate that maximum ChE inhibition may occur 30–60 days or more after exposure begins, after which it levels off or even shows recovery. In the Bucci et al. (1992a) study, both plasma and RBC-AChE levels exhibited signs of recovery at week 13, especially for the lower doses (Tables 5–8). Therefore, increased ChE inhibition is not expected to occur at longer exposure periods. However, an uncertainty factor of 3 is used because studies are not available to verify that adverse effects would not occur following chronic exposures.

A LOAEL-to-NOAEL uncertainty factor of 3 is used instead of 10 because the endpoint, cholinesterase inhibition, was not associated with signs of toxicity.

The database for GD lacks chronic oral studies in two species, and studies assessing reproductive/developmental effects. Because studies on other organophosphate cholinesterase inhibitors, including a multigeneration study on agent VX, indicate that reproductive/developmental effects are unlikely, a full uncertainty factor of 10 is not warranted.

Therefore,

$$\text{Oral RfD} = \frac{0.0125 \text{ mg/kg/day}}{10 \times 10 \times 3 \times 3 \times 3} \quad (3)$$

$$\text{Oral RfD} = 0.000004 \text{ mg GD/kg/day} \quad (4)$$

$$\text{Oral RfD} = 0.004 \text{ µg GD/kg/day} \quad (5)$$

4.3 Comparison of RfD with Toxicity Data

Only limited data regarding exposure to GD are available for comparison to the proposed RfD. An oral LD_{50} of 5–20 mg/kg for humans was estimated by Somani et al., (1992). The proposed RfD of 0.005 μg/kg is considerably lower than this value. The proposed RfD is more than 2 orders of magnitude below the ED_{50} dose (0.97 μg/kg) shown to produce performance decrements in rhesus monkeys after five consecutive days of dosing (Blick et al., 1994). Nieminen et al. (1990) reported behavioral effects concurrent with reduced blood AChE levels in rats given single i.p. injections of GD at doses of 4 or 20 μg/kg.

5. CARCINOGENICITY ASSESSMENT FOR GD

The potential carcinogenicity of agent GD *cannot be determined.* Data are inadequate for performing a quantitative assessment of agent GD.

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APPENDIX A

Comparison of RfDs, ChE Inhibition and Toxicity Data for GA, GB, GD and VX

Endpoint	GA (μg/kg/day)	GB (μg/kg/day)	GD (μg/kg/day)	VX (μg/kg/day)	Ref.
RfD	0.04	0.02	0.004	0.0006	This report
Estimated no-effect level for RBC-AChE inhibition	-	1.0	-	0.24	**GB**[d] VX[a]
27–33% inhibition of RBC-AChE in humans/oral dose	-	2.3 (3 days)	-	0.2-2.0	**GB** - Grob and Harvey, 1958; **VX**-this report
RBC-AChE inhibition in humans/ i.v. dose	-	-	1.5-2.0 (30%)	1.0 (50%)	DA, 1974; Sidell and Groff, 1974
50–60% RBC-AChE inhibition in humans/oral dose	-	10	-	2.4	**GB** - Grob and Harvey, 1958; **VX**-Sidell and Groff, 1974
50% brain ChE inhibition *in vitro*	1.5×10^{-8} (c)	0.3×10^{-8} (c)	-	-	Grob and Harvey, 1958
Acute toxic effects in humans/oral dose	-	20–30	-	2–4.5	**GB** - Thienes and Haley 1972; Grob and Harvey, 1958; **VX**-Sidell and Groff, 1974
human oral LD_{50} (estimated)	25–50[b]	5–20[b]	5–20	3–10[b]	Somani et al., 1992
rat oral LD_{50}	3700	870–1060 600	400	77–128	DA, 1974 Grob & Harvey, 1958
monkey i.v. LD_{50}	50	20	-	6–11	DA, 1974
rat i.v. LD_{50}	70	45–63	50	6.9–10.1	Dacre, 1984
rat i.p. LD_{50}	490, 800	250 218	-	37–55	DA, 1974 RTECS, 1995

[a] Based on ratio of oral to i.v. doses (2.4 and 1.0 μg/kg, respectively) required for 50% RbC-ChE inhibition and the estimated i.v. no effect dose of 0.1 μg/kg

[b] Values were estimated from animal data.

[c] Molar concentration

[d] Estimated from RBC-ChE_{50} values for GB and VX.

APPENDIX D

Health Risk Assessment for The Nerve Agent VX

HEALTH RISK ASSESSMENT FOR THE NERVE AGENT VX

DRAFT REPORT

September 1996

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Material Chemical Risk Assessment Working Group

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Environmental Risk Assessment Program

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PREFACE

This report assesses the potential non-cancer and cancer effects of chemical agent VX (CAS No. 50782-69-9).

This document supports the activities of the Material/Chemical Risk Assessment Working Group of the Environmental Risk Assessment Program, a cooperative endeavor of the Department of Defense, Department of Energy, and Environmental Protection Agency. This working group is developing toxicity values for selected chemicals of concern at federal facilities. Toxicity values will be submitted for consideration by the EPA's IRIS Consensus Process for inclusion on IRIS (EPA's Integrated Risk Information System). The Material/Chemical Risk Assessment Working Group consists of Drs. Jim Cogliano (chair) and Harlal Choudhury (U.S. EPA), Dr. Bruce Briggs (Geo-Centers); Lt. Cmdr. Warren Jederberg and Dr. Robert L. Carpenter (U.S. Naval Medical Research Institute); Dr. Elizabeth Maull and Mr. John Hinz (U.S. Air Force Occupational and Environmental Health Directorate); Drs. Glenn Leach and Winnie Palmer (U.S. Army Center for Health Promotion and Preventive Medicine); Drs. Robert Young and Po-Yung Lu (Oak Ridge National Laboratory).

This document was written by Dr. Dennis M. Opresko, Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN. Internal peer review was provided by Dr. Robert Young, Dr. Annetta Watson, and Mr. Robert Ross. External review of the toxicity data was provided by Dr. Thomas J. Bucci, Integrated Services, White Hall, AR and Dr. I.K Ho of the U. of Mississippi Medical Center, Jackson MS. External review of the derivation of the RfDs was provided by Drs. Michael Dourson and Susan Velazquez of Toxicology Excellence for Risk Assessment, Cincinnati, OH, and Dr. William Hartley of Tulane Medical Center, New Orleans LA. Additional reviews were provided by Mr. Joe King, Dr. Jack Heller, Ms. Veronique Hauschild, Ms. Bonnie Gaborek, Mr. Maurice Weeks, Maj. Robert Gum, and Mr Kenneth Williams of the U.S Army.

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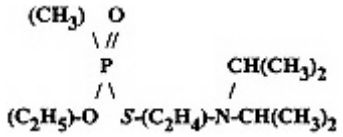
1. INTRODUCTION

Military nerve agents are organophosphate compounds containing either a fluorine, sulfur, or cyanide substituent group (Dacre, 1984). VX contains a sulfur substituent group (for comparison GB contains fluorine and GA contains a cyanide group). The chemical synonyms, Chemical Abstract Service (CAS), Army identification numbers (DA, 1974, 1992; Dacre, 1984), and chemical formula for VX are as follows:

Phosphonothioic acid, methyl-, *S*-[2-[*bis*(1-methylethylamino)ethyl] *O*-ethyl ester;
Phosphonothioic acid, methyl, *S*-(2-(diisopropylamino)ethyl) *O*-ethyl ester;
O-Ethyl *S*-(2-diisopropylaminoethyl) methylphosphonothiolate;
S-2-Diisopropylaminoethyl *O*-ethyl methylphosphonothiolate;
O-Ethyl *S*-(2-diisopropylaminoethyl) methylthiolphosphonate;
TX60;
CAS No. 50782-69-9;
Edgewood Arsenal No. 1701



1.1. PHYSICAL/CHEMICAL PROPERTIES

Agent VX is a colorless to straw-colored liquid with a molecular weight of 267.4 (DA, 1974, MacNaughton and Brewer, 1994); it has a vapor density of 9.2 (air = 1) and a liquid density of 1.0083 g/ml at 25°C (DA, 1974). The vapor pressure of VX is 0.0007 mm Hg at 25°C; its water solubility is 30 g/L per 100 g at 25°C and 7.5 g per 100 g at 15°C (DA, 1974).

1.2. ENVIRONMENTAL FATE

1.2.1 Air

The volatility of agent VX is relatively low (vapor pressure 0.0007 mm HG (DA, 1974; MacNaughton and Brewer, 1994). A vapor concentration of 10.5 mg/m^3 has been reported for a temperature of 25°C (DA, 1974) (although not adequately described in the reference, this is presumably the saturation concentration above a pure liquid). Because VX does not absorb UV radiation above 290 nm (Rewick et al., 1986), photodegradation is not a significant environmental fate process. Based on structure-activity relationships, VX is predicted to react in the troposphere with photochemically produced hydroxyl radicals, with a half-life estimated to be 0.24 days (Atkinson, 1987).

1.2.2 Water

VX has a water solubility of 3 g per 100 g solvent at 25°C and 7.5 g per 100 g solvent at 15°C (DA, 1974). It's Henry's Law Constant has been estimated to be 3.5×10^{-9} atm m^3/mol, indicating a low potential for evaporation from water (MacNaughton and Brewer, 1994); its evaporation rate is about 1/1,500 that of water (Rosenblatt et al., 1995). The agent is relatively resistant to hydrolysis (Franke, 1982); reported half-lives in water at 25°C and pH 7 range from 400 to 1000 hours (Clark, 1989); half-life increases under acidic conditions [(100 days at pH 2–3 (DA, 1974)]. Although solubility is increased at lower temperatures, low temperatures decrease the rate of hydrolysis (Clark, 1989). VX in surface waters may sink and be adsorbed by sediment (Trapp, 1985).

1.2.3 Soil

VX is moderately persistent on bare ground and may remain in significant concentrations for varying time periods, depending on temperature, organic carbon content of the soil, and moisture (Sage and Howard, 1989). Its volatility potential (slope of the vapor pressure vs. concentration in soil organics) of 3.0×10^{-11} mm Hg/mg/kg and its air-soil partition coefficient (for a soil density of 1.4 g/cm^3) of 1.5×10^{-8} mg/m^3 (MacNaughton and Brewer, 1994), indicate that relatively little will evaporate into air. In the laboratory, unstabilized VX of 95% purity decomposed at a rate of 5% per month at 22°C (DA, 1992). In contrast, VX in soils from Carroll Island, MD (a chemical agent test site) decreased to 2.5–7.2% of initial levels (10 mg/g soil) after 14 days storage at room temperature in closed containers (studies reviewed by Small, 1984). In similar studies conducted with soil from Dugway Proving Ground, VX levels (initially 1 mg/g of soil) decreased 79% after 3 days and 90% after 15 days. In other laboratory studies, a VX concentration of 0.2 mg/g in humic sand decreased by 78% after one day, and the same concentration in humic loam and clayey peat decreased by 98% in one day; only 0.1% of the applied amount was detected after 3 weeks in either soil type (Kaaijk and Frijlink, 1977; Verweij and Boter, 1976).

Degradation of VX in soil has also been evaluated in several field studies (see review by Small, 1984). At Carroll Island, MD, VX sprayed on soil decreased by about three orders of magnitude within 17 to 52 days. In an area of Dugway Proving Ground, where VX soil levels prior to 1969 were as high as 6 mg/g, no VX was detected (detection limit 0.4 μg/g) 10 years later. The degradation product, methyl phosphonic acid, was detected at concentrations ranging from 14.9 to 23 μg/g. Approximately three weeks after an accidental release of VX near the Dugway Proving Ground snow samples contained 7–9 ng VX per 400–500 gm of water and grass samples contained 4 μg VX per 900 gm of solid material [estimates based on an assumed 100% extraction efficiency (Sass et al., 1970)].

2. MECHANISM OF ACTION

Nerve agents are inhibitors of acetylcholinesterase (AChE), an enzyme responsible for deactivating the neurotransmitter acetylcholine at some neuronal synapses and myoneural junctions. By a mechanism of phosphorylation, nerve agents act as substrates for the enzyme, thereby preventing deactivation of acetylcholine. The organophosphate-inhibited enzyme can be reactivated by dephosphorylation, but this occurs at a rate that is slower than the rate of reactivation of acetylcholine. Consequently, there is a depletion of acetylcholinesterase and a buildup of acetylcholine. In addition, the nerve agent-enzyme complex can also undergo an "aging" process (thought to be due to a loss of an alkyl or alkoxy group),

whereby it becomes resistant to dephosphorylation (see review by Munro et al., 1994). Differences in rates of aging and reactivation may be important in evaluating toxicity data especially when extrapolating from animal studies to humans. *In vitro* tests conducted by Grob and Harvey (1958) indicate that both GA and GB combine with cholinesterase almost irreversibly during the first hour of their reaction. Sidell and Groff (1974) reported that the GB-ChE complex ages very rapidly *in vivo,* with 45–70% completion by 5 hours after infusion. In contrast, the complex formed between ChE and the nerve agent VX does not age significantly, and the rate of spontaneous reactivation can be as fast as 1%/hr in humans (Sidell and Groff, 1974).

2.1 Effects of Organophosphate Compounds on the Nervous System

The anticholinesterase effects of the organophosphate nerve agents can be characterized as being muscarinic, nicotinic, or central nervous system (CNS)-related. Muscarinic effects occur in the parasympathetic system (bronchi, heart, pupils of the eyes; and salivary, lacrimal and sweat glands) and result in signs of pulmonary edema, bradycardia, miosis, tearing, and sweating. Nicotinic effects occur in somatic (skeletal/motor) and sympathetic systems, and result in muscle fasciculation, muscle weakness, tachycardia, and diarrhea. Effects on the CNS by organophosphates are manifested as giddiness, anxiety, emotional lability, ataxia, confusion, and depression (O'Brien, 1960).

Although the inhibition of cholinesterase within neuro-effector junctions or the effector itself is thought to be responsible for the major toxic effects of organophosphate chemical agents, these compounds can apparently affect nerve-impulse transmission by more direct processes as well. In addition to cholinesterase inhibition, VX reacts directly with ACh receptors and receptors of other neurotransmitters (e.g., norepinephrine, dopamine, gamma-aminobutyric acid) (Zhao et al., 1983; Ho and Hoskins, 1983; Chen and Chi, 1986; Idriss et al., 1986). According to Somani et al. (1992), the direct action of nerve agents on nicotinic and muscarinic ACh receptors may occur when concentrations in the blood rise above micromolar levels, whereas at lower levels the action is mainly the result of inhibition of AChE; however, nanomolar blood concentrations of VX"may directly affect a small population of muscarinic ACh receptors that have a high affinity for [^{3}H]-*cis*-methyldioxalane binding" (Somani et al., 1992). VX may also counteract the effects of ACh by acting as an open channel blocker at the neuromuscular junction, thereby interrupting neuromuscular function (Rickett et al., 1987).

Exposure to some organophosphate cholinesterase inhibitors results in a delayed neuropathy characterized by degeneration of axons and myelin. This effect is not associated with the inhibition of acetylcholinesterase, but rather with the inhibition of an enzyme described as neuropathy target esterase (NTE); however, the exact mechanism of toxicity is not yet fully understood (Munro et al., 1994). For some organophosphate compounds, delayed neuropathy can be induced in experimental animals at relatively low exposure levels, whereas for others the effect is only seen following exposure to supralethal doses when the animal is protected from the acute toxic effects caused by cholinesterase inhibition. Although there is the potential for nerve agents to have direct toxic effects on the nervous system, there is no evidence that such effects occur in humans at doses lower than those causing cholinesterase inhibition. For the purpose of evaluating potential health effects, inhibition of blood cholinesterase is generally considered the most useful biological endpoint.

2.2 Effect on Blood Cholinesterases

Acetylcholinesterase is a natural component of human blood, where it is found on the surface of red blood cells (RBC-AChE). RBC-AChE activity, as well as the activity of a second type of cholinesterase found in blood plasma (butyrylcholinesterase, or plasma cholinesterase), have been used to monitor exposure to organophosphate compounds (pesticides and nerve agents). Both RBC-AChE and plasma-ChE have been used as bioindicators of potential toxic effects of organophosphate cholinesterase inhibitors. There is some evidence that RBC-AChE is as sensitive as brain ChE to the effects of nerve agents. Grob and Harvey (1958) reported that the *in vitro* concentrations producing 50% depression of brain-ChE and RBC-AChE activity were the same in the case of GA (1.5×10^{-8} mol/L), and only slightly different (3×10^{-9} mol/L and 3.3×10^{-9} mol/L) in the case of GB. However, *in vivo* animal studies indicate a poor correlation between brain and RBC-AChE in cases of acute exposures (Jimmerson et al., 1989), and this is reflected in the fact that blood cholinesterase activity may not always be correlated with exposure or with signs and symptoms of toxicity (Holmstedt, 1959). Acute exposures to high concentrations may cause immediate toxic effects before significant changes occur in blood ChE activity, and repeated exposures over a period of several days may result in a sudden appearance of symptoms due to cumulative effects (Grob and Harvey, 1958). Conversely, blood ChE activity can become very low without overt signs or symptoms during chronic exposures to low concentrations of organophosphates. This may be due to a slower rate of recovery of RBC-ChE compared to tissue ChE, or to a noncholinesterase-dependent recovery pathway for neural tissue (Grob and Harvey, 1958). Sumerford et al. (1953) reported that orchard workers exposed to organophosphate insecticides had RBC-AChE values as low as 13% of preexposure values without any other signs or symptoms of exposure. Animal studies have demonstrated that chronic exposures to low concentrations of organophosphate insecticides can also result in increased tolerance levels (Barnes, 1954; Rider et al., 1952; Dulaney et al., 1985). Similarly, Sumerford et al. (1953) reported increased levels of tolerance to organophosphate insecticides in people living near orchards subject to insecticide applications. Such adaptation may result from increased rates of formation of blood ChE, or from increased rates of detoxification. Additional information on the development of tolerance to organophosphate cholinesterase inhibitors can be found in a review paper by Hoskins and Ho (1992).

The blood cholinesterases may, to some degree, provide a protective effect by binding with some fraction of the anticholinesterase compound (Wills, 1972). However, not all nerve agents bind equally well with all cholinesterases. In tests conducted on dogs, Holmstedt (1951) found that GA affected RBC and plasma cholinesterase to a nearly equal degree. In contrast, VX preferentially inhibits RBC-ChE; 70% compared with about 20% inhibition of plasma ChE (Sidell and Groff, 1974). Rodents (but not humans) have other enzymes in the blood, termed aliesterases, which can bind with organophosphates, thereby reducing the amount available for binding with acetylcholinesterase (Fonnum and Sterri, 1981). Agent GB binds with aliesterases; however, according to Fonnum and Sterri (1981), VX has a quaternary ammonium group which prevents it from being a substrate for aliesterases. The strong specificity of agent VX to AChE may account, in part, for the fact that it is more acutely toxic than agents GA, GB, or GD (see Appendix A).

2.2.1 Intra- and Interspecies Variation in Blood Cholinesterase Activity

Although blood cholinesterase activity is used as a measure of exposure to organophosphate compounds, baseline activity levels can vary between individuals and between species. According to Wills (1972), both plasma- and RBC-ChE activity are generally lower in women than in men. Sidell and Kaminskis (1975) reported that, for a test population of 22 human subjects, the highest coefficient of

variation of RBC-ChE was 4.1% per single subject; the average range of variation was ± 2.1% for men and ± 3.1% for women. In individuals studied for one year, the RBC-ChE activity varied by 11% in men and 16% in women. Yager et al. (1976) reported a 10.0% intra-individual coefficient of variation for RBC-ChE and 14.4% for plasma-ChE. Callaway et al. (1951) estimated that with only one pre-exposure measurement, the smallest measurable decrease was 15% of the baseline value for RBC-ChE activity and 20% of the baseline for plasma-ChE.

A small subpopulation of men and women have a genetic defect causing their blood cholinesterase activity to be abnormally low (Evans et al., 1952; Harris and Whittaker, 1962). For homozygous individuals, the activity can be as low as 8–21% of the normal mean (Bonderman and Bonderman, 1971). Morgan (1989) suggests that these individuals may be unusually sensitive to organophosphate anticholinesterase compounds.

Data compiled by Ellin (1981) reveal that the RBC-ChE activity for humans is slightly higher than that for monkeys and much higher than that for rats and other laboratory animals (Table 1).

Table 1. RBC-ChE activity in different species

Species	RBC-ChE activity (μmol/mL/min)	Optimum substrate[a] concentration (M)
Human	12.6	2×10^{-3}
Monkey	7.1	2×10^{-3}
Pig	4.7	1×10^{-3}
Goat	4.0	2×10^{-3}
Sheep	2.9	2×10^{-3}
Mouse	2.4	2×10^{-3}
Dog	2.0	2×10^{-2}
Guinea pig	2.7	2×10^{-3}
Rabbit	1.7	5×10^{-3}
Rat	1.7	5×10^{-3}
Cat	1.5	5×10^{-3}

Source: Ellin, 1981

[a] Acetylthiocholine iodide concentration for maximum RBC-ChE activity.

These differences in RBC-ChE activity may affect a species' sensitivity to a particular organophosphate compound. At the same time, the relative amount of plasma cholinesterase and other compounds in the blood that can bind to the organophosphate agents must also be considered. As noted above, rodents, but not humans, have high levels of aliesterases in the blood (Cohen et al., 1971). These compounds may provide rats and mice with a higher level of resistance to anticholinesterase compounds to which they bind, such as GB, but not to others such as VX (Fonnum and Sterri, 1981).

2.2.2 Potency of Nerve Agents as Cholinesterase Inhibitors

The potency of the anticholinesterase activity of nerve agents and other organophosphates is measured by either the bimolecular rate constant (k_i) for the reaction of the phosphate compound with the enzyme or by the molar concentration causing 50% inhibition of the enzyme (I_{50}) *in vitro*. I_{50} data for several organophosphate nerve agents have been tabulated by Dacre (1984). The pI_{50} (negative log of the molar concentration causing 50% inhibition) for VX was reported to be 8.8. The relationship between I_{50} and k_i as a function of time (t) is expressed by the following equation (Eto, 1974):

$$I_{50} = \frac{0.693}{t \times k_i}$$

Relative potency of nerve agents can also be expressed in terms of the *in vivo* dose necessary to produce the same level of cholinesterase inhibition by a specific exposure route. As would be expected, the effectiveness of the agents in inhibiting cholinesterase is closely correlated with their acute toxicity (see Appendix A).

2.2.3 Cholinesterase Inhibition by VX

The potency of VX in a given species can be expressed in terms of the dose necessary to produce 50% inhibition of AChE ($AChE_{50}$). In humans, the RBC-$AChE_{50}$ for VX is 0.001 mg/kg for an intravenous (i.v.) dose (Sidell and Groff, 1974), 0.0023 mg/kg for an oral dose (Sidell and Groff, 1974), and 0.034 mg/kg (12 hr) and 0.029 mg/kg (24 hr) for a dermal dose of an undiluted liquid (Sim and Stubbs, 1960). These data indicate that an oral dose about 2 times greater than an i.v. dose is needed to produce the same amount of AChE inhibition in the blood. Similiar information is available from animal studies. Goldman et al. (1988) dosed Sprague-Dawley rats with 4 μg/kg VX by various routes of exposure and measured RBC-AChE activity after 3 and 24 hr. The i.v. and s.c. routes resulted in the greatest decreases in RBC-AChE. RBC-AChE levels (expressed as fraction of control values) were 0.14 ± 0.07 at 3 hr and 0.20 after 24 hr for i.v., and 0.13 ± 0.07 at 3 hr and 0.20 after 24 hr for s.c. In contrast, RBC-AChE levels after intragastric administration were 0.48 ± 0.14 of controls at 3 hr and 0.46 after 24 hr, and after intraperitoneal (i.p.) administration 0.35 ± 0.19 at 3 hr and 0.35 after 24 hr. These data indicate that the s.c. and i.v. routes produced a similar level of inhibition, with an estimated RBC-$AChE_{50}$ of about 1 μg/kg. The intragastric route was much less effective in reducing RBC-AChE, possibly due to hydrolysis and detoxification in the stomach and/or limited absorption through the gastrointestinal tract.

3. TOXICOLOGY

3.1 Introduction

Health and environmental impacts of nerve agents and related compounds (i.e., organophosphate insecticides) have been reviewed by O'Brien (1960), Matsumura (1976), Dacre (1984), Carnes and Watson (1989), Watson et al. (1989), and Munro et al. (1994). A brief discussion of the general toxicology of nerve agents and related organophosphate pesticides is given below.

Nerve agents are toxic by all routes of exposure. Initial symptoms of acute poisoning are fatigue, headache, mild vertigo, weakness, and loss of concentration. Moderate exposures result in miosis and excessive sweating, tearing, and salivation. Acidosis and hyperglycemia may also occur in addition to muscular weakness, muscular twitching, lacrimation, urination, and defecation. Acute poisoning can result in prostration, clonic convulsions (rapid repetitive movements), and tonic convulsions (limbs stretched and rigid) (Matsumura, 1976). Exposures sufficiently high to cause convulsions have resulted in brain lesions and cardiomyopathy in laboratory animals (Singer et al., 1987).

In addition to the immediate toxicity of the nerve agents, there is concern that exposures may lead to chronic neurological effects similar to those reported for some related organophosphate insecticides. Included among these possible effects are organophosphate-induced delayed neuropathy (OPIDN), EEG changes, and long-term psychological disturbances (Munro et al., 1994). OPIDN, which appears 5–30 days after exposure, manifests itself as muscle weakness, tingling, and twitching followed by paralysis (Munro et al., 1994). Histopathological changes, which consist of degeneration of axons and myelin of the nervous system, can be correlated, not with inhibition of acetylcholinesterase, but rather with inhibition of an enzyme described as neuropathy target esterase (NTE); however, the exact mechanism of toxicity is not yet fully understood (Munro et al., 1994). There is no clinical or experimental evidence to suggest that VX causes OPIDN in humans. In addition, in studies conducted with antidote-protected animals dosed with supralethal amounts of VX no signs of OPIDN were observed (see Section 3.5). The available data, therefore, indicate that OPIDN is unlikely to occur in humans exposed to VX.

Acute exposures to nerve agents are known to result in EEG changes and psychological effects (Grob and Harvey, 1958; Sidell, 1992). Some studies have indicated that changes in EEG patterns may persist for long periods of time after exposure (Metcalf and Holmes, 1969; Burchfiel et al., 1976; Duffy et al., 1979; Duffy and Burchfiel, 1980); however, the reported changes have been considered to be clinically insignificant and not correlated with behavioral or physiological changes (DHHS, 1988). Although acute exposures can also induce neuropsychological changes, there is no evidence of these effects persisting for months or years as has been reported for some organophosphate insecticides (Savage et al., 1988; Gershon and Shaw, 1961; Mick, 1974; Rodnitzky, 1974; Wagner, 1983; Tabershaw and Cooper, 1966). The available data for the organophosphate insecticides suggest that chronic neuropsychological effects (excluding OPIDN) do not occur in the absence of significant changes in blood cholinesterase. The same conclusion may apply to the organophosphate nerve agents.

3.2 Acute Toxicity

Limited information is available on the oral toxicity of VX to humans. In clinical studies conducted by Sidell and Groff (1974), single oral doses of 2–4.5 μg VX/kg produced gastrointestinal symptoms in 5 of 32 test subjects. Regression analysis of the dose-response data indicated that the RBC

ChE_{50} was 2.3 μg/kg. Sim et al. (1964) reported no signs of toxicity in human volunteers receiving 1.43 μg VX/kg/day for seven days (in four daily doses of 500 mL drinking water); however, average RBC-ChE activity was reduced 60% (to 40% of baseline values).

Data compiled by Sidell (1992) revealed that for individuals exposed to VX dermally, gastrointestinal symptoms (vomiting) occurred in 0.6% (1/166) when RBC-ChE activity was 50% of control values, and in 8% (2/24), 33% (9/27), 45% (19/42) and 67% (16/24) when RBC-ChE levels were 40–49%, 30–39%, 20–29%, and less than 20% of control values, respectively. Sim (1962) reported that a dose of 5 μg VX/kg applied to the cheeks or ear lobes resulted in symptoms of systemic toxicity in about half of the test subjects.

Several studies have been conducted in which human volunteers were injected intravenously with VX. Kimura et al. (1960) reported that a 30-sec i.v. injection of 0.04 μg/kg in one adult test subject caused headaches, tiredness and irritability, but no change in RBC or whole blood cholinesterase activity. A subsequent 30-sec i.v. injection of 0.08 μg/kg 3.5 hr later resulted in headaches, lightheadedness and abdominal cramps as well as an increase in airway resistance, a decrease in respiratory rate, a decrease in pulse rate and an increase in minute volume, but no change in cholinesterase activity. A single 30-sec i.v. dose of 0.225 μg/kg resulted in a 27% decrease in baseline RBC-ChE activity within 15 min and frontal retrobulbar headaches in one test subject. Six subjects receiving 1 μg VX/kg by i.v. infusion over 1.75–4 hr periods exhibited 50–60% depression in cholinesterase activity but no signs of toxicity (except for one 84 kg individual who reported headaches). Sidell and Groff (1974) reported that an i.v. dose of 1.5 μg VX/kg in 18 test subjects resulted in dizziness, nausea, and vomiting in 11, 4, and 6 individuals, respectively; RBC-ChE was depressed 55–90% from baseline values (average about 75%). Regression analysis of dose response data (doses of 1.2–1.7 μg/kg) indicated that the ChE_{50} was 1.1 μg VX/kg. Using the data provided by Kimura et al. (1960), McNamara et al. (1973) concluded that an i.v dose of 0.1 μg/kg would have no effect on RBC-ChE activity.

Based on inhalation data for agent GB, McNamara et al. (1973) calculated the no-effect dose for VX-induced tremors in humans to be 0.34 μg/kg. Carnes et al. (1986) suggested that the threshold for muscular tremors in sensitive subpopulations, such as infants, may be 0.16 μg/kg. McNamara et al. (1973) estimated that the human LD_{50} and no-death levels for VX were 7.5 μg/kg and 0.94 μg/kg, respectively. These estimates were based on extrapolations of LCt_{50} data for GB.

The short-term toxicity of VX to Sprague-Dawley rats was investigated by Goldman et al. (1988). In one series of tests, a single subcutaneous (s.c) injection of 1 μg VX/kg resulted in a 50% inhibition of RBC-AChE relative to controls and a dose of 4 μg/kg resulted in a 87% inhibition relative to controls. In a second test series, male rats were dosed with 4 μg VX/kg by different exposure routes and RBC-AChE levels relative to controls were determined at 3 hr and at 24 hr. High variability in response was seen in animals dosed by intratracheal instillation, intragastric lavage, and i.p. injection. RBC-AChE levels were reduced a similar amount for s.c. injections (13 ± 7% of control values at 3 hr and 21% at 24 hr) and i.v. injections (14 ± 0.07% of control values at 3 hr and 20% at 24 hr). In another pilot study, male and female rats (8–10 weeks old) were injected subcutaneously with 0, 0.25, 0.63, 1.56, 3.91, 9.77, or 14.65 μg VX/kg/day, 5 days/week for 14 days. Each dose group consisted of eight males and eight females except for the high-dose group which had two males and two females. All the animals in the two highest dose groups died as a result of the exposures, but none of the animals in the three other dose groups died as a result of the exposures. Of the remaining animals, one-half was sacrificed at 7 days and the other half at 14 days. RBC-AChE activity levels were depressed in all dose groups in a dose-dependent manner (the results for the 0.25 and 1.56 μg/kg/day dose levels are included in Table 3).

3.3 Subchronic Toxicity

Rice et al. (1971) fed VX to healthy yearling ewes (Columbian, or Columbian crossed with Shropshire, or Rambouillet) for 56 days. The dose levels were 0, 3, 9, or 15 μg/day (five animals per dose group and ten controls). The agent was mixed with Pillsbury 16% rabbit pellets and hand-fed to the test animals. The animals were checked periodically during the feeding period for clinical signs of toxicity (i.e., slowed pupil response, slowed pain reflex, and profuse salivation). There were no reported data identifying changes in clinical chemistry (except whole blood cholinesterase), hematology, body and organ weights, or gross or microscopic pathology. Whole blood ChE activity levels were monitored prior to the first exposure (one determination per animal) and then 16 times during the 56-day test period. Each whole blood ChE determination was first normalized to the average control value for the same time period to give an adjusted daily value. The normalized values for each time period were then compared to the average normalized pre-exposure (baseline) ChE value for each dose group (Table 2). According to Rice et al. (1971), whole blood ChE was significantly depressed at all dose levels (statistical analysis and levels of significance not reported). It was also reported that at the lowest dose (3 μg/day) the decrease in ChE was statistically significant by the twenty-first day. Whole blood ChE stabilized at about 62% of the adjusted baseline by the thirty-first day and remained at this level for the remainder of the 56-day feeding period (see Appendix B). The sheep used in this study had an average weight of 52.7 kg; therefore, the weight-normalized dose for 3 μg/day was 0.06 μg/kg/day. None of the sheep dosed with 3, 9, or 15 μg/day exhibited any physical signs of clinical toxicity, even though the whole blood ChE in the highest dose group was reduced to 5% of the normalized baseline values for the last three weeks of the feeding period. In additional studies (also reported in Rice et al., 1971), physical signs of VX toxicity (not

Table 2. RBC-ChE activity in sheep fed VXa

	VX dose					
Time (hr)[a]	**3 μg/day**		**9 μg/day**		**15 μg/day**	
	Fraction control[b]	Fraction baseline[c]	Fraction control[b]	Fraction baseline[c]	Fraction control[b]	Fraction baseline[c]
0	1.15	1.00	1.03	1.00	1.17	1.00
24	1.05	0.91	0.84	0.81	0.86	0.74
240	1.16	1.01	0.58	0.57	0.51	0.44
360	0.92	0.80	0.55	0.53	0.29	0.24
744	0.72	0.62	0.26	0.25	0.09	0.07
912	0.73	0.64	0.12	0.11	0.05	0.04
1320	0.72	0.63	0.20	0.19	0.08	0.07

Source: Rice et al., 1971

[a] Selected data points presented

[b] Data expressed as a fraction of the control value ChE.

[c] Data expressed as fraction of adjusted baseline ChE

described in detail) were observed in "culled" or weakened ewes dosed with 30 μg VX/day for about 4 weeks and in healthy sheep dosed with 75 μg VX/day for about 3 weeks. Rice et al. (1971) noted that the culled animals surviving the exposure recovered fully without developing any permanent signs of toxicity. Because of the significant reduction in whole blood ChE (38% relative to pre-exposure values), the test dose (3 μg/day) in the 8-week study is considered a lowest-observed-adverse-effect level (LOAEL). Alternately, based solely on the reported physical signs of clinical toxicity, the dose of 75 μg/day from the 3-week study is a LOAEL (for healthy animals), and 15 μg/day (from the 8-week study) is a no-observed-adverse-effect level (NOAEL), even though there was significant reduction in whole blood ChE at this dose (i.e., 96% reduction relative to pre-exposure values by day 38).

The subchronic toxicity of VX to animals was also investigated by Goldman et al. (1988) who injected rats (25/sex/dose group) subcutaneously with VX 5 days/week, for up to 90 days. The administered doses were 0 (saline controls), 0.25, 1.0, or 4.0 μg VX/kg/day. Five animals of each sex were sacrificed at 30, 60, and 120 days (includes a 30-day recovery period), and 10 animals of each sex were sacrificed at 90 days. RBC-AChE activity levels were monitored in 2 of 5 or in 3 of 10 animals per sex at each of the sacrifice times; blood chemistry was evaluated in the remaining animals. The tissues of all sacrificed animals were processed for histological analysis. Urinalysis were conducted on samples collected during weeks 8 and 12. Animals in the highest dose group exhibited body weight loss and behavioral changes (increased irritability and aggressiveness by week 2, followed by decreased grooming and lethargy at week 8). There were periodic cases of diarrhea in this group. By week 5, some of the animals dosed with 1.0 μg/kg/day exhibited some irritability.

Relative brain weight (ratio of brain to body weight) was elevated in the 4.0 μg/kg/day group. There were no significant changes in clinical chemistry or urinalysis parameters that were dose-related. Histopathological examination did not indicate a VX-associated pathology. In a separate 3-generation study in which hematological parameters were evaluated in rats maintained for 120 days under the same exposure protocol, there were no significant effects in male rats; however, in F_0 females dosed with 4.0 μg/kg/day, statistically significant decreases occurred in hemoglobin, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin (Goldman et al., 1988).

Plasma cholinesterase was significantly reduced at 30 days ($p < 0.05$) in males and females given 1.0 μg/kg/day and at 30, 60, and 90 days for males and females given 4.0 μg/kg/day. RBC-AChE levels in males and females were reduced in a dose-dependent manner when compared to control values for the same time period (Table 3); however, the study did not include baseline or preexposure RBC-AChE levels for each test group. Goldman et al. (1988) reported that the observed decreases were significant but the level of statistical significance was not reported. The 30-day data for both males and females were reanalyzed using ANOVA and Dunnett's and Scheffe's Comparisons, and RBC-AChE activity in all dose groups for both sexes was significantly lower ($p < 0.05$) than control values for the same time period (Appendix C). RBC-AChE levels in both males and females returned to 83–98% of control values in the test groups allowed a 30-day recovery period. Daily exposure to VX by s.c. injection for 30 days resulted in statistically significant depression of RBC-AChE at all dose levels.

3.4 Chronic Toxicity

Data on the chronic toxicity of VX were not found in the available literature.

Table 3. RBC-ChE activity in rats injected subcutaneously with VXa

			VX dose (μg/kg/day)[b]			
Time of sacrifice	**Sex**	**No.**	**0.25**	**1.0**	**1.56**	**4.0**
7 days[c]	M	4	0.85 ± 0.06	—	0.46 ± 0.07	0.31 ± 0.03[d]
	F	4	0.90 ± 0.05	—	0.36 ± 0.09	0.34 ± 0.13[d]
14 days[c]	M	4	0.72 ± 0.07	—	0.34 ± 0.12	0.29 ± 0.03[d]
	F	4	0.64 ± 0.12	—	0.28 ± 0.13	0.31 ± 0.08[d]
30 days[e]	M	2	0.46 ± 0.04	0.22 ± 0.00	—	0.04 ± 0.02
	F	2	0.48 ± 0.06	0.20 ± 0.01	—	0.10 ± 0.05
60 days[e]	M	2	0.33 ± 0.17	0.23 ± 0.06	—	0.14 ± 0.00
	F	2	0.53 ± 0.04	0.34 ± 0.02	—	0.23 ± 0.02
90 days[e]	M	3	0.34 ± 0.02	0.37 ± 0.03	,:—	,,0.23 ± 0.08
	F	3	0.64 ± 0.13	0.51 ± 0.17	—	,:0.27 ± 0.07
120 days[f]	M	5	0.91 ± 0.07	0.88 ± 0.11	,:—	,,0.83 ± 0.11
	F	5	0.98 ± 0.12	0.93 ± 0.13	—	,:0.88 ± 0.16

Source: Goldman et al., 1988

[a] Data expressed as fraction of control value; mean ± SD; includes data from 14-day pilot study and 90-day study.

[b] Dosing schedule once per day, five days per week.

[c] Fourteen-day study.

[d] Dose was 3.91 μg/kg/day.

[e] Ninety-day subchronic study

[f] Includes recovery period of 30 days.

3.5 Nervous System Toxicity

Sidell and Groff (1974) reported that volunteers dosed with VX (1.5 μg/kg, i.v.) exhibited a significant decrement in performance on a number facility test within 1 hr after treatment. Bowers et al. (1964) reported anxiety, psychomotor depression, intellectual impairment, and unusual dreaming in volunteers exposed to VX dermally and in whom RBC-ChE was depressed 70% or greater.

No clinical or experimental evidence is available to indicate that VX causes delayed neuropathy in humans (Munro et al., 1994). Chickens injected subcutaneously with supralethal doses of VX (10, 100, or 150 μg/kg, following treatment with antidotes to protect against acute toxicity) exhibited no signs of a delayed neurotoxic response (Goldman et al., 1988). However, QL, a chemical intermediate of VX, has been reported to cause delayed neurotoxic effects in hens dosed at •635 mg/kg (Olajos et al., 1986). The available data indicate that delayed neuropathy in humans exposed to VX is unlikely.

3.6 Developmental and Reproductive Effects

In studies conducted by Schreider et al. (1984), pregnant rats were dosed with 0.25, 1.0, or 4.0 μg VX/kg by s.c. injection on days 6–15 of gestation. The animals were sacrificed on day 20 of gestation. The examined fetuses showed no evidence of malformations. Fetal body weight, litter size, and sex ratio were within normal limits.

The effects of VX on the development and reproduction of sheep were evaluated by Van Kampen et al. (1970) following an accidental release of the nerve agent VX in Skull Valley, Utah. Of some 6,300 affected animals, about 4,500 died or were killed (Van Kampen et al., 1970). Seventy-nine surviving animals that had been pregnant at the time of exposure and their lambs were evaluated for changes in RBC-AChE activity and for signs of toxicity over a 6-month post-exposure period. RBC-AChE activity in the ewes remained significantly depressed for about four months and then returned to normal. Ewes that were sacrificed at 2-week intervals had no gross or microscopic evidence of damage to the central nervous system. Torticollis (wryneck) developed in one ewe one week following exposure and persisted for nine months (a similar effect was seen in 1 of 38 ewes dosed in the laboratory with an undisclosed amount of VX). Of the lambs born 2–3 months after exposure of the ewes, only one (total number examined not reported) exhibited deformities (extra oral opening below the right ear), but these were not considered to be agent related. None of the lambs displayed neurotoxic signs or symptoms, and their whole blood cholinesterase activity was not reduced even when suckling from exposed and affected ewes. Five months after exposure, the ewes exposed in the field as well as ewes dosed with an undisclosed amount of VX four months previously, were mated to unexposed males. Examination four months later indicated that fetal growth and development were normal except for one fetus that appeared stunted (total number examined not reported). The investigators concluded that VX had little or no effect on fetal growth or development.

Goldman et al. (1988) administered VX subcutaneously to Sprague-Dawley rats on days 6–15 of gestation. The administered doses were 0, 0.25, 1.0, or 4.0 μg/kg/day. Body weight, frequency of visceral and skeletal abnormalites, litter size, and sex ratios were evaluated. There was no statistical evidence that VX affected any of the parameters studied. Blood cholinesterase levels were not monitored.

Goldman et al. (1988) administered s.c. doses of 0, 0.25, 1.0 and 4.0 μg VX/kg/day to New Zealand white rabbits on days 6–19 of gestation. Animals were also observed daily for signs of toxicity. The does were sacrificed on day 29 of gestation. Body weight, fetal weights, fetal deaths, frequency of visceral and skeletal abnormalites, litter size, and sex ratios were evaluated. There was no statistical evidence that VX affected any of the parameters studied. Blood cholinesterase levels were monitored in a 7-day pilot study which also included a dose of 8 μg/kg. The 8 μg/kg dose was severely toxic to the rabbits (1/3 died, 2/3 ataxic). The dose of 0.25 μg/kg resulted in a level of RBC-AChE inhibition equal to 0.71 of the control value, but produced no signs of toxicity.

In a modified dominant lethal study, Goldman et al. (1988) administered VX by subcutaneous injection to male and/or female Sprague-Dawley rats and observed the effects on various parameters including terminal body weight, testes weight, testicular histopathology, maternal weight, implantation sites, resorptions, and total corpora lutea. The test animals were dosed with 0 (saline control), 0.25, 1.0, or 4 μg VX/kg/day for ten weeks. Triethylenemelamine was used as a positive control. Exposure to VX produced no significant changes in body or organ weights. VX had no adverse effects on preimplantation losses as evaluated by number of implants, live fetuses, dead fetuses, and resorptions. Microscopic examination of the testes did not reveal any abnormalities that could be attributed to VX exposure.

In a 3-generation study, male and female Sprague-Dawley rats were dosed subcutaneously with 0 (saline controls), 0.25, 1.0, or 4.0 μg VX/kg/day, 5 days/week (Goldman et al., 1988). The F_0 generation (11–12 males and 24 females per dose group) was dosed for about 105 days after which they were mated and the dosing continued through gestation and weaning (total duration of dosing 21–25 weeks). Dosing of the F_1 generation began after weaning and continued for approximately 126 days after which they were mated and dosing continued through gestation and weaning (total duration 24–27 weeks). Five males and 5 females of each dose group of the F_2 generation were sacrificed at weaning. The study included analysis of pup mortality in each of the generations, body and organ weight changes and hematological parameters in the F_0 generation, and histopathological examination of tissues (including nervous system, reproductive system, gastrointestinal tract, lung, liver, and kidney) of the F_1 parental males and females, the F_1 weanlings, and the F_2 weanlings. Blood cholinesterase activity levels were not monitored during the study. VX exposure had no adverse effect on the number of pups born in the F_1 or F_2 generation. Perinatal mortality (i.e., percent of pups born dead or dying within 24 hr of birth) was not significantly different among dose levels for both generations; however, perinatal mortality in the high-dose group (5.7%) was considerably higher than that in the lower dose groups (1.2%). Pup mortality from birth to weaning was significantly ($p < 0.01$) related to VX exposure primarily for the F_1 generation pups in the 4.0 μg/kg/day dose group. Goldman et al. (1988) attributed this increase to the effect of VX on the dams which resulted in the increased incidence of cannibalism of the pups; the investigators concluded that under the conditions of the test, there was no evidence of direct VX reproductive toxicity. The hematological studies conducted on dosed males of the F_0 generation revealed no significant VX-associated effects. In females dosed with 4.0 μg VX/kg/day, statistically significant decreases occurred in hemogloblin, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin. Body and organ weight analysis and histopathological examination revealed three effects that may have been dose related - changes in brain weight, incidence of eosinophilic gastritis, and incidence of pituitary cysts; however, Goldman et al. (1988) attributed the first two effects to statistical chance and considered the third as not being biologically significant. The overall conclusion of the investigators was that there were no organ weight or microscopic changes that could be attributed specifically to the action of VX.

3.7 Carcinogenicity

No information is available regarding the potential carcinogenicity of VX in humans. Standard long-term carcinogenicity studies have not been conducted on laboratory animals exposed to VX. Neoplastic lesions were not observed in male and female CD rats injected subcutaneously with up to 0.25, 1.0, or 4.0 μg VX/kg/day for 90 days (Goldman et al., 1988). No other animal data are available to assess the potential carcinogenicity of VX.

3.8 Genotoxicity

No information is available regarding the genotoxicity of VX in humans. In tests on microorganisms and mammalian cell cultures, VX was not found to be mutagenic or was only weakly mutagenic (Goldman et al., 1988). VX did not induce biologically significant increases in mutations when tested in the Ames Salmonella assay using five revertant strains (TA135, TA100, TA98, TA1537, and TA1538) with and without metabolic activation (Goldman et al., 1988). In tests using the yeast Saccharomyces cerevisiae, VX did not induce recombinants following exposures to concentrations as high as 100 μg/mL (Goldman et al., 1988). VX also failed to induce forward mutations when tested on mouse L5178Y lymphoma cells at concentrations less than 50 μg/mL (Goldman et al., 1988). Although doses of 50 and 100 μg VX/mL resulted in increased numbers of mutations; these were not more than 1.5 times the control level (a 2-fold increase was considered the minimum required to establish a positive result).

4. ORAL REFERENCE DOSE FOR VX

4.1 Cholinesterase Inhibition as an RfD Endpoint

The critical endpoint for defining a maximum acceptable exposure level for nerve agents such as VX is considered to be the level at which no significant depression in blood cholinesterase activity occurs. In humans, 15% inhibition is generally considered to be the minimal change that can be observed with any statistical reliability (Callaway et al., 1951). Existing human response data (Marquis, 1988) indicate that human RBC-AChE inhibition of as much as 20% is not associated with adverse clinical signs or symptoms and should be considered only as evidence of organophosphate exposure. This contention is supported by the U.S. EPA (1995a) which reports scientific agreement that statistically significant inhibition of cholinesterase in multiple organs and tissues accompanied by clinical effects constitutes a hazard; however, in the absence of clinical effects, such inhibition may not be of biological significance. It is generally agreed that inhibition of RBC and/or plasma cholinesterase contributes to the overall hazard identification of cholinesterase inhibiting agents by serving as biomarkers (U.S. EPA, 1995a). In addition, animal data have shown that exposure to low doses of nerve agents for extended periods of time can result in low blood ChE activity levels without signs of toxicity. Bucci et al. (1992) found no evidence of toxicity in rats dosed i.p. with GA (up to 112 μg/kg), even though RBC-AChE activity was reduced about 37% in females (relative to controls). In oral toxicity studies conducted on GB, Bucci and Parker (1992) found that gavage doses of 0.3 mg/kg/day to rats caused nearly a 50% reduction in RBC-AChE activity without signs of toxicity. Goldman et al. (1988) observed no physical signs of clinical toxicity in Sprague-Dawley rats dosed subcutaneously with 1.0 μg VX/kg/day over 30 days, even though RBC-AChE activity was reduced 78–80% relative to controls. Rice et al. (1971) reported that whole blood cholinesterase of sheep dosed orally with 15 μg VX/day was reduced to 5% of the normalized baseline values (during the last 3 weeks of a 8-week dosing period) without any physical signs of clinical toxicity. Rice et al. (1971) also found that sheep showing signs of toxicity at higher dose levels recovered fully after the exposures ended. Further complicating the evaluation is the extreme variability in ChE levels of individual animals and different sexes and ages of the same species (Halbrook et al., 1992). Possible changes in blood ChE that may occur with increasing age of the animals requires comparisons with concurrent controls, because the absence of a significant difference from pre-exposure value may be due to age-related increases in ChE in the dosed animals.

Blood ChE activity has been used by EPA as the critical endpoint in the establishment of oral RfDs for organophosphate insecticides. In the case of malathion (U.S. EPA, 1995b), the no-observed-effect level (NOEL) was identified as the highest oral dose level at which no significant change in RBC-AChE or plasma-ChE activity was recorded in five human volunteers who received the compound orally for 47 days (Moeller and Rider, 1962). The next highest dose was associated with a depression of about 25% in both RBC-AChE and plasma-ChE, but no clinical signs of toxicity. The EPA approach, also used for other organophosphate pesticides, is, therefore, to identify the lowest-effect level (LEL) as the dose at which statistically significant decreases in ChE levels (RBC-AChE, plasma-ChE, or brain-ChE) occur, and then to base an RfD on the dose level where the change in ChE is not statistically significant. This approach is also used in this report so that the RfDs developed for the nerve agents will not be disproportionally different from those for organophosphate insecticides; however, it should be emphasized that these values may be overly conservative. Furthermore, in evaluating the experimental data for the nerve agents, added weight was given to those cases where significant changes in ChE occurred relative to both control and pre-exposure values and where there was evidence of a dose-response relationship.

4.2 Derivation of the Oral RfD for VX

For the derivation of an oral RfD, chronic or subchronic human oral exposure data are preferred; however, the only available human dose-response data for VX pertain to acute exposures. Although such data can be used to establish short-term exposure limits, they are generally not used for establishing subchronic or chronic reference doses (for comparative purposes, an RfD for VX was derived using short-term human exposure data; see Appendix D).

No chronic animal toxicity studies have been conducted on VX; however, there are two subchronic studies which can be used for developing an RfD. In one study, rats were dosed by s.c. injection 5 days per week for 90 days (Goldman et al., 1988). In the second study, sheep received daily doses of VX in feed for 56 days (Rice et al., 1971). Both of these studies identify blood cholinesterase as the most sensitive endpoint. Data are available indicating that sheep are more sensitive than rats to the toxic effects of VX. Ivanov et al. (1993) reported that the oral LD_{50} in sheep is 6 μg/kg whereas that for rats is 66 μg/kg. In addition, Ivanov et al. (1993) suggested that this increased susceptibility in sheep may be due, in part, to the lower concentration of catalytic sites for serum ChE in sheep (7.098×10^{-10} mol/L vs. 1.704×10^{-9} mol/L in rats). The Rice et al. (1971) study is selected here for deriving an oral RfD because it utilized an exposure route that is more relevant for an oral RfD, and also because the experimental evidence indicates that sheep are the more sensitive of the species tested.

Rice et al. (1971) fed VX to healthy yearling ewes for 56 days and measured changes in whole blood cholinesterase over this time period (see Section 3.3 for a more complete discussion of this study). The dose levels were 0, 3, 9, and 15 μg VX/day. Whole blood ChE was significantly depressed at all dose levels (levels of significance not reported) without any physical signs of clinical toxicity in any dose group. At the lowest dose, the decrease in whole blood ChE was statistically significant by the twenty-first day. Because of this significant reduction in ChE (38% relative to pre-exposure values), this dose (3 μg/day) is considered a LOAEL. The sheep used in this study had an average weight of 52.7 kg; therefore, the weight-normalized dose for 3 μg/day is 0.06 μg/kg/day.

The LOAEL of 0.06 μg/kg/day can be used to estimate a human oral reference dose (RfD) by using the following formula:

$$\text{RfD} = \frac{\text{LOAEL } (\mu\text{g/kg/day})}{UF_1 \times UF_2 \times UF_3 \times UF_4 \times UF_5 \times MF}$$

where:

LOAEL	=	0.06 μg/kg/day
UF_1	=	10 (sensitive subpopulations)
UF_2	=	1 (animal to human extrapolation)
UF_3	=	3 (extrapolation from subchronic to chronic exposures)
UF_4	=	3 (LOAEL to a NOAEL extrapolation)
UF_5	=	1 (data base completeness)
MF	=	1 (Modifying factor)

An uncertainty factor of 10 for sensitive subpopulations is considered necessary because some individuals have a genetic defect causing their blood cholinesterase activity to be abnormally low (Evans et al., 1952; Harris and Whitaker, 1962). For homozygous individuals, the activity can be as low as 821% of the normal mean (Bondenan and Bonderman, 1971). These individuals may be unusually sensitive to organophosphate anticholinesterase compounds (Morgan, 1989).

An uncertainty factor is not used to extrapolate from the animals to humans because there is sufficient evidence that humans are not more sensitive to VX than sheep. The following evidence is available to support this position:

1). Sheep have a much lower RBC-AChE activity level compared to humans, 2.9 μmol/mL/min versus 12.6 μmol/mL/mi (see Table 1). If ChE activity in the blood acts as a buffer to the effects of anticholinesterase compounds, than the lower activity level in sheep may cause them to have a higher susceptibility to agents such as VX.

2). In humans, a daily oral dose of 1.43 μg/kg for seven days resulted in a 60% reduction in RBC-AChE activity (Sim et al., 1964), whereas in sheep a nearly equivalent reduction in whole blood ChE (56% inhibition) resulted from a dose of only 0.28 μg/kg/day administered for 8–13 days (Rice et al., 1971). [Note: in sheep, about 90% of the blood ChE activity is in the RBC fraction (Osweiler et al., 1985); therefore, sheep whole blood ChE measurements can be reasonably compared to human RBC-AChE values].

3). The whole blood ChE_{50} in sheep is about 2.4 μg VX/kg (estimated from data presented in Rice et al., 1971), and the oral RBC-$AChE_{50}$ in humans is about 2.3 μg/kg (Sidell and Groff, 1974).

4). The similarities in VX sensitivity between sheep and humans may, in part, be accounted for by similarities in rates of metabolic detoxification of the agent. The latter can be estimated from a comparison of body surface areas (based on body weight raised to the 3/4ths power) as is done for animal-to-human extrapolations used in EPA cancer risk assessments(U.S. EPA, 1996). Using this approach, the human equivalent dose for the 0.06 μg/kg/day sheep LOAEL in the Rice et al. study can be calculated as:

$$LOAEL_{human} = LOAEL_{sheep} \times \frac{1}{\left(\frac{BW_{human}}{BW_{sheep}}\right)^{0.25}}$$

where:

BW_{human}	= default body weight of 70 kg for humans
BW_{sheep}	= average body weight of 52.7 kg for sheep in the Rice et al. (1971) study
$LOAEL_{sheep}$	= the experimental dose of 0.06 μg/kg/day for sheep

therefore:

$$LOAEL_{human} = 0.06\ \mu g/kg/day \times \frac{1}{\left(\frac{70\ kg}{52.7\ kg}\right)^{0.25}} = 0.056\ \mu g/kg/day$$

This calculation indicates that the human dose to produce a similar level of effect would not be substantially different from that in sheep. Considered together with the blood ChE activity and toxicity values mentioned above, the evidence is considered sufficient to support the use of an Uncertainty Factor of 1 for animal-to-human extrapolation.

An uncertainty factor of 3 is used to extrapolate from a subchronic to chronic exposure. In the derivation of oral RfDs for other organophosphate compounds, EPA has used NOAELs for cholinesterase inhibition following short-term exposures without adjustment for a more prolonged exposure period because of the unlikelihood that the endpoint would change over time (i.e., a subchronic-to-chronic UF of 1 was used). In addition, animal data indicate that maximum ChE inhibition may occur 30–60 days or more after exposure begins after which it levels off or even shows signs of recovery. This pattern can be seen in the data for the Rice et al. (1971) study (see Appendix B). However, an uncertainty factor of 3 is used here because chronic studies are not available to verify the unlikelihood that additional effects would occur following chronic exposures.

A LOAEL-to-NOAEL uncertainty factor of 3 is used instead of 10 because the endpoint, cholinesterase inhibition, was not associated with any physical signs of clinical toxicity. Furthermore, regression analysis of the Rice et al. (1971) data (see Table 4) indicates that 30% inhibition of ChE which is considered to be the threshold for a biological significant level of inhibition by EPA (pers. commun. H. Choudhury) would have occurred at about 2 μg/kg/day, substantially above the NOAEL value of 0.3 μg/kg/day that would result from applying a full UF of 10 to the LOAEL.

The data base requirements have been met in that there are subchronic toxicity studies in two species (sheep and rats), teratology studies in two species (rats and rabbits), a modified dominant lethal study in rats, a delayed neuropathy study in chickens, and a multigeneration study in rats. In addition, there are substantial human data supporting the RfD. The uncertainty associated with the absence of a chronic toxicity study is accounted for in UF_3 above.

No modifying factor is required in the derivation of the RfD for VX.

The RfD for VX is therefore:

$$RfD = \frac{0.06\ \mu g/kg/day}{10 \times 1 \times 3 \times 3 \times 1 \times 1}$$

$$RfD = 0.0006\ \mu g/kg/day$$

Table 4. Regression analysis of Rice et al. (1971) data for sheep dosed with VX

	Linear Analysis		Log Transformation	
Time (hr)[a]	**Mean Dose[b] for 30% ChE Inhibition** (μg/kg/day)	r^2	**Mean Dose[b] for 30% ChE Inhibition** (μg/kg/day)	r^2
744	0.88	0.961742	1.94	0.993047
912	1.69	0.836172	2.26	0.940522
1176	0.97	0.930412	1.97	0.980956
1320	1.21	0.90184	2.08	0.969214
Overall mean[c]	1.33		2.15	
Standard deviation	0.46		0.24	
Lower 95% CL	0.76		1.86	
Upper 95% CL	1.90		2.45	

[a] Data analysis based on four time points at which ChE inhibition stabilized
[b] Based on %ChE inhibition at 3, 9 and 15 μg/kg/day (see Appendix B)
[c] Derived from means for 744, 912, 1176, and 1320 hr

4.3 Overall Confidence in the RfD

Study: Medium
Data Base: High
RfD: High

The data base for VX consists of subchronic studies in sheep and rats, teratology studies in rats and rabbits, a modified dominant lethal study in rats, a delayed neuropathy study in chickens, and a multigeneration study in rats. In addition, there are also data available evaluating the effects of VX in humans following acute and short-term exposures. Although the principal study did not report on clinical chemistry, hematology, body and organ weight changes, or gross or histological pathology, there are supporting studies to indicate that cholinesterase inhibition is the appropriate endpoint. There is also evidence that sheep are one of the most sensitive species in their response to cholinesterase inhibitors. Therefore, the overall confidence in the RfD is high.

4.4 Comparison of RfD with Human Toxicity Data

The proposed RfD is compared to the available human toxicity data in Table 5. One study in humans indicated that an oral dose of 1.43 μg/kg/day for 7 days resulted in a 60% RBC-AChE inhibition but no toxic effects (Sim et al., 1964). This dose is over 2000 times greater than the RfD. For an adverse effect level (i.e., mild toxic effect at 2–4.5 μg/kg/day), the "margin of safety" would be larger. The results of the Sim et al. (1964) study indicating a LOAEL of 1.43 μg/kg/day for a 7-day exposure, were used to calculate an oral RfD for comparison with the RfD derived from the animal data (see Appendix D). Using a UF of 10 for sensitive subpopulations, a UF of 10 for a LOAEL-to-NOAEL extrapolation (10 is chosen because the dose of 1.43 μg/kg/day produced a 60% inhibition of RBC-ChE, which could be close to the toxic effect level), and a UF of 3 for protecting against longer exposures (animal data indicate that maximum ChE inhibition may occur 30–60 days after exposure begins and the Sim et al. study was for only a 7-day duration), the resulting estimated oral RfD is 0.005 μg/kg/day, a value approximately one order of magnitude greater than the RfD of 0.0006 μg/kg/day estimated from the Rice et al. (1971) sheep data.

Table 5. Comparison of RfD with human toxicity data of VX

Dose (μg/kg)	**Exposure Route**	**Endpoint**	**References**
0.0006[a]	oral	RfD - no inhibition of RBC-ChE	This report
0.1	intravenous	Estimated no effect level for RBC-ChE inhibition	McNamara et al., 1973
0.24	oral	Estimated no effect level for RBC-ChE inhibition, based on ration of oral to i.v. doses required for 50% RBC-ChE inhibition.	This report
0.34	inhalation	Estimated threshold for tremors based on inhalation data for GB	McNamara et al., 1973
1.0	intravenous	50% inhibition of RBC-ChE	Sidell and Groff, 1974
1.43	oral; once per day for 7 days	60% inhibition of RBC-ChE; no signs or symptoms of toxicity	Sime et al., 1964
2.4	oral	50% inhibition of RBC-ChE	Sidell and Groff, 1974
2-4.5	oral	Gastrointestinal symptoms in 5/32	Sidell and Groff, 1974

[a] Daily dose for chronic exposure

5. CARCINOGENICITY ASSESSMENT

The potential carcinogenicity of VX cannot be determined. Data are inadequate for performing a quantitative assessment of agent VX. The results of tests on bacteria, yeast and mammalian cell cultures (see Section 3.8) indicate that VX is not mutagenic or is only weakly mutagenic. These data provide supporting evidence that VX is not likely to be carcinogenic.

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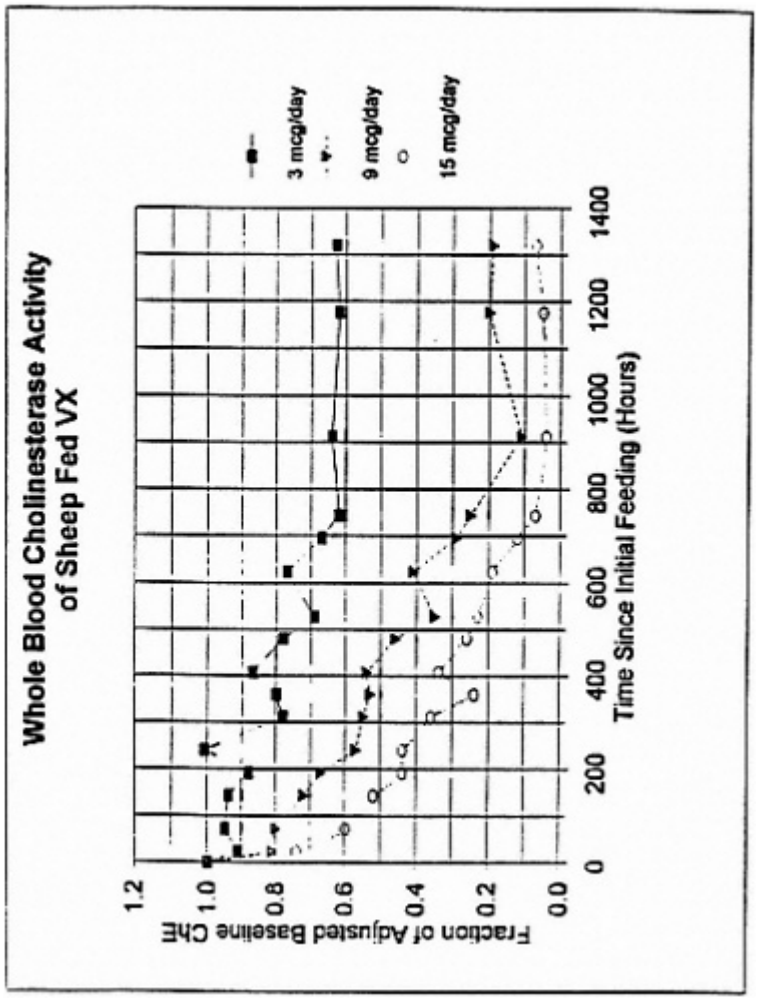
APPENDIX A COMPARISON OF RFDS, CHE INHIBITION AND TOXICITY DATA FOR GA, GB, GD AND VX

Endpoint	GA (μg/kg/day)	GB (μg/kg/day)	GD (μg/kg/day)	VX (μg/kg/day)	Ref.
RfD	0.04	0.02	0.004	0.0006	This report
Estimated no-effect level for RBC-AChE inhibition	-	1.0	-	0.24	**GB**[d] **VX**[a]
27–33% inhibition of RBC-AChE in humans/oral dose	-	2.3 (3 days)	-	0.2–2.0	**GB** - Grob and Harvey, 1958; **VX**-this report
RBC-AChE inhibition in humans/ i.v. dose	-	-	1.5–2.0 (30%)	1.0 (50%)	DA, 1974; Sidell and Groff, 1974
50–60% RBC-AChE inhibition in humans/oral dose	-	10	-	2.4	**GB** - Grob and Harvey, 1958; **VX**-Sidell and Groff, 1974
50% brain ChE inhibition *in vitro*	1.5×10^{-8} (c)	0.3×10^{-8} (c)	-	-	Grob and Harvey, 1958
Acute toxic effects in humans/oral dose	-	20–30	-	2–4.5	**GB** - Thienes and Haley 1972; Grob and Harvey, 1958; **VX**-Sidell and Groff, 1974
human oral LD_{50} (estimated)	25–50[b]	5–20[b]	5–20	3–10[b]	Somani et al., 1992
rat oral LD_{50}	3700	870–1060 600	400	77–128	DA, 1974 Grob & Harvey, 1958
monkey i.v. LD_{50}	50	20	-	6–11	DA, 1974
rat i.v. LD_{50}	70	45–63	50	6.9–10.1	Dacre, 1984
rat i.p. LD_{50}	490, 800	250 218	-	37–55	DA, 1974 RTECS, 1995

[a] Based on ratio of oral to i.v. doses (2.4 and 1.0 μg/kg, respectively) required for 50% RbC-ChE inhibition and the estimated i.v. no effect dose of 0.1 μg/kg.
[b] Values were estimated from animal data.
[c] Molar concentration
[d] Estimated from RBC-ChE_{50} values for GB and VX.

APPENDIX B GRAPHICAL ANALYSIS OF RICE ET AL. (1971) DATA FOR SHEEP DOSED WITH VX



APPENDIX C STATISTICAL ANALYSIS OF RBC-ACHE INHIBITION IN MALE RATS DOSED WITH VX

Study: Goldman et al., 1988
Species/sex: Sprague-Dawley rats/males
Endpoint: RBC-cholinesterase inhibition
Analysis: Comparisons made with controls (0 μ/kg/day) at 30 days

Dose (μ/kg)	0	0.25	1.0	4.0		
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Mean	3.36	1.546	0.74	0.13		
Std	0	0.06	0.0007	0.003		
N	2	2	2	2		
Bartlett's Test for homogeniety indicates the data is suitable for ANOVA.						
ANOVA						
SS Between	11.807	df =	3		F =	4361.593
SS Among	0.004	df =	4		p =	<0.001
MS Among	3.936					
MS Between	0.001					
Scheffe's Comparison						
Comparison with:		Group 2	Group 3	Group 4		
Group 1		p<0.05	p<0.05	p<0.05		
Group 2			p<0.05	p<0.05		
Group 3				p<0.05		
Dunnett's Comparison						
Comparison with:		Group 2	Group 3	Group 4		
Group 1		p<0.05	p<0.05	p<0.05		
Group 2			p<0.05	p<0.05		
Group 3				p<0.05		

APPENDIX C STATISTICAL ANALYSIS OF RBC-ACHE INHIBITION IN FEMALE RATS DOSED WITH VX

Study: Goldman et al., 1988
Species/sex: Sprague-Dawley rats/females
Endpoint: RBC-cholinesterase inhibition
Analysis: Comparisons made with controls (0 μg/kg/day) at 30 days

Dose (μg/kg)	0	0.25	1.0	4.0		
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Mean	2.86	1.37	0.57	0.29		
Std	0	0.08	0.006	0.014		
N	2	2	2	2		
Bartlett's Test for homogeniety indicates the data is suitable for ANOVA.						
ANOVA						
SS Between	7.977	df =	3		F =	1603.729
SS Among	0.007	df =	4		p =	<0.001
MS Among	2.659					
MS Between	0.002					
Scheffe's Comparison						
Comparison with:	Group 2	Group 3	Group 4			
Group 1	p<0.05	p<0.05	p<0.05			
Group 2		p<0.05	p<0.05			
Group 3			p<0.05			
Dunnett's Comparison						
Comparison with:	Group 2	Group 3	Group 4			
Group 1	p<0.05	p<0.05	p<0.05			
Group 2		p<0.05	p<0.05			
Group 3			p<0.05			

APPENDIX D CALCULATION OF ORAL RFD FROM HUMAN DATA

Study: Sim et al., 1964
Dose: 1.43 μg/kg/day orally for 7 days
Effect: 60% RBC-ChE inhibition but no toxic effects
LOAEL: 1.43 μg/kg/day Reference Dose:

$$\text{Oral RfD} = \frac{1.43\ \mu\text{g/kg/day}}{UF_1 \times UF_2 \times UF_3 \times UF_4 \times UF_5 \times MF}$$

where:

UF_1	=	10 (sensitive subpopulations).
UF_2	=	1 (animal to human extrapolation), not needed.
UF_3	=	3 (extrapolation from 7-day exposure to subchronic exposures). Animal data suggest that ChE effects may increase over the first 30–60 days of exposure and then follow a slow rate of recovery.
UF_4	=	10 (LOAEL to a NOAEL extrapolation); 60% inhibition is near a level where physical signs of clinical toxicity may occur.
UF_5	=	1 the data base requirements are met and cholinesterase inhibition is considered to be the mechanism of toxicity.
MF	=	1 no modifying factor is needed.

therefore:

$$\text{Oral RfD} = \frac{1.43\ \mu\text{g/kg/day}}{10 \times 3 \times 10}$$

$$\text{Oral RfD} = 0.005\ \mu\text{g/kg/day}$$

Appendix E

Health Risk Assessment for Sulfur Mustard (HD)

HEALTH RISK ASSESSMENT FOR SULFUR MUSTARD (HD)

DRAFT REPORT

September 1996

(editorial corrections made April 1997)

Prepared for

U.S. Department of the Army

Army Environmental Center

under Interagency Agreement No. 1769-1769-A1

Prepared by

Life Sciences Division

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Oak Ridge, Tennessee 37831

Submitted to

Material/Chemical Risk Assessment Working Group

Advisory and Coordinating Committee

Environmental Risk Assessment Program

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DISCLAIMER

This document is an internal review draft for review purposes only and does not constitute U.S. Government policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

PREFACE

This report assesses the potential non-cancer and cancer effects of sulfur mustard (HD) (CAS No. 505-60-2).

This document supports the activities of the Material/Chemical Risk Assessment Working Group of the Environmental Risk Assessment Program, a cooperative endeavor of the Department of Defense, Department of Energy, and Environmental Protection Agency. This working group is developing toxicity values for selected chemicals of concern at federal facilities. Toxicity values will be submitted for consideration by the EPA's IRIS Consensus Process for inclusion on IRIS (EPA's Integrated Risk Information System). The Material/Chemical Risk Assessment Working Group consists of Drs. Jim Cogliano (chair) and Harlal Choudhury (U.S. EPA), Dr. Bruce Briggs (Geo-Centers); Lt. Cmdr. Warren Jederberg and Dr. Robert L. Carpenter (U.S. Naval Medical Research Institute); Dr. Elizabeth Maull and Mr. John Hinz (U.S. Air Force Occupational and Environmental Health Directorate); Drs. Glenn Leach and Winnie Palmer (U.S. Army Center for Health Promotion and Preventive Medicine); Drs. Robert Young and Po-Yung Lu (Oak Ridge National Laboratory).

This document was written by Drs. Dennis M. Opresko and Rosmarie Faust, Life Sciences Division, Oak Ridge National Laboratory (ORNL), Oak Ridge, TN. Internal peer review was provided by Dr. Robert Young, Dr. Annetta Watson, and Mr. Robert Ross. External review of the toxicity data was provided by Dr. Thomas J. Bucci, Integrated Services, White Hall, AR and Dr. I.K Ho of the U. of Mississippi Medical Center, Jackson MS. External review of the derivation of the RfDs was provided by Drs. Michael Dourson and Susan Velazquez of Toxicology Excellence for Risk Assessment, Cincinnati, OH, and Dr. William Hartley of Tulane Medical Center, New Orleans LA. Additional reviews were provided by Mr. Joe King, Dr. Jack Heller, Ms. Veronique Hauschild, Ms. Bonnie Gaborek, Mr. Maurice Weeks, Maj. Robert Gum, and Mr Kenneth Williams of the U.S Army.

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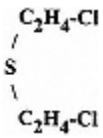
1. INTRODUCTION

Sulfur mustard (HD) is a chemical vesicant capable of causing severe skin and eye damage at very low concentrations. The chemical name, synonyms, identification codes, molecular formula and structural formula for this agent are as follows:

Sulfur mustard
bis(2-chloroethyl)sulfide
1,1'-thiobis(2-chlorethane)
1-chloro-2-(2-chloroethylthio)ethane
Distilled mustard
Agent HD
CAS No. 505-60-2
$C_4H_8Cl_2S$



1.1. PHYSICAL/CHEMICAL PROPERTIES

Pure sulfur mustard (HD) is a colorless, odorless, oily liquid with a molecular weight of 159.08 (MacNaughton and Brewer, 1994). Commercial products, however, have a yellow-brown color and sweet odor due to contaminants (MacNaughton and Brewer, 1994). Sulfur mustard has a vapor density of 5.5 (air = 1), a liquid density of 1.27 g/mL at 25°C, a vapor pressure of 0.11 mm Hg at 25°C, and a water solubility of 0.092 g per 100 g at 22°C (DA, 1974).

1.2. ENVIRONMENTAL FATE

1.2.1 Air

The vapor pressure of sulfur mustard is 0.11 mm Hg at 25°C, indicating moderate volatility. A vapor concentration of 920 mg/m^3 has been reported for a temperature of 25°C (DA, 1974) (although not adequately described in the reference, this presumably is the saturation concentration above a pure liquid). Information on the half-life of HD in air under various environmental conditions was not found in the available literature.

1.2.2 Water

The water solubility of sulfur mustard has been reported as 0.092 g per 100 g water at 22°C (DA, 1974), and 5×10^{-3} M at room temperature (MacNaughton and Brewer, 1994). In dilute aqueous solutions sulfur mustard hydrolyzes almost completely to thiodiglycol and hydrochloric acid (Papirmeister et al., 1991). For dissolved HD, the hydrolysis half-life ranges from about 4 to 15 min for temperatures of 20–25°C; however, bulk HD may persist in water for up to several years (Small, 1984). Small (1984) reported that it would take 15 days for the mass of a 1 cm droplet of HD in quiescent water to decrease by one half.

The Henry's Law Constant for HD has been estimated to be 2.1×10^{-5} atm m^3/mol (MacNaughton and Brewer, 1994), indicating a moderate potential for evaporation from water.

1.2.3 Soil

Sulfur mustard can be very persistent in soil (Rosenblatt et al., 1995). Persistence depends on the soil type, pH, moisture content, and whether the agent is at the soil surface or buried. Small (1984) reported that when HD was applied to the soil surface, volatilization would be the main route of HD loss (half-life about 30 min), but if the soil was wet, hydrolysis would be the main loss pathway. When sprayed onto soil, a vesicant action was still apparent after about 2 weeks; when the agent leaked into the soil, however, a vesicant action was still present after 3 years (DA, 1974). Rosenblatt et al. (1995) state that the persistence of sulfur mustard in soil is due to the formation of oligomeric degradation products that coat the surface of the mustard agent and that are resistant to hydrolysis.

Sulfur mustard has a log K_{ow} of 1.37 and a K_{oc} of 133, indicating that binding to soil organics would limit transport through soil to groundwater (MacNaughton and Brewer, 1994). MacNaughton and Brewer (1994) calculated a leaching index of 7.2 for HD, (i.e., the number of leachings required to reduce the HD soil concentration to one-tenth of the original amount, assuming that for each leaching one kilogram of soil is in equilibrium with one liter of water).

2 MECHANISM OF ACTION

The acute toxic effects of mustard vesicants are usually attributed to the consequences of alkylation reactions with organic compounds including nucleoproteins such as DNA. Alkylation reactions can result in physiological and metabolic disturbances as well as genotoxic effects. Several hypotheses have been advanced concerning the primary cause of cell death following acute exposures. As reviewed by Papirmeister et al. (1991), the three major hypotheses are:

1. Poly(ADP-ribose) polymerase (PADPRP) hypothesis. - In this theory DNA is the initial target of the mustard agent. Alkylated DNA purines undergo spontaneous and enzymatic depurination, leading to the production of apurinic sites which are cleaved by apurinic endonucleases to yield DNA breaks. Accumulation of DNA breaks leads to activation of the chromosomal enzyme PADPRP, which utilizes nicotinamide adenine dinucleotide (NAD^+) as a substrate to ADP-ribosylate and a variety of nuclear

proteins, causing severe lowering of cellular NAD^+. Depletion of NAD^+ results in the inhibition of glycolysis, and stimulation of the nicotinamide adenine dinucleotide phosphate ($NADP^+$)-dependent hexose monophosphate shunt (HMS) pathway follows as a result of the accumulation of glucose-6-phosphate, a common precursor for both glycolysis and the HMS. Induction and secretion of proteases is stimulated as a result of enhanced HMS activity, and this leads to pathological changes in the cell.

2. Thiol-Ca^{+2} peroxidation hypothesis. The first step in this process is thought to be the alkylation of glutathione (GSH) by the mustard agent. Depletion of GSH subjects protein sulfhydryl groups to damage from the agent or from reactive cellular oxidants. Proteins most susceptible to damage include Ca^{2+} translocases (Ca^{2+}-stimulated, Mg^{2+}-dependent ATPase) which are dependent on thiol groups to maintain cellular Ca^{2+} homeostasis, and microfilamentous proteins, where loss of sulfhydryl groups could result in disruptions of the cytoskeletal and structural integrity of the plasma membrane.
3. Lipid peroxidation hypothesis. According to this hypothesis the mustard agent causes depletion of GSH which, in turn leads to the buildup of highly toxic oxidants, usually through H_2O_2-dependent reaction sequences. The oxidizing agents react with membrane phospholipids to form lipid peroxides, initiating a chain reaction of lipid peroxidation which can lead to alterations in membrane fluidity, loss of membrane protein function, and loss of membrane integrity.

3. TOXICOLOGY

3.1 Introduction

Sulfur mustard vesicants are acutely toxic by direct contact. Edema, ulceration, and necrosis of the skin and respiratory tract epithelium can occur, as well as conjunctivitis and blindness. General symptoms of systemic toxicity include nausea, vomiting, fever, and malaise (ITII, 1975). Delayed effects which may occur following acute exposures include: eye lesions, chronic bronchitis, and cancers of the respiratory tract and skin. However, information on adverse effects following long-term exposures to less-than acutely toxic concentrations is very limited. Health effects of sulfur mustard agents have recently been reviewed by ATSDR (1992), Somani (1992), Sidell and Hurst (1992), Watson and Griffin (1992), and the Institute of Medicine (1993). The following is a brief summary of the most important toxicological data for sulfur mustard.

3.2 Acute Toxicity

Acute exposures to sulfur mustard can result in skin and eye damage, gastrointestinal irritation, and depressed myelopoiesis (resulting in leukopenia and anemia) (Vogt et al., 1984). Damage to the respiratory tract, which is the principal cause of mortality in the first few days to weeks after exposure to sulfur mustard, involves acute edema, inflammation, and destruction of the airway epithelial lining (Institute of Medicine, 1993). Infection of the respiratory tract resulting in bronchopneumonia is a common complication of exposure to sulfur mustard.

The skin and eyes are especially sensitive to the toxic effects of sulfur mustard. When applied to human skin, about 80% of the dose evaporates and 20% is absorbed (Vogt et al., 1984). About 12% of the amount absorbed remains at the site and the remainder is distributed systemically (Renshaw, 1946). Doses up to 50 $\mu g/cm^2$ cause erythema, edema, and sometimes small vesicles. Doses of 50-150 $\mu g/cm^2$ cause bullous-type vesicles, and larger doses cause necrosis and ulceration with peripheral vesication. Droplets of liquid sulfur mustard containing as little as 0.0025 mg may cause erythema (Ward et al., 1966). Eczematous sensitization reactions were reported in several early studies and may occur at concentrations below those causing direct primary irritation (Rosenblatt et al., 1975). In humans, the LCt_{50} (estimated concentration x exposure period lethal to 50% of exposed individuals) for skin exposures is 10,000 mg-min/m^3 (DA, 1974) (for masked personnel; however, the amount of body surface area exposed was not reported). The ICt_{50} (estimated concentration x exposure period incapacitating to 50% of exposed individuals) for skin exposures is 2000 mg-min/m^3 at 70–80°F in a humid environment and 1000 mg-min/m^3 at 90°F in a dry environment (DA, 1974, 1992). The ICt_{50} for contact with the eyes is 200 mg-min/m^3 (DA, 1974, 1992). The LD_{Lo} for skin exposure is 64 mg/kg and the LD_{50} is estimated to be about 100 mg/kg (DA, 1974, 1992).

Repeated exposure to 1.4 mg-min/m^3 produced no eye irritation or injury to laboratory animals (Rosenblatt et al., 1975). In humans, a Ct of ≤12 mg-min/m^3 is considered a no-effect dose for eye irritation (McNamara et al., 1975) at ambient temperatures. At higher temperatures (•32°C), threshold and other biological effects occur at lower concentrations. Cts of 12–70 mg-min/m^3 cause mild reddening of the eyes (McNamara et al., 1975); Cts of 40–90 can cause eye irritation and conjunctivitis after a latency period of 2 to 48 hr; and Cts of 90–100 mg-min/m^3 produce moderately severe burns, ulcers, opacity, and perforation after a latency period of 2 to 10 hr (Doull et al., 1980). In some cases there may be a recurrent vascularization and ulceration many years after the initial exposure.

The LCt_{50} for inhalation exposures in humans has been estimated to be 1500 mg-min/m^3 (DA, 1992). In animals, median lethal Ct values for sulfur mustard range from 600 to 1900 mg-min/m^3 for 10-min exposures (see Rosenblatt et al., 1975 for review). An LC_{Lo} (lowest lethal concentration) of 189 mg/m^3/10 min has been reported for mice (Lewis and Sweet, 1984), and a 5-min LC_{Lo} of 77 ppm has been reported for dogs (ITII, 1975).

Information on the acute oral toxicity of sulfur mustard is quite limited. The oral LD_{Lo} for humans has been estimated to be 0.7 mg/kg (DA, 1992). The oral LD_{50} for rats is 17 mg/kg (DA, 1974). Rats treated with 2.5 mg/kg/day for 14 days developed inflammation, petechial hemorrhage, thickening, and sloughing of the gastric mucosa (Hackett et al., 1987).

3.3 Subchronic Toxicity

In a subchronic study conducted by Sasser et al. (1989a), Sprague-Dawley rats (12/sex/group) were dosed by gavage with 0, 0.003, 0.01, 0.03, 0.1 or 0.3 mg sulfur mustard (in sesame oil)/kg body weight/day, 5 days/week, for 13 weeks. No mustard-related mortality occurred at any dose level. Body weights were significantly decreased in animals in the high-dose group. Epithelial hyperplasia of the forestomach occurred in 5/12 males and 5/12 females of the high-dose group and in 1/12 males receiving 0.1 mg/kg/day, but not in any other treatment group. Forestomach lesions were not seen in any of the control animals. No other treatment-related pathological lesions, clinical chemistry changes, or hematological abnormalities were reported.

3.4 Chronic Toxicity

3.4.1 Human Data

The U.S. Department of the Army (DA, 1992) states that chronic exposure to sulfur mustard can cause sensitization and chronic lung impairment (cough, shortness of breath, chest pain); however, specific information on dose-response functions for these effects was not found in the available literature. Limited information on the chronic toxicity of sulfur mustard comes from studies of workers at chemical agent manufacturing and weapons plants. Morgenstern et al. (1947) reported that many workers in a munitions plant handling sulfur mustard developed chronic bronchitis which in some cases developed into bronchiectasis. Wada et al. (1962a, b) reported that a large proportion of workers at a Japanese plant manufacturing mustard, as well as Lewisite and several other agents, exhibited productive cough, irregular fever, chronic bronchitis, emphysematous changes, and pleural adhesions. It is likely that in this case the reported effects were due to concentrations of sulfur mustard sufficiently high to cause acute toxic effects; exposure levels were estimated to reach as high as 50–70 mg/m^3 at times (Inada et al., 1978).

3.4.2 Animal Studies

McNamara et al. (1975) exposed male and female SDW rats (140), A/J mice (140), rabbits (12), guinea pigs (30), and dogs (6 initially) to a sulfur mustard vapor concentration of 0.001 mg HD/m^3 for 24 hr/day, 5 days/wk, for varying exposure durations up to one year (Note: the investigators reported that the experimental protocol involved a continuous exposure, implying that it was for 7 days/wk; however, in several places in the report it is specifically mentioned that the exposures were for only 5 days/wk). The same number of animals of each species were exposed to 0.1 mg HD/m^3 for 6.5 hr followed by 0.0025 mg HD/m^3 for 17.5 hr per day, 5 days/week for up to one year. The latter exposure is equivalent to a 5 day/wk time-weighted average concentration of 0.029 mg/m^3. Unexposed controls consisted of 10 dogs, 7 rabbits, 20 guinea pigs, 100 rats and 120 mice. Exposed animals were sacrificed periodically during the study **and were replaced with new animals.** One hundred ICR mice were added to the test chambers about 6 months after the tests began, and 50 A/J mice were added to the chambers about 3 months later.

Signs of toxicity that could be attributed to the sulfur mustard exposure occurred only in rats and dogs. Of 39 rats exposed to 0.001 mg HD/m^3 for 12 months, 5 exhibited chronic keratitis, a condition that McNamara et al. (1975) reported could possibly have been agent-related; however, this effect was not observed in any rats exposed to 0.1 mg HD/m^3. No signs of toxicity were seen in any of the dogs exposed to 0.001 mg HD/m^3; however, it should be noted that only 2 animals were exposed for the full 52-week period and only 4 animals were exposed for 32 weeks. The major signs of toxicity seen in the dogs exposed to 0.1 mg HD/m^3 were ocular changes consisting of corneal opacity, pannus, vascularization, pigmentation, keratitis, and granulation. McNamara et al. (1975, p. 12) state that chronic keratitis and conjunctivitis occurred in 3 of 10 dogs exposed for 7.5 or 12 months. The tabulated data presented by McNamara et al. (see Tables 1 and 2) indicate that chronic keratitis was also seen in some animals as early as 16 weeks after exposure began, and may have occurred in as many as 5 of 10 animals exposed for 32, 40 or 52 weeks. McNamara et al. (1975) concluded that it was "possible" that these effects were agent-related. Pneumonitis occurred in several of the dogs exposed to 0.1 mg HD/m^3, but this condition was also seen in the control animals, and because no other respiratory tract lesions were found, McNamara et al. (1975) indicated that the observed pneumonitis was not agent-related. There were no changes in

blood chemistry of the exposed dogs except for a possible increase in serum glutamic oxaloacetic transaminase after 12–28 weeks of exposure to 0.1 mg/m^3. As shown in Table 2, two dogs exposed to 0.1 mg HD/m ^{3} for 12 months also exhibited anaphylactic syndrome, gastroenteritis, and petechia.

Table 1. Ocular effects of sulfur mustard in dogsa

Concentration (mg/m^3)	Exposure Period (wk)	No. of dogs affected	Eye effects
0.001	4	0/10	NE
0.001	8	0/8	NE
0.001	16	0/6	NE
0.001	32	0/4	NE
0.001	52	0/2	NE
0.1 (1st group)	4	0/6	NE
0.1	8	0/4	NE
0.1	16	0/2	NE
0.1	28	2/2	Vascularization and pigmentation
0.1	40	1/2	Corneal opacity, pannus, chronic keratitis, granulation
0.1	40	1/2	Vascularization and pigmentation
0.1	52	2/2	Corneal opacity, pannus, chronic keratitis, granulation
0.1 (2nd group)	4	0/4	NE
0.1	8	0/4	NE
0.1	16	2/4	Corneal opacity, pannus, chronic keratitis, granulation
0.1	16	2/4	Vascularization and pigmentation
0.1	32	2/2	Corneal opacity, pannus, chronic keratitis, granulation
0.1	52	2/2	Corneal opacity

SOURCE: McNamara et al., 1975, Table A-18, p. 34
NE= No adverse effects
[a] Exposures were for 5 days/wk

Table 2. Toxicity of sulfur mustard to dogsa

No. Animals	Exposure (months)	Post-exposure (wk)	Gross findings	Microscopic findings
4	2	5	See micro.	Splenic infarct, 1/4; Pneumonia, granulomatous, 1/4; Pneumonitis, chronic 1/4
1	4	4	NSL[b]	NSL
1	4 (control)	4	NSL	NSL
1	4	4	NSL	NSL
1	7.5	4	NSL	Keratitis, pigmentary; Pneumonitis, chronic
1	7.5	4	NSL	Keratitis, chronic
1	7.5 (control)	4	NSL	Pneumonitis, chronic
1	7.5 (control)	4	NSL	Pneumonitis, chronic, active
1	12	10	Gastroenteritis; Multiple petechiae; Anaphylactic syndrome	Congestion, liver, spleen, lung; Hemorrhage, pancreas; Ulcerative colitis; Keratitis, chronic; Conjunctivitis, lymphocytic
1	12	10	Anaphylactic syndrome	Gastroenteritis, hemorrhagic; Heart, petechia; Keratitis, acute

SOURCE: McNamara et al., 1975, Table A-37, p. 53

[a] 0.1 mg HD/m^3 for 6.5 hr followed by 0.0025 mg HD/m^3 for 17.5 hr per day, 5 days/week

[b] NSL= no significant lesions

Although these effects were considered by McNamara et al. (1975) to be unrelated to the exposure to sulfur mustard, they are consistent with the known vesicant and sensitization actions of the agent. It is possible that the HD condensed on the fur of the animals and was subsequently ingested as a result of grooming behavior. Gastroenteritis could then have resulted from direct contact of the vesicant with the gastrointestinal epithelium.

3.5 Delayed Toxicity

Acute exposures to sulfur mustard can also result in long-term respiratory damage manifested as asthma-like conditions, emphysematous bronchitis, and increases in incidence of secondary respiratory infections (bronchopneumonia and tuberculosis) (see review by Watson and Griffin, 1992). Beebe (1960) evaluated the occurrence of respiratory tract disease among a group of World War I soldiers. Soldiers who had been exposed to mustard gas exhibited greater mortality from tuberculosis and pneumonia than either of two reference groups. Manning et al. (1981) reported a significantly increased incidence of mortality from pneumonia among 428 former workers of a sulfur mustard manufacturing facility. The ratio of observed to expected cases was 2 ($p < 0.05$). Some individuals exposed to sulfur mustard concentrations that are damaging to the eyes are susceptible to relapsing keratitis (delayed keratopathy) (see review by Watson and Griffin, 1992). The condition may reappear 8 to 40 years after recovery from the initial exposure (Dahl et al., 1985).

3.6 Developmental and Reproductive Effects

Azizi et al. (1995) investigated changes in serum concentrations of reproductive hormones and sperm counts in men who had been exposed to sulfur mustard during wartime. In 16 individuals, serum free and total testosterone and dehydroepiandrosterone were markedly decreased in the first five weeks after exposure; but levels returned to normal by 12 weeks. In 28 of 42 men evaluated one to three years after exposure, sperm counts were less than 30 million cells/mL and follicle-stimulating hormone was increased compared to controls having sperm counts above 60 million cells/mL. Testicular biopsy of the test subjects revealed partial or complete arrest of spermatogenesis.

In a study conducted by Hackett et al. (1987), sulfur mustard (dissolved in sesame oil) was administered by intragastric intubation to rats and rabbits on gestation days 6–15 (rats) or 6–19 (rabbits). Female rats were dosed with 0, 0.2, 0.4, 0.8, 1.6, 2.0 or 2.5 mg/kg/day in a range-finding study (3–9 animals per dose group of which 2–7 per dose group were pregnant) and with 0, 0.5, 1.0, or 2.0 mg/kg/day in a teratology study (25–27 animals per dose group of which 20–26 per dose group were pregnant). Maternal and fetal toxicity was observed at all dose levels (see Table 3). In the range-finding study significant ($p < 0.05$) maternal effects included mortality (1/3) at the highest dose; severe gastric lesions (petechial hemorrhage and sloughing of gastric mucosa) at 2.0 and 2.5 mg/kg/day; and inflamed mesenteric lymph nodes at doses of 0.4 mg/kg/day and higher. Significant decreases in body weight and decreased extragestational weight occurred at 1.6 mg/kg/day and decreased hematocrit at 0.8 mg/kg/day. There were no adverse effects on fetal weight and no evidence of morphological abnormalities in the fetuses. In the rat teratology study, maternal toxicity was evidenced by gastric inflammation at 2.0 mg/kg/day, and inflamed mesenteric lymph nodes at doses of 0.5 mg/kg/day and higher. Decreased body weight and decreased extragestational weight occurred at 0.5 mg/kg/day; decreased hematocrit at 1.0 mg/kg/day; and decreased weight of the placenta and gravid uteri at 2.0 mg/kg/day. Fetal effects included decreased weight in females and hydroureter at 0.5 mg/kg/day; decreased weight of males at 1.0 mg/kg/day; increased incidences of supernumerary ribs, misaligned sternebrae, and reduced ossification of sternebrae at 2.0 mg/kg/day. The investigators reported that the study did not reveal any evidence for a sulfur mustard-induced teratogenic effect in rats because all of the observed fetal changes occurred at dose levels that also produced maternal toxicity. The NOAEL for maternal and fetal toxicity was reported to be <0.5 mg/kg/day.

Table 3. Lowest doses of sulfur mustard causing maternal and fetal effects in rats and rabbits

Effects		Rat studies		Rabbit studies	
		Range-finding (mg/kg/day)	Teratology (mg/kg/day)	Range-finding (mg/kg/day)	Teratology (mg/kg/day)
Maternal Effects:	mortality	2.5	—	1.0	0.8
	gross lesions:				
	major[a]	2.0	—	1.0	0.4
	minor[b]	0.4	0.5	0.5	0.4
	decreased weight:				
	body	1.6	0.5	2.0	0.8
	extragestational	1.6	0.5	—	—
	extragestational gain	0.4	0.5	—	0.8[c]
	gravid uterus	—	2.0	—	—
	decreased hematocrit	0.8	1.0	—	0.8
	resorptions	0.4[d]	—	—	—
Fetal Effects:	decreased weights:				—
	female fetuses	—	0.5	2.0	—
	male fetuses	—	1.0	2.0	—
	placenta	—	2.0	—	—
	fetal morphology				
	misaligned sternebrae	—	2.0[e]	—	—
	supernumerary ribs	—	2.0[e]	—	—
	reduced ossification				
	vertebrae	—	0.5[d,e]	—	—
	sternebrae	—	2.0[e]	—	—
	hydroureter	—	0.5[d,e]	—	—

Source: Hackett et al., 1987
[a]Gastric lesions or infections
[b]Inflamed mesenteric lymph nodes in rats; enlarged Peyer's patch in rabbits
[c]Significantly different from lowest dose group, but not from controls
[d]Not significant in the highest dose group
[e]Significance based on fetal unit
[f]Significance based on litter unit

In the second part of the Hackett et al., (1987) study, rabbits were dosed with 0, 0.5, 1.0, 2.0, and 2.5 mg/kg/day in a range-finding study (7–8 per dose group), and with 0, 0.4, 0.6, or 0.8 mg/kg/day in the teratology study (7–8 per dose group). Dose levels of 0.8 mg/kg/day or higher were lethal to the dams. Damage to the gastric mucosa and enlarged Peyer's patches were observed in animals that received the lowest dose (0.4 mg/kg/day). Depressed body weight, depressed extragestational weight gain, and depressed hematocrit values occurred at 0.8 mg/kg/day. In the range-finding study a significant depression in fetal body weights occurred at a dose level of 2.0 mg/kg/day; however, in the teratology study no significant effects were observed on intrauterine survival, placental and fetal body weights, or incidence of fetal abnormalities. The investigators concluded that the study provided no evidence that sulfur mustard induced a teratogenic effect in rabbits. The NOAELs for maternal and fetal toxicity were reported to be <0.4 mg/kg/day and >0.8 mg/kg/day, respectively.

In a two-generation reproductive toxicity study conducted by Sasser et al. (1989b), groups of Sprague-Dawley rats (27 females and 20 males/group/generation) were gavaged with 0, 0.03, 0.1 or 0.4 mg/kg/day. The animals were treated according to the following exposure protocol: male and female rats were dosed 5 times/ week for 13 weeks prior to mating and during a 2-week mating period; female rats were dosed daily throughout the 21-day gestation and parturition period; and females were dosed 4–5 times/week during the 21-day lactation period. Males who had mated with females were sacrificed at the birth of their pups; dams who had given birth were sacrificed when the pups were weaned. Male and female F_1 pups received sulfur mustard until they were mated, the females became pregnant, and gave birth. At this point, F_1 males were sacrificed and F_1 dams continued on the dosage schedule until weaning, at which point the study was terminated. Thus, two generations of rats received subchronic exposure to sulfur mustard, with each generation going through a mating cycle. Similarly, two generations of pups were born to parents who had received sulfur mustard. Body weight gain was significantly ($p < 0.05$) lower than control values in the F_1 rats of both sexes born to parents who had received the highest dose of sulfur mustard. There were no significant adverse effects on reproductive parameters at any dose level. However, dose-related lesions of the squamous epithelium of the forestomach (acanthosis and hyperplasia) occurred in both sexes of each treatment group. The lesions were described as mild at the lowest dose level, 0.03 mg/kg, compared with the higher dose groups. The incidence and severity of acanthosis was 0/94 in the controls, 71/94 in the low-dose group, 89/94 in the mid-dose group, and 94/94 in the high-dose group. Benign neoplasms of the forestomach occurred in 8/94 animals in the 0.1 mg/kg group and in 10/94 animals of the 0.4 mg/kg group. The results of this study indicate that lowest dose tested (0.03 mg/kg/day) is a LOAEL for maternal toxicity.

McNamara et al. (1975) reported no increased fetal mortality rate when groups of 10 rat dams were exposed by inhalation to 0.001 mg HD/m^3, 24 hr/day or to 0.1 mg HD/m^3 for 6.5 hours followed by 0.0025 mg HD/m^3 for 17.5 hours during the first, second, or third week, or for the entire period of gestation. In another study, groups of 10 unexposed female rats were bred to male rats which had been exposed to the same exposure concentrations of HD for 1, 2, 4, 8, 24, 36, or 52 weeks to gain information on dominant lethal mutagenesis. There was no evidence of mutagenesis and fetal mortality was considered within normal limits. Both studies had a number of short-comings; in particular, the authors stated that the fetuses were examined, but they did not indicate whether there were fetal abnormalities.

3.7 Carcinogenicity

Several studies on workers occupationally exposed to sulfur mustard have revealed elevated risks of respiratory tract and skin tumors after long-term exposure. In addition, animal studies, mutagenicity studies, genotoxicity data, and the fact that sulfur mustard is a potent DNA alkylating agent, all provide supporting evidence for the carcinogenicity of this chemical agent.

The International Agency for Research on Cancer (IARC) has classified "mustard gas" as a Group 1 carcinogen (IARC, 1987), and the National Toxicological Program (NTP) includes "mustard gas" in the category of **Substances or groups of substances, occupational exposures associated with a technological process, and medical treatments that are known to be carcinogenic** (NTP *Annual Report on Carcinogens,* 1994). The State of Maryland also considers "mustard gas" as a "known human carcinogen" (a Class I.A. Toxic Air Pollutant as defined by the Code of Maryland Regulations, CMR Title 26 Subtitle 11, as amended).

3.7.1 Human Data

IARC (1975), Waters et al. (1983), Watson et al. (1989), and the Institute of Medicine (1993) have summarized the epidemiological evidence concerning the potential carcinogenicity of sulfur mustard in humans. Much of this information has come from studies of soldiers exposed during World War I as well as from studies of workers at chemical agent manufacturing facilities.

Case and Lea (1955) reported 29 deaths from cancer of the lungs and pleura among a sample of 1267 World War I veterans who had been exposed to sulfur mustard, 80% of whom also suffered from chronic bronchitis. In comparison, 14 cases would have been expected in a population of that size based on the mortality rates for the male population of England and Wales. The mortality ratio (207) indicated a significantly elevated risk for respiratory tract neoplasms (p between 0.0001 and 0.01). A similar tumor incidence rate and mortality ratio were found in a population of veterans who had never been exposed to mustard but who were suffering from bronchitis. Case and Lea (1955) concluded that the evidence did not support the view that sulfur mustard was a direct carcinogen. IARC (1975), however, noted that the high tumor rate in the group not exposed to mustard may have been due, in part, to smoking habits (a significantly higher proportion of men injured by mustard gas had given up smoking by the age of 40).

Beebe (1960) evaluated the occurrence of respiratory tract cancers among a group of 2718 American soldiers exposed to sulfur mustard during World War I and found that the ratio of observed to expected cases was 1.47 (based on U.S. mortality rates) compared with 1.15 for wounded soldiers not exposed to sulfur mustard, and 0.81 for soldiers who had pneumonia, but who had not been exposed to mustard. Norman (1975) evaluated the same group of soldiers after a 10-year follow-up period (study completed in 1965) and found that the exposed men had a 40% excess of lung cancer mortality, with an estimated relative risk of 1.3 (95% confidence limits of 0.9–1.9) compared to a control group consisting of wounded soldiers without exposure to mustard. The latency period was estimated to be 22–37 years. Norman (1975) also reported that in a limited subgroup of veterans, the relative risk of lung cancer mortality among cigarette smokers who were exposed to mustard agents was approximately equal to that of veterans exposed to mustard who stated that they did not smoke (4.3 vs 4.4). Norman (1975) concluded that there was no evidence in this limited data set that mustard exposure and cigarette smoking had a synergistic effect on lung cancer mortality.

Retrospective studies of Japanese workers who had been employed at a chemical agent manufacturing plant from 1929 to 1945 have revealed that these individuals have an increased risk of developing respiratory tract cancers. Although sulfur mustard was the main product of the facility, lewisite, diphenylarsine, hydrocyanic acid, phosgene, and chloroacetophenone were also produced there (Inada et al., 1978), and it is not known to what degree these other chemicals contributed to the observed effects. The concentration of mustard in the workplace was estimated to be as high as 50–70 mg/m^3 (Nakamura, 1956), and reportedly, the workers frequently exhibited signs of mustard toxicity including acute conjunctivitis, acute rhinitis, acute bronchitis, and acute dermatitis with blister formation. Studies completed in the 1950's documented individual cases of bronchial and laryngeal carcinoma in this population of workers (Yamada et al., 1953, 1957). Yamada (1963) reported that 16.3% of 172 deaths of former workers were due to cancers of the respiratory tract and oropharynx. The incidence rate among 5030 non-exposed inhabitants from the same geographic area was reported to be of 0.4% (Yamada, 1963). Mortality rates among the former factory workers during the years 1952–1967 were studied by Wada et al. (1968) who found that the incidence of mortality due to respiratory tract cancer was 33/495 (30 confirmed by histological evaluation) compared to an expected 0.9, based on national mortality rates for males with the same age distribution as the mustard workers. Of 930 former factory workers not directly involved in the mustard production process, three had died of respiratory tract cancer compared to 1.8 expected.

Neoplasms occurred in the tongue, pharynx, sphenoidal sinus, larynx, trachea, and bronchi; only one occurred peripherally in the lung. The median length of employment was 7.4 years, and the median interval between first employment and death from cancer of the respiratory tract was 24.4 years (Wada et al., 1968). Additional studies of this population of workers were conducted by Nishimoto et al. (1983, 1988) who incorporated histopathological and mortality data gathered between 1952 and 1986. For 1632 of these workers, the standardized mortality ratio (SMR) for respiratory tract tumors was 3.9 (70 observed vs. 17.8 expected, $p<0.001$, based on data for the Japanese male population) and the SMR for all malignant tumors was 1.2 (173 observed vs. 142 expected, $p<0.01$). These individuals were divided into three groups; (A) those directly involved in the manufacture of sulfur mustard or lewisite; (B) those not involved in mustard or lewisite manufacture, but who experienced some exposure; and (C) those engaged in the manufacture of other gases and those who were never exposed. The SMR for groups A and B (1.6 and 1.9) were also significantly elevated ($p<0.001$) whereas that for group C was not. Nishimoto et al. (1988) also showed that the SMR was about 2.7 for individuals who had worked at the factory 0.5 to 5 years, but 7.17 for individuals who had been employed for more than 5 years. The SMR was not significantly elevated for individuals who had worked at the factory for 7 months or less. SMRs were also calculated for each of six age groups. For individuals 30–39 years old the SMRs for respiratory tract cancer were not significantly elevated; however, the SMRs for the 40–49, 50–59, 60–69, and 70–79 yr olds were 10.3, 3.9, 4.4, and 2.5, respectively; all statistically significant at $p<0.01$ or $p<0.001$.

There is some evidence that these former factory workers may have also suffered elevated rates of digestive tract and skin tumors. Histopathological studies conducted by Yamada (1974, as reported by Inada et al., 1978) on 94 autopsy cases and 8 surgical cases revealed 17 cases of digestive tract cancers among these workers (no comparisons with control groups were reported). Of 488 former workers examined dermatologically, 115 had abnormal pigmentation and 22 had skin tumors of which 8 were cases of Bowen's disease (Inada et al., 1978). Pigmentation disorders were present in 57 cases out of 109 engaged only in the production of mustard and in only 1 of 16 cases engaged only in the production of lewisite. Hyperkeratotic skin lesions such as Bowen's disease, basal cell carcinomas, and hyperkeratotic papular eruptions, were present in 14 cases out of 109 engaged only in mustard production and in 1 case out of 16 engaged only in lewisite production. No abnormalities were observed in 77 former factory workers who had no exposure to chemical agents (Inada et al., 1978). It was also observed that the longer an individual had been exposed to mustard, the more marked the skin lesions tended to become (Inada et al., 1978).

The studies of Nishimoto et al. (1988), Yamada (1974) and Inada et al., (1978) provide strong evidence for a causal link between chemical agent exposure and cancer; however, because the workers were exposed to multiple chemicals, it is not possible to state conclusively that the cancers were due solely to sulfur mustard. Furthermore, it should be noted that several possible confounding factors, such as tobacco smoking habits, pre-existing health conditions, and post-exposure occupational histories of the workers, were not evaluated. In addition, SMRs themselves may not provide an accurate estimate of relative cancer risk if they do not correlate with tumor incidence rates in exposed and control groups (i.e., if social/economic or other differences between control and exposed groups result in differences in health care which affect survival rates).

Weiss and Weiss (1975) conducted studies evaluating the health of 271 workers employed for varying lengths of time between 1935–1945 at a munitions depot where the production, testing and destruction of sulfur and nitrogen mustard (as well as bromoacetone, phosgene, chloropicrin and organic arsenicals) had occurred. Ninety percent of the group had chronic health problems and 114 had died by the end of 1974. Thirty-five percent died from cancer of which 38% were bronchial cancers. The total number of deaths from cancer was significant ($p<0.01$) and the number of bronchial cancers was also

significant (11 observed vs. 5 expected for the population of the geographic region where the facility was located). The number of cancers of the gastrointestinal tract was 35% greater than expected. The average tumor induction time was 21.6 years. IARC (1975) notes that the study was limited to workers with available medical records, which "raises the possibility that the proportion with cancer may have been inflated, since medical records or autopsy records would more likely have been preserved for workers with cancer". Furthermore, IARC (1975) does not indicate whether smoking habits and other confounding factors were accounted for in the study of Weiss and Weiss (1975).

According to Klehr (1984), German workers involved in the dismantling of a sulfur mustard facility developed multiple skin lesions including basal cell carcinomas, Bowen's disease, Bowen's carcinomas, and carcinoma spinocellulare. The incidence rate for all tumors (including skin tumors) was 34% in 53 workers evaluated.

Manning et al. (1981) evaluated the incidence of cancer among former workers of a British mustard manufacturing facility (1939–1945). As of 1974, the number of deaths from all neoplasms combined (45) was slightly greater than that expected from national death rates, but the increase was not statistically significant. Two deaths were attributed to cancer of the larynx and one to carcinoma of the trachea, compared with an expected number of 0.40 ($p<0.02$; relative risk 7.5). Seven individuals were known to have developed cancer of the larynx, compared with 0.75 expected ($p<0.001$; relative risk 9.3). Lung cancer deaths were also elevated (21 observed vs. 13.43 expected) but not to significant levels (relative risk 1.6). In follow-up investigations of this group of workers, Easton et al. (1988) evaluated the mortality records of 3354 individuals and found greater numbers of cancer deaths when compared to national mortality rates. Significant increases were observed in deaths from cancer of the larynx (11 observed, 4.04 expected, $p = 0.003$), pharynx (15 observed, 2.73 expected, $p<0.001$), and all other buccal cavity and upper respiratory sites combined (12 observed, 4.29 expected, $p = 0.002$). There were also 200 deaths from lung cancer compared with 138.39 expected ($p<0.001$). It was also reported that the risks of developing cancer of the lung and pharynx were significantly related to the duration of employment. Significant excess mortality was also observed for cancers of the esophagus (20 observed vs. 10.72 expected) and stomach (70 observed vs. 49.57 expected) but there was no correlation with time since first exposure or duration of exposure.

Manning et al. (1981) concluded that it was very likely that the observed cancers of the pharynx, larynx and other upper respiratory sites were due to exposure to sulfur mustard because the excesses were too large to be accounted for by confounding factors (the effects of smoking, however, were not evaluated), increased with increasing duration of employment, and were limited to the period more than 10 years after first employment. Evidence for a causal relationship between sulfur mustard exposure and other cancers, including lung cancer, was not considered to be as strong.

Although a large number of American military personnel were exposed to sulfur mustard in chamber and field tests conducted during World War II, the morbidity and mortality records of this cohort have not been adequately evaluated to document long-term health risks (Institute of Medicine, 1993).

3.7.2 Animal Studies

Information on the potential carcinogenicity of sulfur mustard is available primarily from studies on rats and mice. McNamara et al. (1975) exposed SDW rats, ICR Swiss albino and A/J mice, rabbits, guinea pigs, and dogs to sulfur mustard vapors for varying exposure durations up to one year. The test animals were exposed to 0.001 mg HD/m^3 continuously or to 0.1 mg HD/m^3 for 6.5 hr followed by

0.0025 mg HD/m^3 for 17.5 hr per day, 5 days/week. In the rat study, 70 males and 70 females were exposed at each of the two concentrations, and 50 of each sex were maintained as controls. No tumors were observed in rabbits, guinea pigs, dogs, or mice; however, skin tumors were seen in the rats and these were considered to be the result of exposure to sulfur mustard. The rats were tested in two separate studies; a "toxicity study" in which the animals were exposed for up to 52 weeks and then followed for 6 months at which time they were sacrificed, and a "carcinogenicity study" in which the animals were exposed for varying times and then observed for varying periods of time before being sacrificed. In both studies skin tumors occurred in animals exposed to the highest concentration, but not in those exposed to the lower concentration. Of the tumors observed in the exposed animals, McNamara et al. (1975) considered basal cell and squamous cell carcinomas, trichoepitheliomas, and keratoacanthomas of the skin to be related to the sulfur mustard exposure; the incidence of these tumors are shown in Tables 4 and 5.

Table 4. Incidences of skin tumors in McNamara et al. (1975) toxicity study[1,2]

		Control		0.001 mg/m^3		0.1/0.0025 mg/m^3	
Exposure duration (months)	**Post-exposure (days)**	**M**	**F**	**M**	**F**	**M**	**F**
2			0/5	0/5	0/5	0/5	
3		0/5	0/5	0/5	0/5	0/5	0/5
4					0/5	0/5	
6		0/5	0/5	0/5	0/5		
8		0/5	0/5	0/5	0/5	0/5	0/5
12		0/5	0/5	0/5	0/5[a]	0/5	
12	70					4/4[4b]	
12	90	0/4	0/4[c]	0/4	0/5	0/1	0/5
12	180	0/7	0/4[a,d]	0/6	0/14[e]	0/6	5/13[4b,f,g]

SOURCE: McNamara et al., 1975; adapted by U.S. EPA, 1991

[1] Superscripts indicate the number and types of tumors: a. subcutaneous fibroma; b. skin, squamous cell carcinoma; c. squamous cell carcinoma of uterus; d. pulmonary adenoma; e. papilloma of the skin; f. basal cell carcinoma of the skin; g. thyroid adenoma

[2] Only tumor types b, and f, were considered by the authors to be related to the HD exposure and only these types are counted in the numerators of this table.

Table 5. Incidences of skin tumors in McNamara et al. (1975) cancer study (Data for both sexes pooled)[1,2]

Exposure duration (weeks)	Post-exposure (months)	Control	0.001 mg/m^3	0.1 mg/m^3
1	13		0/1	
1	15			0/1[a]
1	21		0/4[b]	0/4
2	20		0/5	0/5[c]
4	16		0/1	0/1
4	20		0/4	0/5
8	15	0/4	0/2	0/4
8	17		0/1	
8	18		0/1[d]	
12	12		0/2	4/5[3f,g]
12	17		0/3[e]	
26	14		0/4	3/4[3f]
26	18		1/1[f]	
39[3]	11		0/3[e]	4/4[4f,h]
52	2			1/1[f]
52	4			1/1[h]
52	6			1/1[f]
52	7			0/1
52	10	0/22[e]	0/17	3/14[3e,2f,i]
52	17	0/1[e]		0/1[e]
52	18			4/4[f]

SOURCE: McNamara et al., 1975; adapted by U.S. EPA, 1991

[1] Superscripts indicate the number and types of tumors:

a. subcutaneous lipoma
b. axillary lipoma
c. subcutaneous fibroma
d. astrocytoma
e. skin, fibroma
f. skin, squamous cell carcinoma
g. skin, basal cell carcinoma
h. skin, trichoepithelioma
i. skin, keratoacanthoma

[2] Only types f, g, h, and i, were considered by the authors to be related to the HD exposure and only these types are counted in the numerators. At 0.1 mg/m^3, one type h tumor co-occured in one animal with a squamous cell carcinoma.

Heston (1950) reported an increase in the occurrence of pulmonary tumors in strain A mice injected intravenously with 0.25 mL of a 1:10 dilution of a saturated solution of HD in water (0.06–0.07%) at 2-day intervals for a total of 4 doses. The tumor incidence was 93.3% with 2.6 tumors/mouse compared with 61% in the controls (0.9 tumors/mouse). In a second test in which a slightly lower dose was used, pulmonary tumors were found in 68% of the surviving treated animals (1.09 tumors/mouse) compared with 13% in the controls (0.13 tumors/mouse) ($p<0.001$). A significant increase in the incidence of pulmonary tumors in strain A mice was also seen in an inhalation study in which the test animals were exposed for 15 min to vapors released from 0.01 mL of HD applied to filter paper (Heston and Levillain, 1953; exposure levels were not otherwise quantified). Eleven months after exposure, lung tumor incidence was 49% (33/67) in the exposed animals and 27% (21/77) in the controls ($p<0.01$).

In another study, Heston (1953) found that subcutaneous injections of HD (0.05 cc of a 0.05% solution at weekly intervals for 6 weeks, or 0.1 cc of a 0.1% solution in olive oil at 2-day intervals for a total of 6 doses) into the mid-dorsal region of mice (strains A, C3H, and C3Hf) resulted in injection-site tumors, whereas injections of vehicle alone did not induce tumor formation. Tumors occurring at the injection site included sarcomas, sarcomas neurogenic in origin, a rhabdomyosarcoma, papillomas, a squamous cell carcinoma, a hemangioendothelioma, and a mammary carcinoma.

3.8 Genotoxicity

IARC (1975), Fox and Scott (1980) and ATSDR (1992) have summarized the available evidence concerning the genotoxicity of sulfur mustard. Because sulfur mustard is a strong DNA alkylating agent, genotoxic effects occur through cross-link formation, inhibition of DNA synthesis and repair, point mutations, and chromosome and chromatid aberrations (ATSDR, 1992). Some of these conditions have been observed in humans following exposure to sulfur mustard, others in various test systems including bacteria, yeast, insects, and mammalian cell cultures.

In studies conducted on a group of 28 former employees of a chemical agent manufacturing plant, Yanagida et al. (1988) found that the frequency of mutations to hypoxanthine-guanine-phosphoribosyl-transferase (HGPRT) deficiency was significantly elevated when compared to two control groups matched for age and smoking status. One control group consisted of healthy men and the other of individuals with bronchitis. The data also showed that the mutations were significantly more frequent in those workers who had longer exposures to sulfur mustard. A chromosome study of 16 former workers of this same factory indicated a significantly higher incidence of sister chromatid exchanges in peripheral lymphocytes when compared to a control group ($p<0.03$) (Shakil et al., 1993). Two individuals with chronic myelocytic leukemia had an almost three-fold higher SCE rate than controls and also a high (12.1%) incidence of chromosome abnormalities (Shakil et al., 1993). In an evaluation of the p53 mutations found in lung tumors of sulfur mustard workers, Takeshima et al. (1994) found that the mutations were similar to those in lung tumors of tobacco smokers (the factory workers were also tobacco smokers), however, the prominence of G:C to A:T transitions and the occurrence of double mutations in two of twelve cases suggested that the exposure to sulfur mustard did contribute to the development of the lung cancers.

Wulf et al. (1985) reported significant ($p<0.001$) increases in sister chromatid exchanges in lymphocytes of eleven fisherman who had accidently been exposed to sulfur mustard in sufficiently high concentrations to cause signs of acute toxicity.

Sulfur mustard has found to be genotoxic and mutagenic in several microbial assays. The agent caused alkylation of DNA in the yeast *Saccharomyces cerevisiae* (Kircher and Brendel, 1983), and

interstrand DNA cross-links (Venitt, 1968) and inhibition of DNA synthesis (Lawley and Brookes, 1965) in *Escherchia coli.* Using the histidine reversion assay, Stewart et al. (1989) found that sulfur mustard induced point mutations in *Salmonella typhimurium* strain TA102 and frameshift mutations in TA 97, but neither type of mutation in strains TA98 and TA100.

Sulfur mustard inhibited DNA synthesis in mouse lymphoma cells (Crathorn and Roberts, 1965), HeLa cells (Crathorn and Roberts, 1966), and L-strain mouse fibroblasts (Walker and Thatcher, 1968). It also induced chromosomal aberrations in cultured rat lymphosarcoma and mouse lymphoma cells (Scott et al., 1974), and chromosomal aberrations and reverse mutations in male BDF[1] mice in a host-mediated assay using murine leukemia L5178Y/Asn cell line as an indicator (Capizzi et al., 1973).

Several studies have also demonstrated that sulfur mustard causes dominant lethal mutations. Rozmiarek et al. (1973) reported a dominant lethal mutation rate of 9.4% (±1.9%) in rats after adult males had been exposed to 0.1 mg HD/m^3 for 12 weeks. Sasser et al. (1990) reported that a dominant lethal effect occurred after male Sprague-Dawley rats were dosed orally with 0.5 mg HD/kg/day 5 days/week for 10 weeks. The observed effects included increases in early fetal absorptions, preimplantation losses, and decreases in total live embryo implants. A significant increase in the percentage of abnormal sperm was also reported. Dominant lethal mutations, as well as chromosome rearrangements, have also been observed in *Drosophila melanogaster* exposed to sulfur mustard (Auerbach and Robson, 1946).

The cytotoxic, clastogenic and mutagenic effects of HD in Chinese hamster ovary cells have also been evaluated by Jostes et al. (1989). Chromosomal aberration frequency increased in a dose-dependent manner over the dose range of 0.0625 to 0.25 μM. Mutation induction at the HGPRT locus was sporadic, but the majority of the exposures resulted in mutation frequencies that were 1.2 to 4.0 fold higher than the spontaneous frequencies.

4. ORAL REFERENCE DOSE

4.1 Selection of the Principal Study

The available toxicity data for sulfur mustard are summarized in Table 6. The experimental study considered most suitable for the derivation of an oral RfD for sulfur mustard is the rat two-generation reproductive toxicity study conducted by Sasser et al. (1989b). This study, extending over 42 weeks, provides the lowest LOAEL. Acanthosis and hyperplasia of the epithelial tissue of the forestomach were the critical endpoints. Gastric lesions have also been observed in other oral toxicity studies on sulfur mustard (see Section 3.6). Some investigators have noted that the absence of a forestomach in humans suggests that the rat may not be a reliable human surrogate for evaluating the toxic effects of sulfur mustard. In addition, effects of gavage treatment in which the tissues are in immediate contact with sulfur mustard dissolved in sesame oil may not be comparable to the more dispersed contact expected when sulfur mustard is administered in drinking water.

Table 6. Summary of toxicity data for sulfur mustard

Study	Type	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Comments
Hackett et al., 1987	rabbit teratology	> 0.8		No fetal toxicity
Hackett et al., 1987	rabbit teratology		0.4	maternal toxicity
Hackett et al., 1987	rat teratology		0.5	maternal and fetal toxicity
Sasser et al., 1989a	rat subchronic	0.1	0.3	Epithelial hyperplasia of the forestomach
Sasser et al., 1989b	rat 2 generation		0.03	maternal toxicity acanthosis and hyperplasia of the forestomach
Sasser et al., 1989b	rat 2 generation	0.4		reproductive effects

4.2 Derivation of the Oral Rfd

In the Sasser et al. (1989b) study, male and female Sprague-Dawley rats were gavaged with 0, 0.03, 0.1, or 0.4 mg/kg/day dissolved in sesame oil (see Section 3.6 for details of the experimental protocol). There was no evidence of adverse reproductive effects at the dose levels tested. There was a significantly ($p < 0.05$) decreased body weight gain in the F_1 rats of both sexes born to parents who had received the highest dose of sulfur mustard. In addition, dose-related lesions of the squamous epithelium of the forestomach (acanthosis and hyperplasia) occurred in both sexes of each treatment group. The lesions were described as mild at the lowest dose level, 0.03 mg/kg, compared with the higher dose groups. The incidence and severity of acanthosis was 0/94 in the controls, 71/94 in the low-dose group, 89/94 in the mid-dose group, and 94/94 in the high-dose group. Benign neoplasms of the forestomach occurred in 8/94 animals in the 0.1 mg/kg group and in 10/94 animals of the 0.4 mg/kg group. The investigators reported that the NOAEL for toxicity was <0.03 mg/kg and the NOAEL for reproductive effects >0.4 mg/kg.

The lowest dose tested, 0.03 mg/kg, can be considered a LOAEL for rats subchronically exposed to sulfur mustard, with epithelial acanthosis and hyperplasia of the forestomach as the critical effect. Using this LOAEL, a human chronic RfD can be derived by adjusting the dose to a 7 day/week exposure protocol and then applying the result to the RfD methodology. Dose adjustments for discontinuous exposure can be made as follows: female rats were gavaged 5 times/week for 15 weeks (75 days), total

dose = 2.25 mg/kg; daily for 3 weeks (21 days), total dose = 0.63 mg/kg; and 4 times/week for 3 weeks (12 days), total dose = 0.36 mg/kg. The combined total dose over the 21-week exposure period, therefore, is 3.24 mg/kg; dividing the combined total dose of 3.24 mg/kg by 147 days (21 weeks) results in an adjusted LOAEL of 0.022 mg/kg/day. The adjusted LOAEL can then be applied to the equation for the derivation of an RfD:

$$RfD = \frac{LOAEL}{UF_1 \times UF_2 \times UF_3 \times UF_4 \times UF_5 \times MF}$$

$$RfD = \frac{0.022\ mg/kg/day}{10 \times 10 \times 3 \times 10 \times 1 \times 1}$$

$$RfD = 0.007\ \mu g\ HD/kg/day$$

where:

LOAEL	=	0.022 mg/kg/day
UF_1	=	10 (to protect sensitive individuals)
UF_2	=	10 (for animal to human extrapolation)
UF_3	=	3 (for estimating the NOAEL from the LOAEL)
UF_4	=	10 (for subchronic to chronic extrapolation)
UF_5	=	1 (data base adequate)
MF	=	1 (no additional modifications needed).

An uncertainty factor of 3000 was applied, accounting for protection of sensitive subpopulations (10), animal-to-human extrapolation (10), LOAEL-to-NOAEL extrapolation (3), and extrapolation from a subchronic to chronic exposure (10).

A LOAEL-to-NOAEL uncertainty factor of 10 is not considered to be necessary because the observed effect was reported to be "mild"; the critical effect may have been enhanced due to the vehicle used (sesame oil in which sulfur mustard is fully soluble) and the route of administration (i.e., gavage is more likely to result in localized irritant effects). Although the target organ (rat forestomach) is of questionable relevance to humans, because sulfur mustard is a direct alkylating agent, tissue damage would be expected to occur at the point of contact, even if it were another part of the gastrointestinal tract.

The data base for sulfur mustard contains two developmental toxicity studies in different species, a reproductive bioassay and a standard subchronic toxicity study in one species. In addition, chronic inhalation studies have been conducted on sulfur mustard using rats, mice, guinea pigs and dogs. The principal study identifies a toxic effect that is consistent with the vesicant properties of sulfur mustard. There is no evidence that any other experimental species would be more sensitive to ingested sulfur mustard; therefore, additional oral toxicity studies in other species are not considered critical.

4.3 Confidence in the RfD

Study: High
Data Base: Medium
RfD: Medium

The principal study is a well-designed and well-conducted reproductive toxicity study in rats. The identified endpoint (gastric lesions), also identified in a subchronic study and in two developmental studies, supports the conclusion that direct contact with epithelial tissue is the primary mechanism of toxicity. However, the principal study did not identify a NOAEL for this effect and the route of administration (gavage) may have led to an enhanced response due to the bolus type of dosing. Consequently, the overall confidence in the RfD must be considered medium.

5. CARCINOGENICITY ASSESSMENT

Inhalation unit risks have been derived for sulfur mustard directly from experimental animal data as well as from an analysis of the relative carcinogenic potency of sulfur mustard in comparison with that of known carcinogens for which there are both long-and short-term data. Epidemiological and long-term animal data are not available to directly derive an oral slope factor for sulfur mustard. Estimates of the oral slope factor are made from the inhalation unit risk and from the relative potency method.

5.1 Inhalation Unit Risk

5.1.1 Inhalation Unit Risk Derived from Experimental Animal Data

U.S. EPA (1991) derived a cancer inhalation unit risk for sulfur mustard based on the results of inhalation animal studies conducted by McNamara et al. (1975, see Section 3.7.2); however, it was emphasized in the EPA report that the studies of McNamara et al. (1975) contained deficiencies which made a quantitative analysis difficult. Conducted in 1970, the studies do not conform to the modern norms of acceptable experimental protocol, and it is likely that there was bias in the assignment of the animals to the test categories (U.S. EPA, 1991). In addition, many of the exposures were very brief, included only a few animals, and many of the animals were sacrificed (and some were replaced) before their capacity to develop late-appearing tumors was fully developed (U.S. EPA, 1991). Despite these shortcomings, it was noted by EPA that the McNamara et al. data are the best available for estimating the carcinogenic potency of sulfur mustard. The authors of the EPA report analyzed two sets of McNamara's data; one from a toxicity study and one from a carcinogenicity study (see Section 3.7.2).

In the toxicity study only those animals tested and observed long enough to exceed the demonstrated minimum latency period for first tumor appearance (70 days post-exposure, see Table 4) were included in the data analysis. The resulting tumor incidence rates are shown in Table 7.

Table 7. Skin tumor dataa (toxicity study) used in EPA quantitative assessment

Group	Control	0.001[b] mg/m^3	0.1/0.0025[b] mg/m^3
Males	0/11	0/10	4/11
Females	0/8	0/19	5/18
Both sexes	0/19	0/29	9/29

SOURCE: U.S. EPA, 1991, derived from data of McNamara et al., 1975

[a] Includes only data for rats surviving longer than the time of first tumor appearance

[b] Low exposure was 0.001 mg/m^3 continuously; high exposure was 0.1 mg/m^3 for 6.5 hr daily and 0.0025 mg/m^3 for the remaining 17.5 hr, d days/wk.

Because the rats were observed for 6 months after the exposures ended, the daily average exposure was estimated as being equal to 2/3 of the nominal concentration for 52 weeks (U.S. EPA, 1991). Therefore, for the group exposed to 0.1/0.0025 mg/m^3, the average concentration for 18 months was 0.019 mg/m^3, and for the group exposed to 0.001 mg/m^3, the average concentration for 18 months was 0.00067 mg/m^3. These data were applied to the GLOBAL 86 computer program for estimating a multistage model dose-response curve. The resulting unit risk was 2.9×10^{-2} per μg/m^3 (U.S. EPA, 1991). The unit risk was then adjusted for a rat lifetime exposure of 24 months by multiplying by the ratio of the lifetime to experimental duration to the third power [i.e., (24 mo/18 mo)^3]. This adjustment resulted in an estimated unit risk of 6.8×10^3 per μg/m^3. The data (including the times that the animals were sacrificed) were also subjected to time-to-tumor analysis using the WEIBULL 82 computer program. Empirically, the latency time estimated from the program was about 2 months, and the lifetime upperbound unit risk was estimated to be 8.5×10^{-2} per μg/m^3 (U.S. EPA, 1991).

The results of the McNamara et al. (1975) carcinogenicity study were also analyzed by EPA using the GLOBAL 86 program (U.S. EPA, 1991). The data were grouped into 17 lifetime tumor incidences (two dose levels times eight exposure durations plus a control group), and the exposures were converted to lifetime average concentrations, assuming a lifespan of 2 years (Table 8). The major assumptions used in EPA calculations were that cancer incidence would be linearly related to the product of HD concentration and exposure duration even at very low concentrations, and that the dose-response relationship in humans would be the same as that in animals, when doses are expressed as lifetime average air concentrations. The linearized upper bound on the low-dose slope (q_1*) estimated from these data by GLOBAL 86 was 9.4×10^{-2} per μg/m^3 (U.S. EPA, 1991).

Considering all the above data, the U.S. EPA (1991) selected the unit risk of 8.5×10^{-2} per μg/m^3, derived from the Weibull time-to-tumor model, as the recommended upper bound estimate of the carcinogenic potency of sulfur mustard for a lifetime exposure to HD vapors. However, U.S. EPA (1991) stated that "depending on the unknown true shape of the dose-response curve at low doses, actual risks may be anywhere from this upper bound down to zero". The Weibull model was considered to be the most suitable because the exposures used were long-term, the effect of killing the test animals before a full lifetime was adjusted for, and the sample size was the largest obtainable from the McNamara et al. (1975) data.

Table 8. Skin tumor data (carcinogenicity study) used in EPA quantitative assessment

Exposure Duration (weeks)	Exposure Concentration[a]	Lifetime[b] average daily exposure (μg/m^3)	Incidence of skin carcinomas
Control		0.0	0/27
1	low	0.0096	0/5
2	low	0.0192	0/5
4	low	0.0385	0/5
8	low	0.0769	0/4
12	low	0.115	0/5
26	low	0.250	0/4
1	high	0.279	0/5
39	low	0.375	0/3
52	low	0.500	0/17
2	high	0.558	0/5
4	high	1.12	0/6
8	high	2.23	0/4
12	high	3.35	4/5
26	high	7.25	4/5
39	high	10.9	4/4
52	high	14.5	10/23

SOURCE: U.S. EPA, 1991, derived from data of McNamara et al., 1975

[a] Low exposure was 0.001 mg/m^3 continuously; high exposure was 0.1 mg/m^3 for 6.5 hr daily and 0.0025 mg/m^3 for the remaining 17.5 hr, 5 days/wk.

[b] A 2-yr lifetime was assumed.

5.1.2 Inhalation Unit Risk Derived from Relative Potency

The inhalation carcinogenicity of sulfur mustard has also been evaluated using relative potency methods. Using the results of studies by Heston (1950, see Section 3.7.2) and Shimkin and McClelland (1949), U.S. EPA (1991) determined that the potency of sulfur mustard to induce pulmonary tumors in strain A mice was equivalent to that of 20-methylcholanthrene (MC). EPA then used the results of studies conducted by Stoner et al. (1984) to determine that MC was 10–13 times more potent than benzo(a)pyrene (BaP) in inducing lung tumors in this same strain of mice. Since the potency of sulfur mustard was

considered to be the same as that for MC, it was estimated that the unit risk for sulfur mustard would be 10–13 times the inhalation cancer unit risk for BaP. The latter was derived from the oral slope factor of 11.5 $(mg/kg/day)^{-1}$ using the standard defaults of 20 m^3/day for ventilation rate and 70 kg for body weight. The resulting inhalation unit risk estimate for sulfur mustard, based on the relative potency method, was 0.033–0.043 $(\mu g/m^3)^{-1}$ (U.S. EPA, 1991).

5.2 Oral Slope Factor

Long-term oral carcinogenicity studies have not been conducted on sulfur mustard. In oral subchronic studies in which the agent was administered by gavage to rats (see section 3..3), epithelial hyperplasia of the forestomach occurred in 5/12 males and 5/12 females dosed with 0.3 mg HD/kg/day (in sesame oil), 5 days/week for 13 weeks. In light of the known carcinogenicity of sulfur mustard, the epithelial hyperplasia is suggestive of a pre-neoplastic condition.

To derive an oral slope factor in the absence of long-term experimental data, two non-standard approaches can be considered. One involves the direct conversion of the inhalation unit risk to an oral slope factor, and the other involves the use of relative potency methods. Both approaches are summarized in the following sections.

5.2.1 Oral Slope Factor Derived from Inhalation Unit Risk

The U.S. EPA (1991) identified an "inhalation" unit risk of 8.5×10^{-2} per $\mu g/m^3$, derived from the Weibull time-to-tumor model, as the most appropriate estimate of the carcinogenic potency of sulfur mustard. This unit risk can be converted to a slope factor by normalizing the value for a 70 kg man inhaling 20 m^3 of air per day. The resulting slope factor is 0.3 $(\mu g/kg/day)^{-1}$.

Although it can be argued that conversion of an inhalation unit risk to an oral slope factor might be acceptable under certain conditions, including cases where 1) the cancer target organ is the same regardless of the route of exposure, 2) where differences in g.i. and pulmonary absorption can be taken into account, and 3) where metabolic activation is not critical or, if it is, the differences in first-pass metabolism in the liver and lung are accounted for. In the case of sulfur mustard, the inhalation unit risk is based on the occurrence of skin tumors in rats following exposure to sulfur mustard vapors. **There is no evidence that the tumors occurred following systemic absorption and distribution to the skin.** Considering the vesicant action of the agent, it is likely that the skin tumors resulted from the direct contact of the vapors or condensation droplets on the skin of the test animals. As mentioned above, for oral exposures the only evidence available suggests that if tumors did occur, they would be localized in the epithelial tissue of the g.i. tract. Therefore, it is unlikely that the target organs would be the same, although it could be argued that in both cases an epithelial surface is the target. Because a dose per unit surface area of skin can not be determined from the McNamara et al. (1975) study, quantitative extrapolation from a skin response to a g.i. tract response is not possible. Furthermore, because sulfur mustard is subject to rapid hydrolysis, the amount ingested that actually reaches the epithelial surface of the g.i. tract may be considerably limited, and, in fact, a significant dose may only be possible through gavage administration in an organic solvent vehicle, as was done in the animal toxicity studies.

As noted in section 3.7.1, there is only limited evidence that sulfur mustard causes digestive tract tumors in humans. Yamada (1974, as reported by Inada et al., 1978) found 17 cases of digestive tract cancers among 94 autopsy cases and 8 surgical cases of former workers at a chemical warfare

manufacturing facility. No comparisons were made with control groups; therefore, the significance of this finding cannot be determined. It is known that many of these workers were exposed to sulfur mustard concentrations thought to be sufficiently high (est. 50–70 mg/m^3) to produce signs of acute toxicity (i.e., acute conjunctivitis, acute rhinitis, acute bronchitis, and acute dermatitis with blister formation) (Nakamura, 1956).

5.2.2 Oral Slope Factor Derived from Relative Potency Estimates

As described in section 5.1.2, U.S. EPA (1991) derived an inhalation unit risk for sulfur mustard using the relative potency method in which sulfur mustard was considered to be 10–13 times more potent than BaP. The oral slope factor for BaP, as currently listed on IRIS, is 7.3 $(mg/kg/day)^{-1}$ (U.S. EPA, 1996). Multiplying this slope factor by the relative potency range of 10–13, results in an oral slope factor of 0.073–0.095 $(\mu g/kg/day)^{-1}$ for sulfur mustard.

Watson et al. (1989) estimated the carcinogenic potency of HD by the "rapid screening of hazard" (RASH) method developed by Jones et al. (1988). This approach compares exposures that produce documented toxic effects of a chemical of interest to exposures of a reference chemical producing a similar effect. The RASH procedure was applied to Heston's intravenous (1950) and subcutaneous injection studies (1953). Comparing the carcinogenicity of HD and the well characterized industrial carcinogen benzo[*a*]pyrene (BaP), Watson et al. (1989) showed that the two compounds are of approximate equivalent carcinogenic potency in experimental animals with a best estimate relative potency of 1.3 for sulfur mustard relative to BaP and an interquartile range (middle 50% of distribution) of 0.6-2.9. Consistent results were obtained when the potency of sulfur mustard was compared to that of *bis*-chloromethyl ether (BCME, CAS No. 542-88-1) another alkylating agent that is a powerful lung and eye irritant, as well as an IARC Class 1 carcinogen (Watson et al., 1989; IARC, 1987)

The estimated comparative carcinogenic potency can be used to derive a slope factor (SF) or q_1^* for sulfur mustard. The slope factor converts the estimated daily intake averaged over a lifetime exposure to incremental risk of an individual developing cancer. Because the slope factor is an upper 95th percentile confidence limit on the probability of response based on experimental animal data, the carcinogenic risk will generally be an upper-bound estimate.

Applying the Watson et al. (1989) estimated relative potency of 1.3 and using the currently accepted oral slope factor of 7.3 per (mg/kg)/day for BaP, a SF for HD can be calculated as follows:

$$SF_{HD} = SF_{BaP} \times \text{relative potency}$$

$$SF_{HD} = 7.3\ (mg/kg/day)^{-1} \times 1.3 = 9.49\ (mg/kg/day)^{-1}$$

$$SF_{HD} = 0.0095\ (ug/kg/day)^{-1}$$

5.3 Confidence in the Quantitative Estimates of Carcinogenic Potency

5.3.1 Inhalation Unit Risk

U.S. EPA (1991) notes that the dose-response estimates derived from the McNamara et al. (1975) study are highly uncertain due to the fact that the study was not of a standard design and too few animals were exposed and followed for a lifetime to give adequate sensitivity for detecting long-term effects. In addition, the uncertainty concerning the experimental conditions was too great to allow confidence about the absolute carcinogenic potency value.

In view of the fact that in the McNamara et al. (1975) study, malignant tumors appeared only at the highest mustard concentration and only late in life, U.S. EPA (1991) observed:

> "perhaps it may exert its carcinogenic activity secondarily through lifelong exposure to its cytotoxic or irritating effects. Under such circumstances, human exposures at low concentrations for limited times may entail much less risk than implied by the unit risk factor estimated from lifetime effects at higher doses. On the other hand, the lack of low-dose responses and early-appearing tumors in the McNamara data may be due simply to the inherent difficulty of detecting low-risk levels in experiments of reasonable size".

Because sulfur mustard is known to be a strong and direct DNA alkylating agent, the likelihood is very high that it functions as a non-threshold carcinogen. Consequently, the risks associated with exposures to low concentrations require evaluation, and the McNamara et al. (1975) study provides the only data set that allows for a quantification of carcinogenic potency following exposure to sulfur mustard vapors.

5.3.2 Oral Slope Factor

Although human and animal data are lacking, there is indirect evidence suggesting that sulfur mustard may be carcinogenic by the oral exposure route. The mechanism of action of sulfur mustard as a direct DNA alkylating agent, its known genotoxicity in exposed humans and in various animal bioassays, its induction of respiratory tract and skin tumors following inhalation exposures, and its induction of forestomach hyperplasia in rats following subchronic gavage dosing (see Section 3.3), all support the conclusion that this compound functions as a point of contact carcinogen on epithelial tissues. Furthermore, the mechanism of action of sulfur mustard would be expected to be similar to that of other known or suspected mustard carcinogens such as nitrogen mustard (sulfur mustard tumorigenicity was determined to be comparable to that of the nitrogen mustard agents HN2 and HN2-HCl and the therapeutic nitrogen mustard compounds melphalan and BCME; see Institute of Medicine, 1993, Appendix I), as well as *bis*-(chloro) ethyl ether (BCEE).

In the absence of human or animal oral dose-response data, the relative potency approaches developed by Watson et al. (1989) and U.S. EPA (1991) are considered to be appropriate methods for estimating the tumorigenic potency of sulfur mustard by the oral route of exposure. The oral slope factor derived by Watson et al. is approximately one order of magnitude less than the one derived from the relative potency estimated by U.S. EPA (1991). In the emerging area of relative potency analysis, a factor of 10 difference represents a good fit. There is no significant difference between the estimates of sulfur mustard carcinogenic potency relative to B(a)P published by Watson et al. (1989) and U.S. EPA (1991).

Although there are dose-response data from an animal inhalation exposure study (McNamara et al., 1975, see Section 5.1.1), route-to-route extrapolation (from inhalation to oral, as calculated in Section 5.2.1) is not considered appropriate because the exposure protocol of McNamara et al. (1975) resulted in rat skin tumors which might have occurred, not a result of systemic uptake, but as a result of dermal contact with sulfur mustard vapor (perhaps trapped by the rat pelt). Therefore, there is no method for estimating the dermal dose of sulfur mustard, or for converting this to an oral dose.

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Appendix F

Health Risk Assessment for Lewisite

HEALTH RISK ASSESSMENT FOR LEWISITE

DRAFT REPORT

September 1996

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Prepared for

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Submitted to

Material Chemical Risk Assessment Working Group

Advisory and Coordinating Committee

Environmental Risk Assessment Program

Strategic Environmental Research Development Program

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PREFACE

This report assesses the potential non-cancer and cancer effects of chemical agent lewisite (CAS No. 541-25-3).

This document supports the activities of the Material/Chemical Risk Assessment Working Group of the Environmental Risk Assessment Program, a cooperative endeavor of the Department of Defense, Department of Energy, and Environmental Protection Agency. This working group is developing toxicity values for selected chemicals of concern at federal facilities. Toxicity values will be submitted for consideration by the EPA's IRIS Consensus Process for inclusion on IRIS (EPA's Integrated Risk Information System). The Material/Chemical Risk Assessment Working Group consists of Drs. Jim Cogliano (chair) and Harlal Choudhury (U.S. EPA), Dr. Bruce Briggs (Geo-Centers); Lt. Cmdr. Warren Jederberg and Dr. Robert L. Carpenter (U.S. Naval Medical Research Institute); Dr. Elizabeth Maull and Mr. John Hinz (U.S. Air Force Occupational and Environmental Health Directorate); Drs. Glenn Leach and Winnie Palmer (U.S. Army Center for Health Promotion and Preventive Medicine); Drs. Robert Young and Po-Yung Lu (Oak Ridge National Laboratory).

This document was written by Dr. Robert A. Young, Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN. Internal peer review was provided by Dr. Annetta Watson, and Mr. Robert Ross. External review of the toxicity data was provided by Dr. Thomas J. Bucci, Integrated Services, White Hall, AR and Dr. I. K. Ho of the U. of Mississippi Medical Center, Jackson MS. External review of the derivation of the RfDs was provided by Drs. Michael Dourson and Susan Velazquez of Toxicology Excellence for Risk Assessment, Cincinnati, OH, and Dr. William Hartley of Tulane Medical Center, New Orleans LA. Additional reviews were provided by Mr. Joe King, Dr. Jack Heller, Ms. Veronique Hauschild, Ms. Bonnie Gaborek, Mr. Maurice Weeks, Maj. Robert Gum, and Mr Kenneth Williams of the U.S Army.

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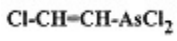
1. INTRODUCTION

In response to the FY Defense Authorization Act [Public Law (PL) 102–484, Sect. 176], review and evaluation of data were conducted to derive RfDs for non-stockpile chemical materiel (NSCM) in an effort to develop control limits for NSCM in soil and water. Lewisite (CAS No. 541-25-3) was identified as a priority chemical at non-stockpile materiel sites.

1.1 PHYSICAL/CHEMICAL PROPERTIES

Lewisite [dichloro(2-chlorovinyl)arsine] is an organic arsenical known for its vesicant properties (Rosenblatt et al., 1975). It has a molecular weight of 207.32, vapor pressure of 0.58 mm HG at 25°C, a liquid density of 1,89 g/cm^3 at 25°C, freezing point of -18°C, boiling point of 190°C, and is negligibly soluble in water (DA, 1974). The chemical structure of lewisite is shown below. Lewisite may occur as a trans-isomer and as a cis-isomer. In aqueous solutions, the cis-isomer undergoes photoconversion to the trans-isomer (Clark, 1989). In the presence of moisture, lewisite is rapidly converted to the more stable but highly toxic lewisite oxide (2-chlorovinylarsenous acid) (Cameron et al., 1946).



Lewisite
[dichloro(2-chlorovinyl)arsine]

1.2 ENVIRONMENTAL FATE

Regardless of the method of lewisite degradation (combustion, hydrolysis, or other environmental degradation), the arsenic component will not be eliminated and, therefore, at least some combustion product or other degradation products may be some form of arsenical. The recognized degradation products of lewisite are listed in Table 1.

1.2.1 Air

Although no data on its fate in the atmosphere are available, UV absorption spectrum of lewisite at 200 to 350 nm indicates that some photodegradation may take place. Rapid hydrolysis may occur in the gas phase (MacNaughton and Brewer, 1994).

1.2.2 Water

Although lewisite is only slightly soluble in water, 0.5 g/L (Rosenblatt et al., 1975), hydrolysis, resulting in the formation of lewisite oxide and HCl is rapid. *Cis*-Lewisite must be heated to over 40°C to react with NaOH to yield vinyl chloride, sodium arsenite, and acetylene (Rosenblatt et al., 1975). In aqueous solution, the *cis* isomer undergoes a photoconversion to the *trans* isomer (Rosenblatt et al., 1975). Upon standing in water, the toxic trivalent arsenic of lewisite oxide is converted to the less toxic pentavalent arsenic (Epstein, 1956).

Table 1. Lewisite Degradation Products

Product	Formula	CAS No.
Hydrolysis Product:		
Chlorovinyl arsenous oxide	C_2H_2ClAsO	3088-37-7
Combustion Products:		
Acetylene	C_2H_2	74-86-2
Acetylene monochloride	C_2HCl	593-63-5
Arsenic trichloride	$AsCl_3$	7784-34-1
Arsenic trioxide	As_2O_3	1327-53-3
Chlorine	Cl_2	7782-50-5
Methyl chloride	CH_3Cl	74-87-3
Vinyl chloride	C_2H_3Cl	75-01-4
Acetylene dichloride	$C_2H_2Cl_2$	540-59-0
Arsenic oxychloride	AsOCl	Not found
Chlorovinyl arsenous oxide	C_2H_2ClAsO	3088-37-7

Sources: DA, 1974, 1988; Small, 1984; HEAST, 1993

1.2.3 Soil

Lewisite applied to soil may rapidly volatilize and/or be converted to lewisite oxide through exposure to soil moisture (Rosenblatt et al., 1975). However, its low water solubility indicates intermediate persistence in moist soil (Watson and Griffin, 1992). Both lewisite and lewisite oxide may be slowly oxidized to 2-chlorovinylarsonic acid (Rosenblatt et al., 1975). Suggested pathways of microbial degradation in soil include epoxidation of the C=C bond and reductive dehalogenation and dehydrohalogenation (Morrill et al., 1985). In addition, residual hydrolysis would result in arsenical compounds. Although lewisite will probably not bioaccumulate through food chains, arsenic (an elemental poison) may (Rosenblatt et al., 1975).

2. MECHANISM OF ACTION

The toxicological effects of lewisite are ultimately due to its interaction with thiol groups of biologically active proteins such as enzymes. The interaction with sulfhydryl groups of enzymes may result in inhibition of enzyme function by the formation of stable cyclic structures with arsenic (As^{+3}) as a result of the reaction of the arsenic with the sulfhydryl groups of organic compounds such as those occurring in dihydrolipoic acid and in reduced keratin (De Bruin 1976). Dihydrolipoic acid is a dithiol cofactor active in several important enzyme systems (required for cellular respiration) including alphaketoacid oxidases such as pyruvate oxidase, 2-oxoglutarate oxidase, and aldehyde dehydrogenase. Lewisite combines with the dihydrolipoic acid to form stable six-member ring structures (cyclic thioarsenite

complexes), thereby inactivating the enzymes. Overall, the end result of these interactions and the ultimate mechanism of lewisite toxicity appear to be energy depletion which, in turn, results in cell death. Organochloroarsines, of which lewisite is an example, are also potent alkylating agents; this feature suggests carcinogenic potential.

3. TOXICOLOGY

3.1 Introduction

Lewisite is a lethal vesicant and systemic poison. The toxicology of lewisite has recently been reviewed by Goldman and Dacre (1989), Watson and Griffin (1992), and Trammell (1992) and will, therefore, only be briefly discussed in this section. Lewisite may be lethal following inhalation or dermal exposure, or by ingestion. Its lethality is due primarily to vapor inhalation, although lewisite is much less potent than neurotoxic chemical warfare agents. Generally, the toxic effects of lewisite are of rapid onset and result from acute exposures. The vesicant properties of lewisite result from direct skin contact; it has been estimated that as little as 2 ml to an adult human (equivalent to 37.6 mg/kg) can be fatal within several hours (Sollman, 1957). Being lipophilic, percutaneous absorption of lewisite is rapid and may be associated with systemic toxicity characterized by pulmonary edema, diarrhea, agitation, weakness, hypothermia, and hypotension (IOM, 1993). The threshold for severe systemic effects in humans following dermal exposure to lewisite is approximately 10 mg/kg (9.1 – 13.4 mg/kg) (Sollman, 1957). It has been hypothesized that fatalities following dermal exposure to lewisite may be due to blood plasma loss resulting from extensive capillary damage (i.e., lewisite shock) (Cameron et al., 1946). Ingestion of trivalent arsenicals may also cause death due to fluid loss resulting from intestinal epithelium damage. The vesicant properties of lewisite are characterized by immediate onset of pain and, for ocular exposure, possible corneal necrosis. Studies in animals have shown that the target tissues and organs for systemic toxicity of lewisite include the liver, gall bladder, urinary bladder, lung, and kidneys (Cameron et al., 1946; Snider et al., 1990). It is important to note that the gaps in knowledge regarding the toxic effects and dose response for lewisite are extensive.

3.2 Short-term Toxicity

Liquid lewisite applied by eye-dropper to the forearms of men caused blanching and discoloration of the skin followed by extensive erythema within 15 to 30 minutes and vesication within 12 hours or less (Wardell, 1941, as cited in Goldman and Dacre, 1989). The pain associated with these dermal exposures reportedly occurred within two minutes and considerable discomfort persisted for about one week. Other tests with human subjects and clinical reports also indicate a similar temporal sequence of events. Exposure to lewisite vapor (0.06 to 0.33 mg/L) caused discoloration and blistering with the maximum effect occurring by 36 to 48 hours after exposure (Wardell, 1941). At a concentration of 0.01 mg/L, lewisite vapor caused inflammation of the eyes and swelling of the eyelids after 15 minutes of exposure, and inhalation of 0.5 mg/L for five minutes is considered to be potentially lethal.

Short-term exposure (10 to 30 minutes) of dogs to lewisite vapor (0.05 to 0.12 mg/L) produced vomiting, urination, defecation, and severe respiratory distress that resulted in the death of 80% of the dogs within 3 to 48 hours (Goldman and Dacre, 1989). It was not reported whether the exposures were whole body or head only.

Acute oral toxicity values for lewisite have been summarized by Watson and Griffin (1992). The only available oral LD_{50} is that for the rat (50 mg/kg). Lethality values for other routes of exposure indicate some species variability but the values differ by less than an order of magnitude for any particular exposure route.

3.3 Subchronic Toxicity

A drinking water exposure study in rats was reported by Leitch et al. (1941). In this study, 10 rats were administered lewisite in drinking water (10 or 16 mg/L) for 19 weeks (133 days). The treatment did not affect consumption of food or water and had no effect on animal growth. Additionally, there were no treatment-related histopathological findings. Based on this report, a lewisite concentration of 16 mg/L drinking water would represent a NOAEL. However, this study has some deficiencies, as noted by Daniels (1990). The study neither defined an effect level, nor monitored the actual concentration of lewisite in drinking water consumed. Additionally, the report did not provide information regarding water consumption by the test animals. These data would be critical in determining an actual or estimated dose of lewisite. It is also possible that the consumed concentration may have varied from the target concentration because of test article degradation. Daniels (1990), however, suggested that these data would probably provide an estimate of a 7-day NOEL equivalent to 1.4 mg lewisite/kg.

In a dose range-finding study for a teratology study in rats and rabbits, lewisite was administered by gavage to rats (10 per group) on gestation days 6–15 at doses of 0, 0.5, 1.0, 2.0, or 2.5 mg/kg, and by gastric intubation to rabbits (8 per group) on gestation days 6–19 at doses of 0, 0.5, 1.0, 1.5, or 2.0 mg/kg. For rats, deaths attributed to lewisite occurred in the 2.5 mg/kg group (2/10) and in the 2.0 mg/kg group (1/10). Dosing trauma deaths were also reported (1/10, 2/10 and 1/10 in the 1.0, 2.0, and 2.5 mg/kg groups). For rabbits, deaths attributed to lewisite were reported in the 1.0 mg/kg group (6/8), 1.5 mg/kg group (5/8) and 2.0 mg/kg group (8/8). Dosing trauma deaths were also noted; 1/8 and 3/8 in the 1.0 mg/kg group and 1.5 mg/kg group, respectively.

A 90-day subchronic toxicity study of lewisite in rats was conducted by Sasser et al. (1989a). In this study, groups of 10 male and 10 female rats were given lewisite in sesame oil by gastric intubation at doses of 0.01, 0.1, 0.5, 1.0, or 2.0 mg/kg. Dosing protocol was 5 days per week for 13 weeks or approximately 65 dosing days. Vehicle controls received sesame oil at a dose of 1.67 ml/kg. Deaths were observed in the three highest dose groups; three males and seven females of the 2.0 mg/kg dose groups, eight males and six females of the 1.0 mg/kg dose group, and two males and three females of the 0.5 mg/kg dose group. Although all of the deaths occurred in the three highest dose groups, the response was not dose-dependent. Forestomach lesions were observed in the two highest dose groups (8/10 males and 4/10 females in the 2.0 mg/kg group, and 1/10 males in the 1.0 mg/kg group) and were attributed to the test article. These lesions were characterized by necrosis of the stratified squamous epithelium accompanied by infiltration of numerous neutrophils and macrophages, hemorrhage, and edema. In some instances, hyperplasia of adjacent areas was noted. There was no evidence that the lesions were precancerous, but the duration of exposure and observation was insufficient to assess carcinogenic responses. Lesions were also present in the glandular stomach but to a lesser degree. The presence of the lesions was consistent with the irritant effect of lewisite. No lesions were observed in the lower dose groups.

Some of the animals died without exhibiting any clinical signs of toxicity; drooling or wetness around the mouth and chin, and labored respiration were noted among other rats immediately preceding death.

Gross pathology findings attributed all deaths, except one, to severe inflammatory lesions characterized by edema and epithelial necrosis of the respiratory tract. Respiratory lesions were most likely due to aspiration of the test material or induced reflux of stomach contents into the pharynx with subsequent aspiration into the airways. Inflammatory lesions observed in the respiratory tract of surviving rats were also indicative of accidental deposition or induced reflux of the test material. No significant treatment-related effects on body weights or organ weights were observed for any of the dose groups.

Clinical chemistry evaluations revealed a significant ($p<0.05$) decrease in total serum protein, serum creatinine, and serum SGOT and SGPT in male rats of the highest dose (2.0 mg/kg) group at 13 weeks. Lowered serum enzyme activity was also observed in male rats of the other lewisite dose groups. Females of the highest dose group exhibited significantly increased lymphocyte and platelet counts; the former at 6 weeks but not at 13 weeks and the latter only at 13 weeks. The biological/toxicological significance of these findings is, however, uncertain. The investigators noted that the no-effect dose was greater than 0.5 mg/kg and less than 1.0 mg/kg. The 0.5 mg/kg dose may be considered an estimate of the NOAEL for short-term oral exposure to lewisite.

3.4 Chronic Toxicity

No human or animal studies examining the effects of lewisite following chronic exposure were located in the searched literature.

3.5 Developmental and Reproductive Effects

In a teratogenicity study by Hackett et al. (1987), lewisite was administered by gavage to pregnant rats on gestation days 6 through 15 at doses of 0.5, 1.0, and 1.5 mg/kg and by gastric intubation to pregnant rabbits on gestation days 6 through 19 at doses of 0.07, 0.2, and 0.6 mg/kg. For rabbits, the mortality rates were 13%, 46%, and 69% for the 0.07, 0.2, and 0.6 mg/kg dose groups, respectively. The mortality rates were corrected for death from other causes (e.g., dose-delivery trauma, accidental delivery of the dose to the lungs, handling trauma, pregnancy complications unrelated to the test article) and, therefore, represent a significant dose-related frank effect. Surviving rabbits in the highest dose group exhibited decreased body weight gain relative to controls and other dose groups. However, the study authors noted more frequent incidences of anorexia in the high-dose rabbits when compared to controls and other dose groups. For those rabbits whose deaths were not attributed to the extraneous causes previously noted, gastric lesions (mucosal inflammation, edema, necrosis, and mucosal sloughing) were observed at all dose levels. The only statistically significant developmental effects were a significant increase in the incidences of fetal stunting and supernumerary ribs in the high-dose (0.6 mg/kg) group. Fetal weight and crown-rump length were somewhat lower in the 0.6 mg/kg dose group but these differences were not statistically significant. Maternal toxicity (13%) was also associated with the low-dose group, thereby indicating a NOAEL for this study to be <0.07 mg/kg/day for maternal toxicity and 0.2 mg/kg/day for developmental toxicity. The LOAEL based on maternal effects is 0.07 mg/kg/day and for developmental effects is approximately 0.6 mg/kg/day. The increased mortality of the does (13%) and the occurrence of gastric lesions in the low-dose group (0.07 mg/kg/day) suggest that the rabbit is the most sensitive of the species for which data are available.

The use of increased mortality as the critical effect for derivation of a reference dose is not appropriate. Furthermore, the intragastric intubation technique used for rabbits in this study concentrates the test article on the gastric mucosa more effectively than simple gavage administration thereby making the apparent increased sensitivity of rabbits more an artifact of administration than actual toxicodynamics. In the discussion of the study, Hackett et al. noted that the fetal toxicity observed in the rabbits appeared to be

occurring at doses above those required to induce increased maternal mortality. The findings of this study are, however, statistically compromised by the low number of pregnant survivors (9/12, 6/11, 5/13, and 3/15 for the control, 0.07, 0.2 and 0.6 mg/kg dose groups, respectively). In a dose range-finding study for this experiment (see Section 3.3.), significant mortality was observed in the 1.0 mg/kg group (6/8), 1.5 mg/kg group (5/8) and 2.0 mg/kg group (8/8).

Another phase of the Hackett et al. (1987) study investigated the potential teratogenicity of lewisite in rats. In this phase of the study, no maternal toxicity or teratogenic effects were observed, thereby identifying 1.5 mg/kg as a NOAEL. However, it must be noted that in a dose range-finding study in rats (Hackett et al., 1987; see Section 3.3), doses of 2.0 mg/kg and 2.5 mg/kg resulted in 10% and 20% maternal mortality, respectively.

A two-generation reproductive study in rats was conducted by Sasser et al. (1989b). In this study, lewisite (in sesame oil) was administered intragastrically at doses of 0.10, 0.25, or 0.60 mg/kg/day to groups of 25 male and 25 female rats five days per week for 13 weeks prior to mating and 7 days per week during gestation (21 days), and at least four days per week during lactation (21 days). The doses were selected based upon the findings of the subchronic toxicity study by Sasser et al. (1989a) which identified a NOAEL between 0.5 and 1.0 mg/kg/day, and the teratogenicity study by Hackett et al. (1987) in which 1.5 mg/kg/day was a NOAEL. In the dose range-finding phase of this report, 20% mortality (corrected for deaths due to dosing trauma) was observed in the 2.5 mg/kg/day group. At the time of birth of the F_1 generation, the F_0 male rats were sacrificed. Dams continued treatment (minimum of four doses per week) throughout lactation (3 weeks). A vehicle control group was given equivalent volumes of sesame oil (1.67 ml/kg). After weaning, 20 male and 25 female offspring were selected for the F_1 phase of the study. The treatment protocol for these animals was as described for the F_0 generation. Mortality was high among both the F_0 and F_1 females. The cause of death for most of these animals appeared to be associated with aspiration of the test article resulting in fatal respiratory tract lesions. Exposure of rats to lewisite did not adversely affect reproductive performance, fertility, or reproductive organ weights. The treatment had no significant effect on litter weights, sex ratio, mean pup weight, or offspring survival for either generation. Although this study revealed no toxic effects, arsenic is known to be embryotoxic and teratogenic, and the possibility exists that inorganic arsenic could be metabolically derived from lewisite.

An unpublished USSR study analyzed by the U.S. Army Research Institute of Chemical Defense (Solana, 1992) provided data indicating that preconception maternal exposure of rats to 0.045 or 0.002 mg lewisite/cm^3,4 hours/day, 5 days/week for 4 months did not affect numbers of corpora lutea or implantations, number and physical dimensions of fetuses, increased intrauterine mortality or ossification of long bones. Approximately 140 litters of rats were used in this study.

Human data regarding reproductive/developmental effects due to lewisite exposure are inconclusive because of confounding factors such as concurrent exposure to other agents such as sulfur mustards and incomplete exposure data. Yamakido et al. (1985) studied workers from the Okuno-jima (Japan) factory where mustard and lewisite were manufactured in the World War II era, and noted no evidence of agent-induced mutations.

3.6 Carcinogenic and Mutagenic Effects

In a long-term follow-up study, Krause and Grussendorf (1978) reported the formation of a malignant lesion at the site of contact eight years following a single, acute dermal exposure to lewisite. A German soldier had been accidentally exposed to liquid lewisite on his lower right leg in 1940. In 1948, the lesion was diagnosed as malignant. Thirty-eight years after exposure, the area around the contact site was still ulcerated and diagnosed as Bowen's disease (intradermal squamous cell carcinoma). Bowen's disease was also diagnosed in workers at a Japanese facility that produced lewisite. These latter findings, however, were not conclusive because these workers were exposed concurrently to diphenylcyanoarsine and mustard agent and no quantitative estimates of dose or exposure rates were available (Inada et al., 1978).

There is only anecdotal evidence for the potential carcinogenicity of lewisite. These data are not definitive and do not support classifying lewisite as a suspected carcinogen. As such, quantitative assessment of the potential carcinogenicity of lewisite is not currently possible. Although the available evidence is not of sufficient quality to label lewisite a suspected carcinogen, the position maintained by CDC (CDC, 1988) that "some evidence suggests that lewisite *might* also be a carcinogen" seems tenable. However, for environmental exposure and remediation concerns, the arsenic component and/or arsenic-containing degradation products would warrant concern.

Although the carcinogenicity of lewisite *per se* is equivocal and cannot be assessed quantitatively, several of its degradation products are known carcinogens. Lewisite combustion produces the inorganic arsenicals arsenic trichloride and arsenic trioxide, as well as vinyl chloride. Inorganic arsenic is carcinogenic in humans and animals and is classified as a Group A carcinogen for both oral and inhalation exposure (U.S. EPA, 1989). Arsenic trioxide and vinyl chloride are both considered Group A carcinogens by the U.S. EPA (U.S. EPA, 1984, 1988) and Group 1 carcinogens by IARC (IARC, 1987). Additionally, compounds such as arsenic trichloride, sodium arsenite (a lewisite hydrolysis product), arsenic oxychloride, and inorganic arsenicals in general are of concern to EPA as potential carcinogens (U.S. EPA, 1988). However, there are no human epidemiologic data or data from animal studies that show organic arsenicals to be carcinogenic. A review by the World Health Organization (WHO, 1981) stated that "There is no conclusive evidence that any of the organoarsenic compounds tested for carcinogenicity in laboratory animals are carcinogenic." IARC (1987) concluded that adequate data were not available for evaluating the carcinogenicity of organic arsenic compounds.

Data from genotoxicity studies do not indicate a carcinogenic potential for lewisite. Genotoxicity studies in *Salmonella typhimurium* strains TA97, TA98, TA100, and TA102 were negative with and without S9 activation at lewisite concentrations <1.0 μg/plate (Stewart et al., 1989). At 1.0 μg/plate and higher, lewisite was cytotoxic. Jostes et al. (1989) reported on the effects of lewisite in one mutation assay (hypoxanthine-guanine phyosphoribosyl transferase [HGPRT] locus) and two cytogenetic assays (chromosomal aberration and sister chromatid exchange [SCE]) using Chinese ovary cells. At concentrations ranging from 0.12 to 2.0 μM, the mutagenic response at the HGPRT locus was not significantly different from control values. The SCE assay resulted in a weakly positive response from 0.25 to 1.0 μM concentrations, but the values were not significantly different from control values. However, chromosome aberrations were induced at 0.50, 0.75, and 1.0 μM that were significantly greater than control values. The investigators concluded that lewisite was cytotoxic and clastogenic but SCE and mutation at the HGPRT locus was insignificant. Assays to determine sex-linked lethal mutations and chromosomal rearrangements in *Drosophila melanogaster* yielded negative results (Auerbach and Robson, 1946, 1947).

A dominant lethal study using CD rats was conducted by Bucci et al. (1993). In this study, male CD rats (20/group) were given lewisite in sesame oil by gavage for five days at doses of 0.375, 0.75, or 1.5 mg/kg. Vehicle controls received an equivalent volume of the vehicle and positive controls were given the vehicle followed by 100 mg ethyl methanesulphonate/kg, i.p. on day five. Each male was mated with two females over the next 10 weeks. With the exception of the positive controls, no significant differences were observed in reproductive indices and there were no histopathologic findings that could be attributed to lewisite treatment. Under the conditions of this study there were no dominant lethal mutations resulting from exposure to lewisite.

4. ORAL REFERENCE DOSE FOR LEWISITE

The U.S. EPA has not adopted any Reference Doses for lewisite; consequently, an RfD for lewisite will be derived using available data. No controlled studies are available that have evaluated the oral toxicity of lewisite in humans; therefore, extrapolation from animal data is necessary.

The effects levels for the available studies are summarized in Table 2. A NOAEL of between 0.5 and 1.0 mg/kg/day was obtained from the rat 90-day oral subchronic toxicity study of Sasser et al. (1989a). The oral teratogenicity study by Hackett et al. (1987) provided data indicating a NOAEL of 1.5 mg/kg for teratogenic effects in rats with maternal toxicity occurring at 2.0 mg/kg/day. The report by Hackett et al. (1987) also reported that gestational exposure of rabbits at a dose 0.6 mg/kg/day resulted in maternal toxicity and fetal stunting. Doses as low as 0.07 mg/kg/day also resulted in 13% maternal toxicity (excluding deaths from extraneous causes) and were accompanied by marked gastric lesions. These data indicate a LOAEL of 0.07 mg/kg based upon gastric lesions and increased mortality. However, the results of this study are statistically compromised by the low numbers of surviving animals. A NOAEL of 0.6 mg/kg was obtained from the multigeneration-reproduction study in rats reported by Sasser et al. (1989b). Although the data from the 90-day subchronic toxicity study by Sasser et al. (1989a) only identified the NOAEL as being between 0.5 mg/kg and 1.0 mg/kg, the 0.5 mg/kg dose would provide a conservative estimate of the NOAEL.

Based upon the limited available data, the rabbit appears to represent the most sensitive species as indicated by the occurrence of gastric lesions concurrent with increased mortality following 14-day administration of lewisite by gastric intubation. The rabbit data are, however, statistically compromised by the small number of survivors in each treatment group. Studies assessing reproductive/developmental endpoints in rats were negative, and the data from rabbits indicated that developmental effects occurred at doses exceeding those that induce significant maternal mortality.

Table 2. Summary of Effect Levels for Lewisite Toxicity Studies

Study Type[a]	Species	NOAEL	LOAEL (Critical Effect)	Reference
Subchronic	rat	1.4 mg/kg (est.)	None	Leitch et al., 1941
90-day	rat	0.5 mg/kg/day	1.0 mg/kg (gastric lesions)	Sasser et al., 1989a
Multigeneration	rat	0.6 mg/kg/day (0.44 mg/kg/day TWA)	None	Sasser et al., 1989b
Developmental[b]	rat	1.5 mg/kg/day	None	Hackett et al., 1987
Developmental[b]	rabbit	<0.07 mg/kg/day	0.07 mg/kg/day (gastric lesions, increased mortality)	Hackett et al., 1987
Range-finding[b]	rat	1.0 mg/kg/day	2.0 mg/kg/day (increased mortality)	Hackett et al., 1987
Range-finding[b]	rabbit	0.5 mg/kg/day	1.0 mg/kg/day (increased mortality)	Hackett et al., 1987

[a] Route of administration is gavage/gastric intubation, except Leitch et al. (1941) which was drinking water.
[b] Test article administered on gestation days 6–15 (rats) and 6–19 (rabbits).

For the derivation of RfD for lewisite, both a 90-day study (Sasser et al., 1989a) and a multigeneration study (Sasser et al., 1989b) in rats were used to identify effect levels. Data from the 90-day study identified a LOAEL of 1.0 mg/kg/day based upon gastric lesions. The accompanying NOAEL from this study was 0.5 mg/kg/day. The multigeneration study represents a chronic exposure situation relative to reproductive/developmental effects but would be considered subchronic duration for systemic effects in the adult animals. It must be noted that the absence of reproductive/developmental effects does not necessarily eliminate the possibility of more sensitive effects in alternate targets. The highest dose (0.6 mg/kg/day) from the multigeneration study of Sasser et al. (1989b) appears to represent the most valid NOAEL and would be the best value for deriving an RfD. However, because of the discontinuous exposure and variable dosing protocol, a time-weighted average dose must be calculated. This adjustment will provide a NOAEL adjusted for discontinuous exposure ($NOAEL_{adj}$) and is based on the following: rats were dosed at 0.6 mg/kg/day × 5 days/7 days for 13 weeks (91 days) = 0.43 mg/kg/day for 13 weeks; females dosed daily (0.6 mg/kg/day) during gestation (21 days) = 0.6 mg/kg/day for 3 weeks, and females dosed at least 4 days per week at 0.6 mg/kg/day during lactation (21 days) = 0.34 mg/kg/day for 3 weeks. The time-weighted average (TWA) dose for this 133-day period is calculated as

$$\text{TWA} = \frac{\Sigma[(0.43\ \text{mg/kg/day} \times 13\ \text{weeks}) + (0.6\ \text{mg/kg/day} \times 3\ \text{weeks}) + (0.34\ \text{mg/kg/day} \times 3\ \text{weeks})]}{19\ \text{weeks}}$$

$$\text{TWA} = 0.44\ \text{mg/kg/}\textit{day}$$

This NOAEL is slightly lower than the NOAEL of 0.5 mg/kg/day from the Sasser et al. (1989a) study and, therefore, is being used as the basis for the RfD for lewisite. The selection of this NOAEL is supported by the available data set with the exception of the rabbit data, the validity of which uncertain. The RfD for lewisite is calculated as follows:

$$RfD = \frac{NOAEL_{adj}}{UF_1 \times UF_2 \times UF_3 \times UF_4 \times MF}$$

$$NOAEL_{adj} = 0.44 \text{ mg/kg/day}$$

$$RfD = \frac{0.44 \text{ mg/kg/day}}{10 \times 10 \times 10 \times 3 \times 1}$$

$$RfD = 0.0001 \text{ mg/kg/day}$$

$$RfD = 0.1 \ \mu\text{g/kg/day}$$

where

NOAEL	=	0.6 mg/kg/day
$NOAEL_{adj}$	=	0.44 mg/kg/day (adjusted for discontinuous exposure and varying dosing protocol)
UF_1	=	10 (sensitive subpopulations)
UF_2	=	10 (interspecies extrapolation)
UF_3	=	10 (extrapolation from subchronic to chronic exposure)
UF_4	=	3 (deficient data base)
MF	=	1 (no additional modifying factor suggested)

Note: EPA recommends that when five uncertainty factors are used, the total uncertainty should not exceed 10,000, and when four uncertainty factors are used, the total uncertainty should not exceed 3,000 (U.S. EPA, 1991).

The derivation of an RfD for lewisite necessitated addressing several issues regarding the available data set: 1) interpretation of toxicity data from gavage/gastric intubation studies and 2) identification of the critical effect. The available data for lewisite toxicity is limited to gavage and gastric intubation administration studies. Although these routes of administration allow for more precise control of the administered dose as opposed to drinking water or feeding studies, in the case of lewisite (or any highly corrosive agent), they impart substantial caveats in data interpretation. Firstly, the use of a sesame oil vehicle and the gavage/gastric intubation administration result in the gastric mucosae being exposed to a bolus of material in a vehicle that limits normal dispersion of the test article in the stomach. The decrease

in dispersion causes an increase in contact time between the corrosive agent and a limited surface mucosal area, thereby increasing the potential for inflammatory responses observed in the described studies. Furthermore, the presence of an oil vehicle will likely affect the physicochemical interactions at the chemical/tissue interface by altering the solubility and distribution of the chemical. Secondly, within the context of the RfD, intake of a corrosive agent as a bolus/oil suspension would not be toxicologically or physiologically analogous to exposure to the chemical agent via environmental media such as water. The critical effect of orally administered lewisite in animals appears to involve gastric lesions (rats and rabbits), possible developmental effects (rabbits), respiratory tract inflammation responses (rats and rabbits), and increased mortality (rats and rabbits). The increased mortality and respiratory tract inflammation responses reported in the available studies appear to be associated more so with dosing errors or simple reflux of the corrosive, irritating lewisite. The reflux and subsequent respiratory tract response would be highly unlikely in an environmental exposure situation (i.e., drinking water contamination). Furthermore, the studies did not provide data affirming the respiratory responses to be a function of systemically mediated lewisite toxicity. Therefore, an RfD based upon available data is tenuous and difficult to verify.

The proposed RfD for lewisite underwent preliminary review (July 10–12, 1996) by the Material/Chemical Risk Assessment (MCRA) Working Group of the Environmental Risk Assessment Program (ERAP). The MCRA Working Group of ERAP represents multiagency (EPA, DoD, and DOE) input by individuals experienced in deriving and validating toxicity values. The MCRA Working Group agreed that the critical toxic effect observed in the lewisite studies (forestomach lesions) appears to be an artifact of administration, and that the overall database for lewisite is not robust. Although it was recognized that the structure of lewisite might imply toxic activity differing from inorganic arsenic, it was the consensus of the MCRA Working Group that the lewisite RfD be considered not verifiable due to data deficiencies, and that the existing RfD for inorganic arsenic (3E-04 mg/kg/day) be used as a surrogate. This is considered a valid and justifiable approach inasmuch as the inorganic arsenic RfD and the proposed lewisite RfD are similar (3E-04 vs 1E-04 mg/kg/day, respectively), and the fact that lewisite in environmental media will be degraded to inorganic arsenic.

5. CARCINOGENICITY ASSESSMENT FOR LEWISITE

Data are inadequate to quantitatively assess the potential carcinogenicity of lewisite. There are inadequate human and inadequate animal data regarding the carcinogenic potential of lewisite. Genotoxicity data are equivocal or negative.

It was recommended by the MCRA Working Group that for risk assessments, the carcinogenic potential of lewisite degradation products be considered.

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Appendix G

Inhibition of Cholinesterases and an Evaluation of the Methods Used to Measure Cholinesterase Activity

ESTERASES

CHOLINESTERASES (ChEs) are enzymes that hydrolyze esters of choline. One is acetylcholinesterase (AChE, acetylcholine hydrolase, EC 3.1.1.7). Another is butyryl cholinesterase (BuChE, acylcholine acylhydrolase, EC 3.1.1.8), sometimes known as nonspecific cholinesterase or pseudocholinesterase. The preferred substrates for AChE and BuChE are acetylcholine (ACh) and butyryl choline or propionylcholine, respectively. AChE and BuChE are inhibited by organophosphate (OP) esters that react at the catalytic site of the enzymes, forming enzyme-inhibitor complexes that block the action of the enzyme. Carboxyesterases (CaE, EC 3.1.1.1) are a third type of enzyme. One CaE, known as neuropathy target esterase (NTE), is an enzyme associated with organophosphate-induced delayed neuropathy (OPIDN) (Johnson 1977). The biochemistry, determination, pharmacology, toxicology, and biology of the ChEs and their inhibitors have been reviewed (Augustinsson 1948; Aldridge and Reiner 1972; Silver 1974; Whittaker 1986; Hoffmann et al. 1989; Gallo and Lawryk 1991; Ballantyne and Marrs 1992; Chambers and Levi 1992; EPA 1992; Ecobichon 1994; Taylor 1994, 1996).

AChEs, BuChEs, and CaEs are specialized carboxylic ester hydrolases classed among the B esterases, enzymes that are inhibited by OPs. Another class of enzymes are the A esterases (e.g., paraoxonase and DFPase) that actively hydrolyze OPs, destroying their toxic potential

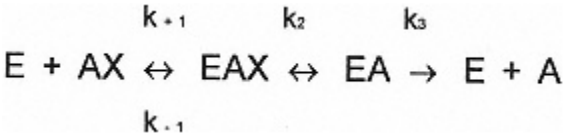
(Aldridge and Reiner 1972). The A esterases have a serine catalytic site. The tertiary structure and amino acid sequences of several AChEs and BuChEs have been established (Taylor 1994). AChEs regulate excitation at cholinergic synapses, destroying the neurotransmitter ACh. AChE is one of the most active enzymes known, cycling within a few milliseconds (Taylor 1996). AChEs are found at synapses and neuromuscular junctions, and in central-nervous-system (CNS) neuron cell bodies, axons, muscles, red blood cells (RBCs), and platelets of ovine and rodent species (Silver 1974; Traina and Serpietri 1984; Hoffmann et al. 1989).

There is AChE-like activity in the plasma of some birds and mammals (Traina and Serpietri 1984; Smucker and Wilson 1990). Plasma ChE of rodents, such as the laboratory rat, is high in both AChE and BuChE activities (Traina and Serpietri 1984). AChE activity in human blood is found mainly in RBCs (Silver 1974). AChE activity also occurs in the serum of developing mammals and birds, decreasing to adult levels after birth (Smucker and Wilson 1990). Together with AChEs, BuChEs are also found at synapses, motor end plates, and muscle fibers. BuChE activity in blood is restricted to serum (Silver 1974)

Substrate preferences for AChEs and BuChEs vary with the species. Both mammal and bird AChEs rapidly hydrolyze ACh and its thiocholine analog acetylthiocholine (AcTh) (Silver 1974). Plasma-BuChE activity in the rat is reported to favor propionyl rather than butyryl substrates (Augustinsson 1948; Hoffmann et al. 1989). AChEs and BuChEs respond differently to increasing substrate concentration. AChEs are inhibited by excess substrate above 1–2 millimolars (mM) (Wilson et al. 1997; Silver 1974). BuChEs are less sensitive. BuChEs are preferentially inhibited by the selective inhibitor iso-OMPA and quinidine, and AChEs by the bisquaternary compound BW284c51.

BIOCHEMISTRY

The action of AChE on ACh is a multistep process, represented below by the formation of a reversible enzyme-substrate complex (EAX), acetylation of the catalytic site (EA), and hydrolysis of the enzyme-substrate complex yielding acetic acid, choline, and the regenerated enzyme (E + A). A similar reaction scheme is applicable to BuChEs.



Scheme 1 Action of AChE on ACh. E, enzyme; AX, substrate (ACh) or inhibitor; EAX, reversible enzyme complex; k, reaction-rate constants.

Reaction of an OP with AChE, BuChE, or other B esterases is similar to the reaction of AChE with ACh, except that the hydrolysis step is much slower or, in some cases, might not occur at all. Its basis is a phosphorylation of the enzyme. A serine hydroxyl at the catalytic site reacts with the phosphorus atom of the inhibitor to form an OP-ChE complex. A side group on the phosphorus atom, known as the leaving group (X), is lost. With some OPs, the phosphorylated enzyme might, in time, reactivate by rehydrolysis (Wilson et al. 1992). Spontaneous reactivation might take hours to days. OP-ChE complexes also undergo a reaction known as aging, in which a second group is lost from the phosphate, stabilizing the OP-ChE complex and blocking its reactivation.

TOXICITIES

The toxicities of OPs and carbamates usually correlate with the extent of their inhibitions of brain AChE. For example, a plot of intraperitoneal LD_{50} versus pI_{50} (50% inhibition) in mice shows the relationship between the toxicity in vivo of 30 directly acting OPs and their inhibition of AChE in vitro (Gallo and Lawryk 1991).

Many of the physiological effects of anti-ChEs are attributable to excess neurotransmitter ACh (Taylor 1996). The precise symptoms and the time course depend on the chemicals and the localization of the receptors affected. Early symptoms of cholinergic poisoning represent stimulation of muscarinic neuro-effectors of the parasympathetic system. Effects include slowing of the heart (bradycardia), constriction of the pupil of the eye, diarrhea, urination, lacrimation, and salivation. Actions at nicotinic skeletal neuromuscular junctions (motor end plates) result in muscle fasciculation (disorganized twitching) and, at higher doses,

muscle paralysis. Anti-ChE actions at the cholinergic junctions of the sympathetic and parasympathetic autonomic ganglia affect the eye, bladder, heart, and salivary glands. Anti-ChEs also affect junctions of the CNS, producing symptoms that include hypothermia, tremors, headache, anxiety, convulsions, coma, and death. Whether exposure to low doses of OPs results in consistent behavioral effects, such as deficits in learning and memory, is a matter of current research. Behavioral effects (performance deficits) in the absence of miosis (usually taken as the most sensitive indicator of airborne exposure to nerve agents), salivation or fasciculation, are described in primates receiving oral GD under controlled conditions (Hartgraves and Murphy 1992).

Excess ACh produced at motor end plates also causes reversible subjunctional myopathy (Dettbarn 1984). ACh opens receptor channels, promoting influx of Ca^{+2} and other ions into the post-synaptic cell. In rats, that activity brings about regions of necrosis in 10–30% of muscle fibers around the motor end plates. Prolonged muscle weakness and muscle damage might last several weeks or longer after exposure to high concentrations of some OPs, including methyl parathion, fenthion, and dimethoate. These experimental observations might correspond to the proximal muscle weakness that sometimes develops in individuals after an organophosphate pesticide-induced cholinergic crisis. That phenomenon has been termed "intermediate syndrome" because it follows the cholinergic crisis and precedes the appearance of peripheral neuropathy (in the event the organophosphate is able to induce neuropathy).

Although most of the effects of OPs are considered to be due to AChE inhibition, there is evidence that anti-ChEs directly affect ACh receptor channels (Rocha et al. 1996; Katz et al. 1997), that anti-AChE pesticides depress the immune system in experimental animals (Casale et al. 1993), and that choline itself might act as an allosteric regulator of nicotinic receptors in the CNS (Alkondon et al. 1997).

A few OPs have been shown to cause OPIDN, a retrograde degeneration of long and large nerve fibers in the spinal cord and peripheral nerves of humans and experimental animals. Some OPs, such as GB, chlorpyrifos, and isofenphos, require very high doses to be acutely neuropathic (WHO 1986; Lotti 1991). Inhibition of approximately 70% or more of the carboxylesterase NTE often is associated with the disorder (WHO 1986; Lotti 1991). Onset of OPIDN is usually 10 days to several weeks after exposure. It is not clear whether OPIDN can occur after long-term exposure to low concentrations of OPs.

Genetic variation between individuals plays a role in the toxicity of anti-ChEs. An example is humans with inherited low concentrations of plasma BuChEs. Although usually symptomless, such persons given succinylcholine (or a similar drug) during surgery to bring about muscle relaxation, cannot speedily destroy the drug, intensifying and prolonging its activity, sometimes with fatal consequences (Whittaker 1986). Studies on experimental animals indicate that depressing ChEs with anti-ChEs intensifies cocaine's effects. Artificially high blood ChEs protect against chemical-warfare agents, and there is some evidence that low blood ChEs enhance their toxicity (Shih et al. 1998).

EVALUATION OF METHODS USED TO MEASURE CHE

The methods used to determine ChE activity were reviewed by the subcommittee with several questions in mind: (1) Were the methods accurate enough to meet the objectives of the study? (2) Were the methods standardized enough to permit comparisons of one study to another? (3) Were the findings suitable for deriving RfDs?

GA, GB, AND GD STUDIES

In its evaluation of the critical studies of GA, GB, and GD, the subcommittee noted that ChE assay kits and instructions developed for humans appeared to be applied without question to studies of experimental animals. Validation of such applications has not been done, and, therefore, ChE measurements reported in the critical studies of GA (Bucci et al. 1992a), GB (Bucci and Parker 1992), and GD (Bucci et al. 1992b) are of questionable accuracy.

In the critical studies of GA, GB, and GD, the manual Boehringer-Mannheim kit or an Encore II centrifugal analyzer using Boehringer-Mannheim conditions were used to measure ChE activity. RBC-AChE activities were calculated by determining the difference between whole-blood and plasma-ChE concentrations using a formula from the reagent manufacturer (Boehringer-Mannheim Diagnostics 1981). Relatively high activity of plasma AChE (Table G-1) and RBC thiol oxidase (Table G-2), which are present in rats but not humans, was not taken into account; resulting in low estimates of blood-AChE (plasma and RBC combined)

activity. Moreover, the studies were conducted with a substrate concentration of acetylthiocholine of 5.4 mM and a pH of 7.2, which have been shown to reduce human blood-AChE activity by approximately 40% (Wilson et al. 1997). Although the results of the individual studies might be consistent internally, the lack of optimal conditions and the lack of correction for the thiol oxidase activity make it difficult to compare the findings between studies.

TABLE G-1 The Ellman Assay of Cholinesterase Activities in Plasma from 18 Rats

Total ChE (mU/mL)	BuChE (mU/mL)	AChE (mU/mL)
(Mean ± SE)	(Mean ± SE)	(Mean ± SE)
452 ± 17	175 ± 12	210 ± 8

Substrates: acetylthiocholine for ChE, butyrylthiocholine for BuChE, and acetylthiocholine and 0.1 mM iso-OMPA.
Abbreviations: AChE, acetylcholinesterase; BuChE, butyrylcholinesterase; ChE, cholinesterase; iso-OMPA, tetraisopropylpyrophosphoramide
Source: Traina and Serpietri (1984).

The problems with the assay methods might have been minimized by Oak Ridge National Laboratory's (ORNL) use of relative rather than

TABLE G-2 Red-Blood-Cell Dithiobisnitrobenzoate (DTNB) Background Reaction in Various Species

Species	Percent Total Activity
Man	<10
Monkey	18–27
Dog	30–60
Rabbit	30–50
Rat	50–70
Mouse	60–75
Brain (All)	<4
Plasma (All)	Not applicable

Transient activity during the first 5–10 min of DTNB by using the Ellman method.
Source: Loof (1992).

absolute values. It is not clear, however, whether the effects on the dose-response curves were sufficient to materially affect the levels at which changes in activity could be detected.

Table G-3 illustrates the effect of a constant high blank that would occur when the thiol oxidase activity of rodent RBCs is taken into consideration using arbitrary values. When the data are presented in absolute units, the slopes of the curves are the same but the intercepts differ by a value equal to the level of the blank. However, when the data are expressed as a percentage of the total activity, the intercepts are little affected, and the higher the blank, the lower the slopes. Regardless, because the dose values are unchanged, the relative value at which the effect of the inhibition appears seems little affected.

TABLE G-3 Effect of a Constant Blank on Acetylcholinesterase Activity

Dose	0-ACT	20-ACT	30-ACT	40-ACT	%-20	%-30	%-40
1	100	120	130	140	100	100	100
2	90	110	120	130	92	92	93
3	70	90	100	110	75	77	79
4	40	60	70	80	50	54	57
5	25	45	55	65	38	42	46
6	8	28	38	48	23	29	34
7	5	25	35	45	21	27	32
8	3	23	33	43	19	26	31
SLOPE	-15.5	-15.5	-15.5	-15.5	-13.0	-11.9	-11.1
INTCPT	112.5	132.5	142.5	152.5	110.6	109.4	109.0
CORR	-0.969	-0.969	-0.969	-0.969	-0.970	-0.968	-0.967

The effect of a constant high blank, such as that occuring with the thiol oxidase activity of rodent red blood cells, is illustrated above. Arbitrary units of blank activity 0, 20, 30, and 40 are added to a 100-unit enzyme activity. When the data are plotted in absolute numbers, the slopes of the curves are the same, but the intercepts differ by a value equal to the level of the blank. When the data are presented as a percent of the total activity, the intercepts are little affected, and the higher the blank, the lower the slopes. Because the dose values are unchanged, the relative value at which the effect of the inhibition appears should be little affected.
Abbreviations: ACT, activity; INTCPT, intercept; CORR, correction.

VX STUDIES

As with its evaluation of the critical studies of GA, GB, and GD, the subcommittee noted that ChE assay kits and instructions developed for humans were applied to sheep (Rice et al. 1971) and rats (Goldman et al. 1988) in experimental studies of VX. Validation of such applications was not done; therefore, the accuracy of the ChE measurements reported in the studies are questionable.

In its evaluation of the methods used to measure AChE activity in the Rice et al. (1971) study of VX in sheep, the subcommittee noted that the study relied on whole-blood samples collected in heparinized tubes and used within 3 days of sampling. No mention was made of the conditions of sample storage (i.e., whether the samples were iced or refrigerated). A pH method was used to determine AChE activity with acetylcholine as a substrate. Substrate-activity curves showed a clear-cut inhibition of activity with excess substrate, providing good evidence that the measurements were mainly of AChE. Other investigators (e.g., Mohammad et al. 1997) reported that sheep blood has little if any pseudocholinesterase, suggesting that AChE was presumably all that was measured by Rice et al. (1971). The investigators reported that they used a substrate concentration that was greater than optimal, meaning that they started their measurements in the inhibitory range. The reason given was to avoid problems of substrate depletion. The data suggest, however, that the reaction time of the assay might have been too long. Because the values were obtained from end points, there is no way of knowing whether samples of high ChE activity yielded values that were too low.

The subcommittee also evaluated the method used to measure AChE in the Goldman et al. (1988) study of VX in rats. Using a Technicon Autoanalyzer II system, the investigators followed an automated procedure for the assay based on the Ellman method devised to measure exposure of individuals applying pesticides (Knaak et al. 1978). The AChE activity of the RBCs was obtained using acetylthiocholine as a substrate. The activity of the plasma from heparin-treated samples was subtracted from the activity of the whole blood to obtain an estimate of what was believed to be the RBC activity. Values were corrected with hematocrits. No corrections were made for the high AChE concentrations in plasma. At best, subtraction of the plasma values presumably yielded an estimated activity of the total AChE activity in the blood, whether it was from RBCs, serum, or platelets. Second, as was true of

the studies on GA, GB, and GD, no correction was made for an indeterminate amount of thiol oxidase activity in rat RBCs (Loof 1992; Wilson et al. 1996).

DETERMINING LOAELS

ORNL focused on establishing lowest-observed-adverse-effect levels (LOAELs) as the first step in deriving reference doses (RfDs) for the chemical-warfare agents. On balance, that choice might have been good. Errors made in the techniques used might not affect determination of the first detectable decrease in enzyme activity as much as determinations of the slope of the dose-response curve and the values derived from it. In this case, the ORNL experiments were not designed to establish a minimal detectable inhibition. Indeed, many of the dose studies started with inhibitions in the 40% range. It is accepted that decreases of approximately 15% can be reliably detected in carefully performed experiments designed for the purpose.

SUMMARY

The critical studies used by ORNL to derive RfDs for the nerve agents and many other studies in the literature have serious flaws in their ChE determinations and interpretations. Those flaws include

- Application of commercial kits developed for humans to experimental animals without validating them for the species to be used.
- Failure to consider RBC-thiol-oxidase activity. Rat RBCs contain high thiol oxidase activity and low AChE activity, which can result in large errors in calculating percentage of inhibition if uncorrected. There was no indication that such corrections were made in the assays used in the critical studies of GA, GB, and GD. For the critical studies on VX, consideration of whether similar thiol oxidase activity occurs in sheep RBCs was not relevant, because the sheep ChEs were assayed with choline and not thiocholine esters.
- Failure to consider high plasma-AChE activity in the rat. Whether the activity is due to platelet AChE or to soluble AChE is not clear, because the distribution of platelet activity would depend on the specifics

of the collection of samples, their storage, and the method of isolation of blood particulates.

- Failure to consider propionyl cholinesterases. Strictly speaking, rat plasma does not contain a BuChE; it is known to favor propionyl esters more than butyryl esters and both of those esters more than acetyl esters. Sheep plasma is said to have little if any ChE activity, although it is known to have platelet AChE.

CONCLUSIONS

A case can be made that the critical studies on GA, GB, GD, and VX were not optimal for the purpose of establishing LOAELs; they were not designed for that purpose and were not performed with assays validated for the species used and the conditions of the experiments. The subcommittee believes, however, that the critical studies are probably sufficiently reliable to permit their use in deriving RfDs. One reason is that the absolute values of the enzyme activities might not be as important as the relative inhibitions because it is the highest dose at which an inhibition can be detected that is important. Another reason is that methods that subtracted plasma activities from whole-blood activities avoided the problem of plasma-AChE activities, so long as the relative changes in total AChE activity were not affected. However, the problem of high thiol oxidase activity in the rat might represent an uncorrectable confounding factor, because its contribution to total enzyme activity becomes greater as more enzyme activity is inhibited.