Acute Exposure Guideline Levels for Selected Airborne Chemicals

Volume 3

Subcommittee on Acute Exposure Guideline Levels

Committee on Toxicology

Board on Environmental Studies and Toxicology

Division on Earth and Life Studies

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Preface

Extremely hazardous substances (EHSs)[1] can be released accidentally as a result of chemical spills, industrial explosions, fires, or accidents involving railroad cars and trucks transporting EHSs. The people in communities surrounding industrial facilities where EHSs are manufactured, used, or stored and in communities along the nation's railways and highways are potentially at risk of being exposed to airborne EHSs during accidental releases. Pursuant to the Superfund Amendments and Reauthorization Act of 1986, the U.S. Environmental Protection Agency (EPA) has identified approximately 400 EHSs on the basis of acute lethality data in rodents.

As part of its efforts to develop acute exposure guideline levels for EHSs, EPA and the Agency for Toxic Substances and Disease Registry (ATSDR) in 1991 requested that the National Research Council (NRC) develop guidelines for establishing such levels. In response to that request, the NRC published *Guidelines for Developing Community Emergency Exposure Levels for Hazardous Substances* in 1993.

Using the 1993 NRC guidelines report, the National Advisory Committee (NAC) on Acute Exposure Guideline Levels for Hazardous Substances—consisting of members from EPA, the Department of Defense (DOD), the Department of Energy (DOE), the Department of Transportation, other federal and state governments, the chemical industry,

[1]As defined pursuant to the Superfund Amendments and Reauthorization Act of 1986.

academia, and other organizations from the private sector—has developed acute exposure guideline levels (AEGLs) for approximately 80 EHSs.

In 1998, EPA and DOD requested that the NRC independently review the AEGLs developed by NAC. In response to that request, the NRC organized within its Committee on Toxicology the Subcommittee on Acute Exposure Guideline Levels, which prepared this report. This report is the third volume in the series *Acute Exposure Guideline Levels for Selected Airborne Chemicals*. It reviews the AEGLs for the nerve agents (GA [tabun], GB [sarin], GD [soman], GF, and VX), sulfur mustard, diborane, and methyl isocyanate for scientific accuracy, completeness, and consistency with the NRC guideline reports.

This report was reviewed in draft by individuals selected for their diverse perspectives and technical expertise, in accordance with procedures approved by the NRC's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its 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 for their review of this report: Mohamed Abou-Donia of Duke University; Janice Chambers of Mississippi State University; and Sidney Green of Howard University.

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations nor did they see the final draft of the report before its release. The review of this report was overseen by David Moore of Battelle Memorial Institute, appointed by the Division on Earth and Life Studies, who was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

The subcommittee gratefully acknowledges the valuable assistance provided by the following persons: Roger Garrett (deceased, March 31, 2003), Paul Tobin, and Ernest Falke (all from EPA); George Rusch (Honeywell, Inc.); Po Yung Lu, Claudia Troxel, Robert Young, Carol Forsyth, Dennis Opresko, and Annetta Watson (all from Oak Ridge National Laboratory). Aida Neel was the project assistant. Kelly Clark

edited the report. We are grateful to James J. Reisa, director of the Board on Environmental Studies and Toxicology (BEST), for his helpful comments. The subcommittee particularly acknowledges Kulbir Bakshi, project director for the subcommittee, for bringing the report to completion. Finally, we would like to thank all members of the subcommittee for their expertise and dedicated effort throughout the development of this report.

Daniel Krewski, *Chair*
Subcommittee on Acute Exposure
Guideline Levels

Bailus Walker, *Chair*
Committee on Toxicology

Dedication

The subcommittee dedicates this series of reports
to our late colleague and director of
the Acute Exposure Guideline Levels program,
Dr. Roger L. Garrett,
whose 27 years of distinguished service with the
U.S. Environmental Protection Agency
in the fields of toxicology and health-risk assessment
contributed significantly to scientific knowledge,
to the development of
the Acute Exposure Guideline Levels program,
and to the protection of public health and safety.

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Acute Exposure Guideline Levels for Selected Airborne Chemicals

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Introduction

This report is the third volume in the series *Acute Exposure Guideline Levels for Selected Airborne Chemicals.*

In the Bhopal disaster of 1984, approximately 2,000 residents living near a chemical plant were killed and 20,000 more suffered irreversible damage to their eyes and lungs following accidental release of methyl isocyanate. The toll was particularly high because the community had little idea what chemicals were being used at the plant, how dangerous they might be, and what steps to take in case of emergency. This tragedy served to focus international attention on the need for governments to identify hazardous substances and to assist local communities in planning how to deal with emergency exposures.

In the United States, the Superfund Amendments and Reauthorization Act (SARA) of 1986 required that the U.S. Environmental Protection Agency (EPA) identify extremely hazardous substances (EHSs) and, in cooperation with the Federal Emergency Management Agency and the Department of Transportation, assist Local Emergency Planning Committees (LEPCs) by providing guidance for conducting health-hazard assessments for the development of emergency-response plans for sites where EHSs are produced, stored, transported, or used. SARA also required that the Agency for Toxic Substances and Disease Registry (ATSDR) determine whether chemical substances identified at hazardous waste sites or in the environment present a public-health concern.

As a first step in assisting the LEPCs, EPA identified approximately 400 EHSs largely on the basis of their "immediately dangerous to life and health" (IDLH) values developed by the National Institute for Occupational Safety and Health (NIOSH) in experimental animals. Although several public and private groups, such as the Occupational Safety and Health Administration (OSHA) and the American Conference of Governmental Industrial Hygienists (ACGIH), have established exposure limits for some substances and some exposures (e.g., workplace or ambient air quality), these limits are not easily or directly translated into emergency exposure limits for exposures at high levels but of short duration, usually less than 1 h, and only once in a lifetime for the general population, which includes infants, children, the elderly, and persons with diseases, such as asthma, heart disease, or lung disease.

The National Research Council (NRC) Committee on Toxicology (COT) has published many reports on emergency exposure guidance levels and spacecraft maximum allowable concentrations for chemicals used by the Department of Defense (DOD) and the National Aeronautics and Space Administration (NASA) (NRC 1968, 1972, 1984a,b,c,d, 1985a,b, 1986a,b, 1987, 1988, 1994, 1996a,b, 2000). COT has also published guidelines for developing emergency exposure guidance levels for military personnel and for astronauts (NRC 1986b, 1992). Because of COT's experience in recommending emergency exposure levels for short-term exposures, in 1991 EPA and ATSDR requested that COT develop criteria and methods for developing emergency exposure levels for EHSs for the general population. In response to that request, the NRC assigned this project to the COT Subcommittee on Guidelines for Developing Community Emergency Exposure Levels for Hazardous Substances. The report of that subcommittee, *Guidelines for Developing Community Emergency Exposure Levels for Hazardous Substances* (NRC 1993), provides step-by-step guidance for setting emergency exposure levels for EHSs. Guidance is given on what data are needed, what data are available, how to evaluate the data, and how to present the results.

In November1995, the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NAC)[1] was established to identify, review, and interpret relevant toxicologic and other scientific data and to develop acute exposure guideline levels (AEGLs) for high-

[1]NAC is composed of members from EPA, DOD, many other federal and state agencies, industry, academia, and other organizations. The roster of NAC is shown on page 8.

priority, acutely toxic chemicals. The NRC's previous name for acute exposure levelsCcommunity emergency exposure levels (CEELs)Cwas replaced by the term AEGLs to reflect the broad application of these values to planning, response, and prevention in the community, the workplace, transportation, the military, and the remediation of Superfund sites.

Three levels—AEGL-1, AEGL-2, and AEGL-3—are developed for each of five exposure periods (10 min, 30 min, 1 h, 4 h, and 8 h) and are distinguished by varying degrees of severity of toxic effects.

The three AEGLs are defined as follows:

AEGL-1 is the airborne concentration (expressed as ppm [parts per million] or mg/m^3 [milligrams per cubic meter]) of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic nonsensory effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.

AEGL-2 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape.

AEGL-3 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.

Airborne concentrations below AEGL-1 represent exposure levels that can produce mild and progressively increasing but transient and nondisabling odor, taste, and sensory irritation or certain asymptomatic, nonsensory adverse effects. With increasing airborne concentrations above each AEGL, there is a progressive increase in the likelihood of occurrence and the severity of effects described for each corresponding AEGL. Although the AEGL values represent threshold levels for the general public, including susceptible subpopulations, such as infants, children, the elderly, persons with asthma, and those with other illnesses, it is recognized that individuals, subject to unique or idiosyncratic responses, could experience the effects described at concentrations below the corresponding AEGL.

SUMMARY OF REPORT ON GUIDELINES FOR DEVELOPING AEGLS

As described in the *Guidelines for Developing Community Emergency Exposure Levels for Hazardous Substances* (NRC 1993) and the NAC guidelines report *Standing Operating Procedures on Acute Exposure Guideline Levels for Hazardous Substances*(NRC 2001), the first step in establishing AEGLs for a chemical is to collect and review all relevant published and unpublished information available on a chemical. Various types of evidence are assessed in establishing AEGL values for a chemical. These include information from (1) chemical-physical characterizations, (2) structure-activity relationships, (3) in vitro toxicity studies, (4) animal toxicity studies, (5) controlled human studies, (6) observations of humans involved in chemical accidents, and (7) epidemiologic studies. Toxicity data from human studies are most applicable and are used when available in preference to data from animal studies and in vitro studies. Toxicity data from inhalation exposures are most useful for setting AEGLs for airborne chemicals because inhalation is the most likely route of exposure and because extrapolation of data from other routes would lead to additional uncertainty in the AEGL estimate.

For most chemicals, actual human toxicity data are not available or critical information on exposure is lacking, so toxicity data from studies conducted in laboratory animals are extrapolated to estimate the potential toxicity in humans. Such extrapolation requires experienced scientific judgment. The toxicity data from animal species most representative of humans in terms of pharmacodynamic and pharmacokinetic properties are used for determining AEGLs. If data are not available on the species that best represents humans, the data from the most sensitive animal species are used to set AEGLs. Uncertainty factors are commonly used when animal data are used to estimate risk levels for humans. The magnitude of uncertainty factors depends on the quality of the animal data used to determine the no-observed-adverse-effect level (NOAEL) and the mode of action of the substance in question. When available, pharmacokinetic data on tissue doses are considered for interspecies extrapolation.

For substances that affect several organ systems or have multiple effects, all end points—including reproductive (in both sexes), develop-mental, neurotoxic, respiratory, and other organ-related effects—are evaluated, the most important or most sensitive effect receiving the greatest attention. For carcinogenic chemicals, excess carcinogenic risk is estimated, and the AEGLs corresponding to carcinogenic risks of 1 in 10,000 (1×10^{-4}), 1 in

100,000 (1×10^{-5}), and 1 in 1,000,000 (1×10^{-6}) exposed persons are estimated.

REVIEW OF AEGL REPORTS

As NAC began developing chemical-specific AEGL reports, EPA and DOD asked the NRC to review independently the NAC reports for their scientific validity, completeness, and consistency with the NRC guideline reports (NRC 1993; NRC in press). The NRC assigned this project to the COT Subcommittee on Acute Exposure Guideline Levels. The subcommittee has expertise in toxicology, epidemiology, pharmacology, medicine, industrial hygiene, biostatistics, risk assessment, and risk communication.

The AEGL draft reports are initially prepared by ad hoc AEGL Development Teams consisting of a chemical manager, two chemical reviewers, and a staff scientist of the NAC contractor—Oak Ridge National Laboratory. The draft documents are then reviewed by NAC and elevated from "draft" to "proposed" status. After the AEGL documents are approved by NAC, they are published in the *Federal Register* for public comment. The reports are then revised by NAC in response to the public comments, elevated from "proposed" to "interim" status, and sent to the NRC Subcommittee on Acute Exposure Guideline Levels for final evaluation.

The NRC subcommittee's review of the AEGL reports prepared by NAC and its contractors involves oral and written presentations to the subcommittee by the authors of the reports. The NRC subcommittee provides advice and recommendations for revisions to ensure scientific validity and consistency with the NRC guideline reports (NRC 1993, 2001). The revised reports are presented at subsequent meetings until the subcommittee is satisfied with the reviews.

Because of the enormous amount of data presented in the AEGL reports, the NRC subcommittee cannot verify all the data used by NAC. The NRC subcommittee relies on NAC for the accuracy and completeness of the toxicity data cited in the AEGLs reports.

This report is the third volume in the series *Acute Exposure Guideline Levels for Selected Airborne Chemicals*. AEGL documents for nerve agents (GA, GB, GD, GF, and VX), sulfur mustard, diborane, and methyl isocyanate are published as an appendix to this report. The subcommittee concludes that the AEGLs developed in those documents are scientifically valid conclusions based on the data reviewed by NAC and are consistent

with the NRC guideline reports. AEGL reports for additional chemicals will be presented in subsequent volumes.

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Appendixes

1

Nerve Agents GA, GB, GD, GF, and VX[1]

Acute Exposure Guideline Levels

SUMMARY

The nerve agents for which AEGL analyses have been performed include the G-series agents (GA [tabun], GB [sarin], GD [soman], and GF) and nerve agent VX. These agents are all toxic ester derivatives of phosphonic acid containing either a cyanide, fluoride, or sulfur substituent group; they are commonly termed "nerve" agents as a consequence of their anticholinesterase properties. These compounds were developed as chemical warfare

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[1]This document was prepared by the AEGLs Development Team comprising Annetta Watson, Dennis Opresko, and Robert Young (Oak Ridge National Laboratory) and John Hinz and Glenn Leach (Chemical Managers) of the National Advisory Committee (NAC) on Acute Exposure Guideline Levels for Hazardous Substances. The NAC reviewed and revised the document and the AEGL values as deemed necessary. Both the document and the AEGL values were then reviewed by the National Research Council (NRC) Subcommittee on Acute Exposure Guideline Levels. The NRC subcommittee concludes that the AEGLs developed in this document are scientifically valid conclusions based on the data reviewed by the NRC and are consistent with the NRC guidelines reports (NRC 1993, 2001).

agents, and one (agent GB, or sarin) was used by terrorists in the 1995 exposure incident that took place in the Tokyo subway system. The chemical names of these five agents are as follow: agent GA, dimethylamido-cyanoethylphosphate (CAS Registry No. 77-81-6); agent GB, isopropyl methylphosphonofluoridate (CAS Registry No. 107-44-8); agent GD, pinacolyl methylphosphonofluoridate (CAS Registry No. 96-64-0); agent GF, O-cyclohexylmethyl-fluorophosphonate (CAS Registry No. 329-99-7); and agent VX, O-ethyl-S-(diisopropylaminoethyl) methyl phosphonothiolate (CAS Registry No. 50782-69-9).

The G agents are all viscous liquids of varying volatility (vapor density relative to air between 4.86 and 6.33) with faint odors ("faintly fruit," or "spicy," odor of camphor). Toxic effects may occur at vapor concentrations below those of odor detection. Agent VX is a amber-colored liquid with a vapor density of 9.2 (air = 1) and is considered odorless. As a consequence, agent VX vapor possesses no olfactory warning properties.

The vapor pressures and acute toxicity of these agents are sufficiently high for the vapors to be rapidly lethal. Within the G-series, GB is considered a greater vapor hazard than agent GD. Agent GA represents a smaller vapor hazard and is expected to present a relevant contact hazard. The vapor density of agent GF is intermediate between that of agents GA and GD. Agent VX, which has a vapor density (9.2) greater that of any G agent under consideration, was deliberately formulated to possess a low volatility; VX is approximately 2,000 times less volatile than nerve agent GB (DA 1990). As a consequence, agent VX is a persistent, "terrain denial" military compound with the potential to off-gas toxic vapor for days following surface application.

Exposure to acutely toxic concentrations of nerve agents can result in excessive bronchial, salivary, ocular, and intestinal secretions and sweating, miosis, bronchospasm, intestinal hypermotility, bradycardia, muscle fasciculations, twitching, weakness, paralysis, loss of consciousness, convulsions, depression of the central respiratory drive, and death. Minimal effects observed at low vapor concentrations include miosis (contraction of the pupils of the eye, with subsequent decrease in pupil area), tightness of the chest, rhinorrhea, and dyspnea (Dunn and Sidell 1989).

The results of agent GB vapor exposure studies conducted with human volunteers indicate that the threshold for miosis and other minimal toxic effects falls in the range of 0.05-0.5 mg/m^3 for 10-30 minute (min) exposures. The findings are based on the results of low-concentration nerve agent exposures of informed volunteers who were under clinical supervision during the periods of exposure as well as for postexposure periods of several months.

A concern associated with symptomatic exposures to anticholinesterase compounds such as the nerve agents is the possibility of chronic neurological effects. There is, at present, no evidence indicating that asymptomatic exposures to any of the nerve agents result in chronic neurological disorders. In general, the available epidemiological data indicate that most clinical signs of toxicity resolve within hours to days; severe miosis can require several months after exposure for resolution. However, several studies have shown that subclinical signs may persist for longer periods. Following the chemical terrorist attacks with nerve agent GB (sarin) that occurred in Japan in 1994 and 1995, clinical signs of agent toxicity were no longer apparent in the surviving victims 3 months (mo) after the exposures had occurred; however, several studies conducted on a small number of asymptomatic individuals 6-8 mo after the attack revealed subclinical signs of neurophysiological deficits as measured by event-related and visual evoked potentials, psychomotor performance, and increases in postural sway.

Small but measurable changes in single fibre electromyography (SFEMG) of the forearm were detectable between 4 and 15 mo following exposure to a concentration of agent GB that produced minimal clinical signs and symptoms in fully informed human subjects who were under clinical supervision in compliance with Helsinki accords (Baker and Sedgwick 1996). The SFEMG effects were not clinically significant and were not detectable after 15-30 mo. In a separate study of workers who had been occupationally exposed to agent GB (sarin), altered electroencephalograms (EEGs) were recorded 1 year (y) or more after the last exposure had occurred. Spectral analysis of the EEGs indicated significant increases in brain beta activity (12-30 Hz) in the exposed group when compared with nonexposed controls, and sleep EEGs revealed significantly increased rapid eye movement in the exposed workers; however, those observations were not clinically significant. Increases in beta activity were also observed in rhesus monkeys 1 y after being dosed with GB at 5 mg/kg. Slight, but nonsignificant, increases in beta activity, without deleterious effects on cognitive performance, were reported for marmosets injected with GB at 3.0 mg/kg and tested 15 mo later. The significance of subclinical neurological effects for the long-term health of exposed individuals has not been determined.

Animal data from vapor and oral exposure studies for the G-series nerve agents and agent VX suggest that agents GB and VX do not induce reproductive or developmental effects in mammals. Oral exposure studies of agent GD in lab animals as well as injection exposure studies of agent GA likewise suggest a lack of reproductive or development effects for

these agents. Neither agent GB nor agent VX were found to be genotoxic in a series of microbial and mammalian assays, but agent GA was reported to be weakly mutagenic. There is no evidence indicating that agents GB, GA, or VX are carcinogenic.

Derivation of G-Agent AEGL Estimates

The base of data for toxicological effects in humans is more complete for agent GB than for any of the other nerve agents under consideration in this analysis. Furthermore, agent GB is the only G agent for which sufficient human data are available to directly derive AEGL-1 and AEGL-2 estimates, and the only G agent for which sufficient laboratory animal data are available for deriving an AEGL-3 value for all five AEGL time periods.

AEGL-1 and AEGL-2 Values for G-series Agents

The AEGL-1 values for agent GB were derived from a well-conducted study on adult female Sprague-Dawley rats exposed whole-body in a dynamic airflow chamber to a range of GB vapor concentrations (0.01 to 0.48 mg/m^3) over three time durations (10 min, 60 min, or 240 min) (total of 283 agent-exposed rats of which 142 were female and 141 were male) (Mioduszewski et al. 2002b). With the inclusion of range-finding experiments and controls (N = 130), a total of 423 rats were used in this well-conducted study, which involved highly credible protocols for GB vapor generation and measurement. Analysis of rat pupil diameters assessed pre- and postexposure allowed determination of EC_{50} values for miosis (defined as a postexposure pupil diameter of 50% or less of the preexposure diameter in 50% of the exposed population). Blood samples collected from tail vein and heart at 60 min and 7 d postexposure indicated no significant change from preexposure baseline in monitored blood RBC-ChE, butyrylcholinesterase (BuChE) or carboxylesterase. No other clinical signs were evident throughout the duration of the study. Gender differences (females more susceptible) were statistically significant at 10 min (p = 0.014) and 240 min (p = 0.023), but not at 60 min (p = 0.054). This is a well-defined animal end point in a susceptible gender, and it is transient, reversible, and nondisabling.

In terms of potential effects on humans, an EC_{50} for miosis is not considered an adverse effect. This degree of miosis is the first measurable change, by modern and reproducible techniques, in the continuum of

response to anticholinesterase compounds. In bright daylight or under bright lighting, a 50% reduction in pupil diameter would result in greater visual acuity among some members of the affected exposed population and no marked reduction in visual acuity for the majority of the affected population. In twilight or dim light conditions, 50% reduction in pupil diameter in some persons would result in reduced visual acuity and less-than-optimal performance of tasks requiring operation of vehicular controls, monitoring or tracking on computer screens, reading of fine text, or shifts in focus between near and far fields. For individuals with central cataracts, the effects would be more pronounced at all illumination levels. During the Tokyo Subway Incident (terrorist release of GB), persons experiencing $50% reduction in pupil diameter were able to self-rescue and to render aid to others.

Data from GB vapor studies of nonhuman primates (marmosets, 5 h exposures to GB vapor concentrations at 0.05 to 150 $\mu g/m^3$) (van Helden et al. 2001, 2002) and human volunteers (minimal and reversible effects of miosis, rhinorrhea, headache, etc., after a 20-min exposure to a GB vapor concentration at 0.05 mg/m^3) (Harvey 1952; Johns 1952) are considered secondary and supportive. The human data of Harvey (1952) and Johns (1952) indicate that some adult humans exposed to concentrations within the exposure range tested by Mioduszewski et al. (2002b) would experience some discomfort (headache, eye pain, nausea, etc.) in addition to miosis corresponding to #50% pupil area decrement but no disability (see definition of AEGL-1 provided in NRC [2001]). Compared to the available human data, the miosis data derived from the study on rats (Mioduszewski et al. 2002b) are considered a more reliable data set because they are based on current and multiple analytical techniques for quantifying exposures and measuring miosis and because they apply an experimental protocol incorporating sufficiently large test and control populations. With the additional knowledge that the EC_{50} exhibited by rats in the study of Mioduszewski et al. (2002b) is transient and reversible, the determination was made that EC_{50} for miosis in female (susceptible gender) SD rats is an appropriate end point for estimating AEGL-1 values. Mioduszewski et al. (2002b) is considered the critical study for derivation of AEGL-1 estimates for agent GB. The weight-of-evidence analysis indicates reasonable concordance among AEGL-1 estimates derived from the female Sprague-Dawley rat, the marmoset, and the human data sets identified above. Application of the Mioduszewski et al. (2002b) rat miosis data did not significantly change the interim values for AEGL-1 (based on the human experimental data of Harvey [1952] and Johns [1952]) but confirmed that

the interim values were representative, protective, and could be retained as final AEGL-1 values.

The AEGL-2 values for agent GB were derived from a study in which miosis, dyspnea, photophobia, inhibition of red blood cell cholinesterase (RBC-ChE), and changes in single fibre electromyography (SFEMG) were observed in human volunteers following a 30-min exposure at 0.5 mg/m^3 (Baker and Sedgwick 1996). The SFEMG changes noted in the study were not clinically significant and were not detectable after 15-30 mo. Baker and Sedgwick considered SFEMG changes a possible early indicator or precursor of the nondepolarising neuromuscular block associated with intermediate-syndrome paralysis in severe organophosphorous insecticide poisoning cases. They concluded that the electromyographic changes were persistent (>15 mo), but that they were reversible and subclinical.

Although not considered debilitating or permanent effects in themselves, SFEMG changes are considered an early indicator of exposures that potentially could result in more significant effects. Selection of this effect as a protective definition of an AEGL-2 level is considered appropriate given the steep dose-response toxicity curve of nerve agents (Aas et al. 1985; Mioduszewski et al. 2000, 2001, 2002a). The concept of added precaution for steep dose-response is consistent with the emergency planning guidance for nerve agents that was developed by the National Center for Environmental Health of the Centers for Disease Control and Prevention (Thacker 1994).

Animals exposed to low concentrations of the G agents exhibit the same signs of toxicity as humans, including miosis, salivation, rhinorrhea, dyspnea, and muscle fasciculations. Studies on dogs and rats indicate that exposures to GB at 0.001 mg/m^3 for up to 6 h/d are unlikely to produce any signs of toxicity.

Because exposure-response data were not available for all of the AEGL-specific exposure durations, temporal extrapolation was used in the development of AEGL values for some of the AEGL-specific time periods. The concentration-exposure time relationship for many systemically acting vapors and gases may be described by $C^n \times t = k$, where the exponent n ranges from 0.8 to 3.5. The temporal extrapolation used here is based on a log-log linear regression of the LC_{01} lethality of GB in female Sprague-Dawley rats (Mioduszewski et al. 2000, 2001, 2002a) and a log-log linear regression of female SD rat miosis data following GB vapor exposure for durations of 10-240 min (Mioduszewski et al. 2002b). Regression analysis of the LC_{01} values yields an n value of 1.93 with an r^2 of 0.9948, and regression analysis of the miosis data yields an n value of 2.00 with an r^2 of 0.4335 (24 data points; see Appendix B). Given that all mammalian

toxicity end points observed in the data set for all nerve agents represent different points on the response continuum for anticholinesterase exposure, and that the mechanism of acute mammalian toxicity (cholinesterase inhibition) is the same for all nerve agents, the experimentally derived n = 2 from the Mioduszewski et al. (2000, 2001, 2002a,b) rat lethality and miosis data sets is used as the scaling function for all the AEGL derivations rather than a default value. An n of 1.16 (r^2 = 0.6704) was calculated for comparison using other data (human volunteer) and other end points (e.g., GB-induced miosis in humans; see Appendix B). However, because of uncertainties associated with some of the exposure measurements in the earlier studies, the Mioduszewki et al. rat data were determined to be the best source of an estimate for n. The n value of 2 was used to extrapolate for exposure time periods for which there were no experimental data. Those included (1) the 8-h AEGL-3 value (extrapolated from experimental data for 6 h); (2) the 30-min and 8-h AEGL-1 values (extrapolated from 10-min and 4-h experimental data; and (3) all of the AEGL-2 values (extrapolated from experimental data for 30 min).

In consultation with experimental investigators at Porton Down (United Kingdom) and the TNO Prins Maurits Laboratory (Netherlands), the analysis has determined that the miotogenic response of mammalian eyes to agent GB vapor exposure is similar across species. The species evaluated include standard laboratory animals (rabbits, rats, guinea pigs), nonhuman primates (marmosets), and humans. As a consequence, the interspecies uncertainty factor (UF) for the critical AEGL-1 end point of miosis is considered equal to 1. To accommodate known variation in human cholinesterase and carboxylesterase activity that may make some individuals susceptible to the effects of cholinesterase inhibitors such as nerve agents, a factor of 10 was applied for intraspecies variability (protection of susceptible populations). A modifying factor is not applicable. Thus, the total UF for estimating AEGL-1 values for agent GB is 10.

The fact that AEGL-2 analyses for agent GB are based on data from human volunteers (Baker and Sedgwick 1996) precludes the use of an interspecies UF. As was the case in the AEGL-1 estimations, a factor of 10 was applied for intraspecies variability (protection of susceptible populations). A modifying factor is not applicable. Thus, the total UF for estimating AEGL-2 values for agent GB is 10.

In comparison to the data set for agent GB, the data sets characterizing the toxicity of agents GA, GD, and GF are less complete. However, the database for the G agents as a group is considered reasonably complete in that there is/are (1) experimental data for multiple species, including humans; (2) documented nonlethal and lethal end points that follow an

exposure-response curve; (3) a known mechanism of toxicity common to all the G agents with all end points representing a response continuum to inhibition of cholinesterase activity; and (4) no uncertainties regarding other toxic end points such as reproductive or developmental effects or carcinogenicity. Because the mechanism of action is the same for all the G agents, data uncertainty is reduced, and target organ effects are expected to be identical, but different in magnitude. Thus, it was possible to develop AEGL estimates for agents GA, GD, and GF by a comparative method of relative potency analysis from the more complete data set for agent GB. This concept has been applied before in the estimation of G-series nerve agent exposure limits, most recently by Mioduszewski et al. (1998).

The AEGL-1 and AEGL-2 values for agents GA, GD, and GF were derived from the AEGL-1 and AEGL-2 values for GB using a relative potency approach based on the potency of the agents needed to induce LOAEL effects of miosis, rhinorrhea, and SFEMG and agent concentration in milligrams per cubic meter. Agents GA and GB were considered to have an equivalent potency for causing miosis; thus, the AEGL-1 values for agents GA and GB are equal in milligrams per cubic meter. Agents GD and GF are considered approximately 2 times as potent as agents GB or GA for these end points, and equipotent to each other for AEGL-1 and AEGL-2 effects. Thus, the AEGL-1 and AEGL-2 concentration values for agents GD and GF are equal to 0.5 times those values derived for agents GA and GB, in milligrams per cubic meter.

AEGL-3 Values for G-Series Agents

AEGL-3 values for agent GB were derived from recent inhalation studies in which the lethality of GB vapor in female Sprague-Dawley rats was evaluated for 10-, 30-, 60-, 90-, 240-, and 360-min time periods (Mioduszewski et al. 2000, 2001, 2002a). Both experimental LC_{01} and LC_{50} values were evaluated. The use of a rat data set resulted in selection of an interspecies UF of 3; the full default value of 10 was not considered appropriate because the mechanism of toxicity in rats and humans is the same, and lethality represents one point on the response continuum for these anticholinesterase compounds. The full default value of 10 for intraspecies uncertainty was considered necessary to protect susceptible populations. Because a modifying factor is not applicable, the composite UF for AEGL-3 determination for agent GB is equal to 30.

The AEGL-3 values for agent GA were derived from the AEGL-3 values for GB using a relative potency approach based on lethality of the

agents; the potency of agent GA was considered to be only one-half that of agent GB for this end point. Thus, the AEGL-3 concentration values for agent GA are equal to 2.0 times the AEGL-3 values for agent GB, in milligrams per cubic meter.

The lethal potencies of agents GD and GF are considered equivalent and equipotent to that of agent GB; thus, the AEGL-3 concentration values for agent GB, GD, and GF are equal in milligrams per cubic meter, and the same composite UF (30) was applied in the derivation of the AEGL-3 values for agents GB, GD, and GF. For comparison, AEGL-3 values for GD were alternately derived from a secondary and short-term GD inhalation study of rat lethality for exposure times #30 min (Aas et al. 1985). As was the case in the derivation of the GB AEGLs, an *n* value of 2 was used for extrapolating to different time periods; however, because of the sparse data set for GD, the full default values for interspecies (10) and intraspecies (10) uncertainty were applied to the Aas et al. (1985) data. Because a modifying factor is not applicable, a composite UF of 100 was used for the Aas et al. (1985) data, whereas in the GB AEGL derivation from the Mioduszewski et al. (2000, 2001, 2002a) rat lethality data, a composite UF of 30 was used. The resulting 10-min AEGL-3 (0.27 mg/m^3) and 30-min AEGL-3 (0.15 mg/m^3) estimates for agent GD from Aas et al. (1985) are very similar to those for GB (0.38 mg/m^3 for 10 min and 0.19 mg/m^3 for 30 min) from Mioduszewski et al. (2000, 2001, 2002a) and support the assumption of lethal equipotency for agents GB and GD.

Derivation of Agent VX AEGL Estimates

Insufficient data are available from which to directly derive AEGL values for VX from human or animal inhalation toxicity studies. The few studies available are historical and are considered nonverifiable because of flawed study design, poor sampling techniques, or suspect contamination of sampling and detection apparatus. Nevertheless, available literature clearly indicates that inhibition of cholinesterase activity is a common mechanism of toxicity shared by the G-series nerve agents and nerve agent VX. Thus, it was possible to develop AEGL estimates for agent VX by a comparative method of relative potency analysis from the more complete data set for nerve agent GB. The concept has been applied before in the estimation of agent VX exposure limits, most recently by Reutter et al. (2000). There are a number of estimates in the literature regarding the potency of VX relative to agent GB; all estimates indicate that vapor toxicity for agent VX is greater than that for agent GB. Comparable RBC-

ChE_{50} data from clinically supervised human volunteers (Grob and Harvey 1958; Sidell and Groff 1974), who were exposed to agents GB and VX during well-conducted studies, are available for estimation of relative potency. The human data indicate that agent VX is approximately 4 times more potent than agent GB for inducing the RBC-ChE_{50} end point, which is considered an early and quantitative measure of the response continuum known for those compounds. Thus, the GB:VX relative potency ratio of 4 is considered an appropriate estimate of GB:VX relative potency for all VX AEGL determinations.

All mammalian toxicity end points observed in the data set for nerve agent VX as well as the G-series agents represent different points on the response continuum for anticholinesterase effects. Further, the mechanism of mammalian toxicity (cholinesterase inhibition) is the same for all nerve agents. In consequence, the experimentally derived $n = 2$ from the Mioduszewski et al. (2000, 2001, 2002a,b) rat miosis and lethality data sets for agent GB are used as the scaling function for the agent-VX AEGL-1, AEGL-2, and AEGL-3 derivations rather than a default value.

By applying the GB:VX relative potency concept outlined above (the relative potency of GB:VX equal to 4), the AEGL-1 analyses for agent VX are derived from miosis data for adult female SD rats exposed to GB vapor for three time durations of significance for AEGLs (10, 60, and 240 min) (Mioduszewski et al. 2002b). Data from a GB vapor study of nonhuman primates (marmosets, 5 h exposures to GB vapor concentrations at 0.05-150 : g/m^3) (van Helden et al. 2001, 2002) and human volunteers (minimal and reversible effects of miosis, rhinorrhea, headache, etc., after a 20-min exposure to a GB vapor concentration at 0.05 mg/m^3) (Harvey 1952; Johns 1952) are considered secondary and supportive. The same UFs and logic applied in the derivation of AEGL-1 and AEGL-2 values for agent GB (e.g., interspecies UF of 1, intraspecies UF of 10) are used here for estimating AEGL-1 and AEGL-2 values for agent VX. With application of a modifying factor of 3 for the sparse VX data set, the total UF for estimating AEGL-1 values for agent VX (from the GB data set of Mioduszewski et al. [2002b]) is 30.

By further application of the GB:VX relative potency concept outlined above, the AEGL-2 values for agent VX were derived from a GB vapor exposure study of human subjects in which miosis, dyspnea, photophobia, inhibition of red blood cell cholinesterase (RBC-ChE) to approximately 60% of individual baseline, and small but measurable changes in SFEMG of the forearm occurred following a 30-min exposure at 0.5 mg GB/m^3 (Baker and Sedgwick 1996).

The fact that AEGL-2 analyses for agent VX are based on data from clinically supervised human volunteers exposed to GB vapor (Baker and Sedgwick 1996) precludes the use of an interspecies UF. With application of a factor of 10 for intraspecies variability and a modifying factor of 3 for the sparse VX data set, the total UF for estimating AEGL-2 values for agent VX (from the GB data set of Baker and Sedgwick [1996]) is 30.

By further application of the GB:VX relative potency concept outlined above, the AEGL-3 values for agent VX were derived from recent inhalation studies in which the lethality of GB to female Sprague-Dawley rats was evaluated for the 10-, 30-, 60-, 90-, 240-, and 360-min time periods (Mioduszewski et al. 2000, 2001, 2002a). Both experimental LC_{01} and LC_{50} values were evaluated. The same UFs and logic applied in the derivation of AEGL-3 values for agent GB (interspecies UF of 3 and an intraspecies UF of 10) are used here for agent VX. With the additional application of a modifying factor of 3 for the sparse VX data set, the total UF for AEGL-3 determination for agent VX is equal to 100.

Research Needs

G-Series Agents

Further data analysis and experimentation is needed to more fully understand gender differences in susceptibility to nonlethal and lethal end points among the test population of SD rats. Interspecies susceptibility could be more fully characterized by determining if similar results can be obtained for the same protocol with different test species (particularly nonhuman primates).

The scarcity of dose-response data for agents GA, GD, and GF forces the AEGL analysis to rely on assumptions of relative potency that need experimental confirmation.

Agent VX

It is noted that additional research to more fully characterize VX is needed in the following areas:

1. The toxicity of VX vapor in whole-animal systems. It is noted that specific experimental focus should be on obtaining data that would reduce uncertainties regarding the relative potency of agents GB and VX, or the potency of agent VX, for critical effects such as miosis, rhinorrhea, and lethality. Such studies could be adequately performed on a limited test population and scale.
2. The emissions profile expected during VX release, especially the generation and yield of VX vapors versus aerosol.
3. Comparative examination of agents GB and VX with regard to noncholinergic mechanisms in an effort to correlate whole-organism toxic responses with those reported for in vitro rat hippocampal cells in culture. The primary goal would be to generate a more refined determination of GB:VX relative potency.

Final AEGL estimates for the G-series nerve agents and VX are given in the summary table below.

1. INTRODUCTION

This evaluation of the AEGL values for the nerve agents GA, GB, GD, and GF is based on studies and data that are documented in the open literature as well as some unclassified documents with limited distribution requirements. Because of the military-specific nature of these compounds, some additional reports from the United States and elsewhere with classified or restricted distribution requirements exist. However, because of the open review process established by the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances, classified and other restricted-distribution reports are not cited in this evaluation, to the best of our knowledge.

TABLE 1-1 Summary of Final AEGL Values for Nerve Agents GA, GB, GD, GF, and VX[a]

Agent	Classification	10-min	30-min	1-h	4-h	8-h	End Point (Reference)
GA	AEGL-1 (Nondisabling)	0.0010 ppm (0.0069 mg/m^3)	0.00060 ppm (0.0040 mg/m^3)	0.00042 ppm (0.0028 mg/m^3)	0.00021 ppm (0.0014 mg/m^3)	0.00015 ppm (0.0010 mg/m^3)	Based on relative potency from GB[b]
	AEGL-2 (Disabling)	0.013 ppm (0.087 mg/m^3)	0.0075 ppm (0.050 mg/m^3)	0.0053 ppm (0.035 mg/m^3)	0.0026 ppm (0.017 mg/m^3)	0.0020 ppm (0.013 mg/m^3)	Based on relative potency from GB[b]
	AEGL-3 (Lethal)	0.11 ppm (0.76 mg/m^3)	0.057 ppm (0.38 mg/m^3)	0.039 ppm (0.26 mg/m^3)	0.021 ppm (0.14 mg/m^3)	0.015 ppm (0.10 mg/m^3)	Based on relative potency from GB[c]
GB	AEGL-1 (Nondisabling)	0.0012 ppm (0.0069 mg/m^3)	0.00068 ppm (0.0040 mg/m^3)	0.00048 ppm (0.0028 mg/m^3)	0.00024 ppm (0.0014 mg/m^3)	0.00017 ppm (0.0010 mg/m^3)	EC_{50} for miosis observed in adult female SD rats exposed to a range of GB vapor concentrations (0.01-0.48 mg/m^3) for 10, 60, and 240 min (Mioduszewski et al. 2002b)and miosis data from secondary and supportive studies with

TABLE 1-1 *Continued*

Agent	Classification	10-min	30-min	1-h	4-h	8-h	End Point (Reference)
							marmosets (van Helden et al. 2001, 2002) and humans (Harvey 1952; and Johns 1952)
	AEGL-2 (Disabling)	0.015 ppm (0.087 mg/m^3)	0.0085 ppm (0.050 mg/m^3)	0.0060 ppm (0.035 mg/m^3)	0.0029 ppm (0.017 mg/m^3)	0.0022 ppm (0.013 mg/m^3)	Miosis, dyspnea, RBC-ChE inhibition, single fibre electro-myography (SFEMG) changes in human volunteers exposed at 0.5 mg/m^3 for 30 min (Baker and Sedgwick 1996)
	AEGL-3 (Lethal)	0.064 ppm (0.38 mg/m^3)	0.032 ppm (0.19 mg/m^3)	0.022 ppm (0.13 mg/m^3)	0.012 ppm (0.070 mg/m^3)	0.0087 ppm (0.051 mg/m^3)	Based on experimental SD rat lethality data (LC_{01} and LC_{50}); whole-body dynamic exposure to concentrations between 2 and 54 mg/m^3 for 3, 10, 30, 60, 90, 240, and 360 min (Mioduszewski et al. 2000, 2001, 2002a)
GD	AEGL-1 (Nondisabling)	0.00046 ppm (0.0035 mg/m^3)	0.00026 ppm (0.0020 mg/m^3)	0.00018 ppm (0.0014 mg/m^3)	0.000091 ppm (0.00070 mg/m^3)	0.000065 ppm (0.00050 mg/m^3)	Based on relative potency from GB[d]

	AEGL-2 (Disabling)	0.0057 ppm (0.044 mg/m^3)	0.0033 ppm (0.025 mg/m^3)	0.0022 ppm (0.018 mg/m^3)	0.0012 ppm (0.0085 mg/m^3)	0.00085 ppm (0.0065 mg/m^3)	Based on relative potency from GB[d]
	AEGL-3 (Lethal)	0.049 ppm (0.38 mg/m^3)	0.025 ppm (0.19 mg/m^3)	0.017 ppm (0.13 mg/m^3)	0.0091 ppm (0.070 mg/m^3)	0.0066 ppm (0.051 mg/m^3)	Based on relative potency from GB; supported by Wistar rat LC_{50}; dynamic chamber exposures at 21 mg/m^3 for three time periods of ≤30 min (Aas et al. 1985)[e]
GF	AEGL-1 (Nondisabling)	0.00049 ppm (0.0035 mg/m^3)	0.00028 ppm (0.0020 mg/m^3)	0.00020 ppm (0.0014 mg/m^3)	0.00010 ppm (0.00070 mg/m^3)	0.000070 ppm (0.00050 mg/m^3)	Based on relative potency from GB[d]
	AEGL-2 (Disabling)	0.0062 ppm (0.044 mg/m^3)	0.0035 ppm (0.025 mg/m^3)	0.0024 ppm (0.018 mg/m^3)	0.0013 ppm (0.0085 mg/m^3)	0.00091 ppm (0.0065 mg/m^3)	Based on relative potency from GB[d]
	AEGL-3 (Lethal)	0.053 ppm (0.38 mg/m^3)	0.027 ppm (0.19 mg/m^3)	0.018 ppm (0.13 mg/m^3)	0.0098 ppm (0.070 mg/m^3)	0.0071 ppm (0.051 mg/m^3)	Based on relative potency from GB[e]

TABLE 1-1 *Continued*

Agent	Classification	10-min	30-min	1-h	4-h	8-h	End Point (Reference)
VX[f]	AEGL-1 (Nondisabling)	0.000052 ppm (0.00057 mg/m^3)	0.000030 ppm (0.00033 mg/m^3)	0.000016 ppm (0.00017 mg/m^3)	0.0000091 ppm (0.00010 mg/m^3)	0.0000065 ppm (0.000071 mg/m^3)	Derived by relative potency from EC_{50} for miosis observed in adult female SD rats exposed to a range of GB vapor concentrations (0.01-0.48 mg/m^3) for 10, 60, and 240 min (Mioduszewski et al. 2002b) and miosis data from secondary and supportive studies of van Helden et al (2001, 2002), Harvey (1952), and Johns (1952) in marmosets and humans, respectively[g]
	AEGL-2 (Disabling)	0.00065 ppm (0.0072 mg/m^3)	0.00038 ppm (0.0042 mg/m^3)	0.00027 ppm (0.0029 mg/m^3)	0.00014 ppm (0.0015 mg/m^3)	0.000095 ppm (0.0010 mg/m^3)	Derived by relative potency from study of GB vapor exposure to exercising human volunteers exposed at 0.5 mg/m^3 for 30 min; miosis, dyspnea, inhibition of RBC-ChE, changes in single fibre electromyography (SFEMG) (Baker and Sedgwick 1996)[h]

AEGL-3 (Lethal)	0.0027 ppm (0.029 mg/m^3)	0.0014 ppm (0.015 mg/m^3)	0.00091 ppm (0.010 mg/m^3)	0.00048 ppm (0.0052 mg/m^3)	0.00035 ppm (0.0038 mg/m^3)	Derived by relative potency from experimental SD rat lethality data (LC_{01} and LC_{50}); whole-body dynamic exposure to GB vapor concentrations between 2 and 54 mg/m^3 for 3, 10, 30, 60, 90, 240, and 360 min (Mioduszewski et al. 2000, 2001, 2002a)[i]

[a]The derived AEGL values are for vapor exposures only. Percutaneous absorption of nerve agent vapors is known to be an effective route of exposure; nevertheless, percutaneous vapor concentrations needed to produce similar adverse effects are greater than inhalation vapor concentrations by several orders of magnitude. (For agent VX, the percutaneous vapor concentrations needed to produce similar adverse effects are greater than inhalation vapor concentrations by an approximate factor of 10.) Thus, the AEGL values presented are considered protective for both inhalation and percutaneous routes of exposure.

[b]Based on relative potency equal to that of agent GB (see Section 4.3 and Mioduszewski et al. [1998]).

[c]Agent GA is considered approximately one-half as potent as GB in lethality; thus, AEGL-3 values for GA are estimated by multiplying each time-specific AEGL-3 value for agent GB by a factor of 2 (see Section 4.3 and Mioduszewski et. al. [1998]).

[d]Agents GD and GF are considered approximately twice as potent as agents GA and GB for causing miosis, and they are equipotent to each other. Thus, AEGL-1 and AEGL-2 values are estimated by multiplying each time-specific AEGL-1 or AEGL-2 value for agent GB by a factor of 0.5 (see Section 4.3 and Mioduszewski et al. [1998]).

[e]Based on a relative potency for lethality of GD = GF = GB and lethality data of Aas et al. (1985) (which provides a 10-min AEGL-3 estimate of 0.27 mg/m^3 and a 30-min AEGL-3 value of 0.15 mg/m^3 and is thus supportive of the GD AEGL-3 estimate derived from relative potency) (see Section 4.3 and Appendix A).

[f]Based on relative potency. Agent VX is considered approximately 4 times more potent than agent GB (see Section 4.3.4, Grob and Harvey [1958], and Sidell and Groff [1974]).

[g]Derived from miosis effects noted in young adult female SD rats exposed to agent GB vapor at concentrations (0.010-0.48 mg/m^3) for 10, 60, and 240 min (Mioduszewski et al. 2002b). VX concentration to achieve same end point estimated by relative potency adjustment presented in footnote *f* above.

[h]Derived from transient effects noted in exercising human volunteers exposed to agent GB vapor at 0.5 $mg \cdot min/m^3$ for 30 min (Baker and Sedgwick 1996). VX concentration to achieve same end point estimated by relative potency adjustment presented in footnote *f* above.

[i]Derived from LC_{01} values for female Sprague-Dawley rats exposed to GB vapor in dynamic exposure chamber (Mioduszewski et al. 2000, 2001, 2002a). VX concentrations to achieve same end point estimated by relative potency adjustment presented in footnote *f* above.

The chemical-warfare agents discussed here are highly toxic organophosphate ester derivatives of phosphonic acid. They are commonly termed "nerve" agents as a consequence of their anticholinesterase properties and subsequent adverse effects on smooth and skeletal muscle function as well as the central nervous system. As a group, nerve agents are divided into the G-series agents ("G" for German, identifying these agents as among those secretly developed by the German Ministry of Defense before and during World War II—they contain a fluorine or cyanide substituent group) and the V agents (which contain a sulfur substituent group) (Sidell 1997). The G agents addressed in the current analysis include GA, or tabun (dimethylamidocyanoethylphosphate; $C_3H_{11}N_2O_2P$); GB, or sarin (isopropyl methylphosphonofluoridate; $C_4H_{10}FO_2P$); GD, or soman (pinacolyl methyl- phosphonofluoridate; $C_7H_{16}FO_2P$); and GF (O-cyclohexyl-methylfluoro-phosphonate; $C_7H_{14}FO_2P$). The V agent discussed in this document is VX (S-(diisopropyl aminoethyl) methyl phosphonothiolate, O-ethyl ester; $C_{11}H_{26}NO_2PS$). Agent VX is a persistent, "terrain denial" compound with a deliberately formulated low volatility; it is designed to contaminate surfaces.

Organophosphate (OP) nerve agents have been specifically designed and formulated to cause death, major injuries, or incapacitation to enemy forces in wartime. They are particularly effective in a military sense because of their potency. Detailed descriptions of nerve agent toxicity can be found in reviews by NRC (1999), Mioduszewski et al. (1998), Opresko et al. (1998), Sidell (1997), Munro et al. (1994), and Watson et al. (1989), among others.

Munitions containing agents GA, GB, and VX are stored at various military installations within the continental United States as part of the domestic unitary chemical warfare agent stockpile, which is undergoing congressionally mandated destruction (Carnes and Watson 1989). "Unitary" (as opposed to binary) munitions are those in which undiluted agents have been placed for immediate release upon firing or detonation.

According to information recently released by the Army at public meetings held in June 2001 in Pueblo, Colorado, the status of the project is as follows:

1. Disposal operations at Johnston Atoll were completed on November 29, 2000. Over one million munitions, containing over 2,030 tons of agents HD, GB, and VX, were destroyed.

2. As of June 6, 2001, the Tooele, Utah, facility had destroyed over 5,100 tons of agent GB, representing 37.4% of the original inventory at

Tooele. The Tooele facility began operations in August 1996. Demilitarization operations there are scheduled for completion in FY04.

3. Agents GB and/or GA are under secure storage and awaiting destruction at military facilities near Anniston, Alabama; Pine Bluff, Arkansas; Richmond, Kentucky; Tooele, Utah; and Umatilla, Oregon.

4. Agent VX is under secure storage and awaiting destruction at military facilities near Anniston, Alabama; Newport, Indiana; Pine Bluff, Arkansas; Richmond, Kentucky; Tooele, Utah; and Umatilla, Oregon.

5. The remaining demilitarization facilities are in various stages of construction.

Small quantities of agent GD are held in research and development facilities in the United States. Agents GA, GB, GD, and VX are listed as materiel thought to be located at some nonstockpile sites (DA 2001; USACMDA 1993a,b) and are being dealt with during installation restoration activities. The Chemical Weapons Convention (April 1997; Convention on the Prohibition of the Development, Production, Stockpiling and Use of the Chemical Weapons and on Their Destruction) has increased the interest in, and pace of, nonstockpile installation restoration.

Agent GF is believed to have been manufactured within Iraq during the Persian Gulf War (1990-1991) when precursors of agent GB (but not GF) were embargoed. Agent GF is currently considered of little strategic interest (Sidell 1997) but is included for completeness. With the possible exception of agent GF, all of the G agents identified above are considered potential military or terrorist threats.

Public and institutional concerns exist regarding potential agent release during unitary stockpile disposal, nonstockpile installation restoration activities, and potential chemical terrorism events (e.g., IOM 1999; Carnes 1989; NRC 1999; FEMA/DA 1996; DHHS 1988). A new dimension was added to consideration of this issue when it was determined that nerve agent GB had been used by a non-state terrorist group in two attacks on civilians in Japan during 1994 and 1995 (Sidell 1997; IOM, 1999). As a consequence, current domestic community emergency planning and preparedness often includes protocols for treating and managing exposure to chemical warfare agents (particularly nerve agents).

Experimental research specifically designed to improve the state of existing data sets quantifying toxic responses of mammals to nerve agent vapor exposure is currently underway and is supported by multiple military services. The AEGL analysis developed in this technical support document makes use of the most recent research findings (Mioduszewski et al. 2000, 2001, 2002a,b; van Helden et al. 2001, 2002; Anthony et al. 2002) from the initiative. As the effort progresses, more of the assumptions necessary for

developing AEGL estimates will be clarified. As new data and results become available in the next several years, assumptions will evolve. It is acknowledged that the current estimates represent a work in progress that will be updated as necessary.

Historical military approaches to chemical warfare (CW) agent protection and treatment of young and healthy soldiers are not necessarily suitable for application to heterogeneous civilian populations, and guidelines are needed for "safe and effective evacuation, decontamination, and other protective action" in the event of CW agent release in a civilian setting (IOM 1999). The development of AEGLs is intended to help address that need.

At present, the only CW agent control limits published in the United States for use in civilian community emergency preparedness planning are those developed by the Department of Health and Human Services (DHHS 1988; Thacker 1994). For the agents GA and GB, the current time-weighted average (TWA) applied as a no-adverse-health-effect level for 24-h continuous exposure to the general population is 3×10^{-6} mg/m^3. For the same agents, the 8-h TWA applied as a no-adverse-health-effect level for 8-h continuous workplace exposure for worker populations is 1×10^{-4} mg/m^3 (53 Fed. Reg. 8504 [1988]; DHHS 1988). Agents GD and GF, which are not part of the unitary stockpile, were not evaluated by DHHS in 1988. For VX, the TWA applied as a no-adverse-health-effect level for 24-h continuous exposure to the general population is 3×10^{-6} mg/m^3; the 8-h TWA applied as a no-adverse-health-effect level for 8-h continuous workplace exposure to worker populations is 1×10^{-5} mg/m^3 (DHHS 1988).

As part of a regularly scheduled review process, the Centers for Disease Control and Prevention (CDC) is currently reevaluating the 1988 agent control limits with application of recent risk assessment models and updated scientific data (67 Fed. Reg. 895 [2002]; DHHS 2002). The review is in progress (as of September 2002), and the CDC has not yet released a final position.

Acute Threshold Effects Levels developed by the CDC (Thacker 1994) are values of cumulative exposure (Ct) (concentration in mg/m^3 multiplied by time in minutes, or mg"min/m^3—Ct does not express the amount retained within the organism [Sidell 1997]). These cumulative exposure values are considered by the CDC to represent "lowest-observed-effect-levels" that "could be exceeded without danger" to the public and form the basis for planning protective actions, such as emergency evacuations, in the Chemical Stockpile Emergency Preparedness Program (CSEPP) of the Federal Emergency Management Agency and the Department of the Army. The Acute Threshold Effect Levels are described by the CDC as protective

of the general population (including consideration of vulnerable subgroups, such as infants, the elderly, and debilitated or ill persons) (Thacker 1994). The value for agent GB is 0.5 mg"min/m^3, a protective cumulative exposure at which miosis is not expected to occur in humans (McNamara and Leitnaker 1971). If projected GB concentrations resulting from a release result in GB Cts > 0.5 mg"min/m^3, then the CDC considers protective measures (such as evacuation or shelter-in-place) warranted as a means of providing maximal protection to the general public. At the time of publication, the CDC has not established similar values for other G agents. The Acute Threshold Effects Level for agent VX is 0.4 mg"min/m^3.

The database for toxicological effects in humans is more complete for agent GB than for the other G agents and for agent VX. Further, agent GB is the only G agent for which sufficient human data are available for use in deriving AEGL-1 and AEGL-2 estimates and the only G-agent for which sufficient laboratory animal data are available for deriving AEGL-1 and AEGL-3 values for all five AEGL time periods. In consequence, estimates for agents GA, GD, GF, and VX are, out of necessity, based on extrapolations of potency relative to the toxicity of agent GB.

Data for the derivation of AEGL-3 values for agent GB are from recent experimental studies of lethality in Sprague-Dawley rats (Mioduszewski et al. 2000, 2001, 2002a). AEGL-3 values for agent GD are derived from relative potency comparison with agent GB and limited inhalation lethality data for experimental exposures to Wistar rats (Aas et al. 1985).

All literature published in this technical support document is unclassified (i.e., not secret at any level, not confidential), including critical studies. Classified material relevant to AEGL assessment for these agents has been reviewed by document developers and has been found to contain no significant data that are not also found in unclassified reports. The technical support document itself was determined to be unclassified following examination by the Intelligence and Security Office of the U.S. Army Soldier and Biological Chemical Command (SBCCOM) (Aberdeen Proving Ground, Maryland) in July 2000.

Given the nature of the compounds under review, military literature is a major source of the relevant toxicity data. In consequence, some of the significant sources possess "limited distribution," which is a separate issue from "classification." Several sources possess a restricted distribution because of treaty restrictions on data access with allies, concerns regarding distribution of engineering information characterizing agent dissemination or vapor generation contained in other sections of the same document, and related issues. To ensure public access to pertinent toxicity data originating

from "limited distribution" materials, pertinent data from those sources have been incorporated into the technical support document. The technical support document itself was "cleared and approved for public access" by the Intelligence and Security Office of the U.S. Army SBCCOM (Aberdeen Proving Ground, Maryland) in July 2000. If additional details are desired, the U.S. Army Center for Health Promotion and Preventive Medicine will assist any request on a one-to-one basis. The point of contact is Ms. Veronique Hauschild (U.S. Army Center for Health Promotion and Preventive Medicine, Environmental Health Engineering, Bldg. E-1675, Aberdeen Proving Ground, MD 21010-5403).

All human exposure studies presented in this evaluation meet the criteria for acceptance for use in the AEGL process (e.g., there is evidence that subjects provided informed consent and that the studies were performed under appropriate clinical supervision) (NRC 2001).

The G agents are all viscous liquids of varying volatility (see Tables 1-2 through 1-5), with faint odors ("faintly fruity" or "spicy," odor of camphor) (DA 1990a,b; Dutreau et al. 1950; McGrath et al. 1953; MODa, unpublished material; all as cited in Marrs et al. 1996). However, these agents are considered odorless in field concentrations for all practical (military) purposes (DA 1990a,b). Odor thresholds are somewhat undefined (DA 1974, 1990a,b, 1992). Agent GA has been reported to have a faintly fruity odor, although it has no odor when pure (DA 1974, 1990a,b, 1992). For agent GB, the odor threshold was reported to be less than 1.5 mg/m^3 (DA 1974, 1990b, 1992; MODa, unpublished material, as cited in Marrs et al. 1996). For agent GD, the odor threshold was reported to be between approximately 1.5 mg/m^3 and 7.0 mg/m^3 (MODa, unpublished material, as cited in Marrs et al. 1996). Approximately 65% of adult subjects ($N = 34$) exposed to GD at 3.3 to 7.0 mg/m^3 exhibited "mild nasal and airway symptoms" (Dutreau et al. 1950); a "median detectable concentration by odor for man is 7 ± 2.4 mg/m^3." However, Dutreau et al. (1950) warn that it is doubtful that an untrained civilian could detect agent GD in sufficient time to avoid a partially incapacitating exposure. Agent GF is reported to have a sweet or musty odor of peaches and has an odor threshold between about 10.4 mg/m^3 and 14.8 mg/m^3 (McGrath et al. 1953, as cited in Marrs et al. 1996; DA 1990b).

As a class, G agents are more volatile and less persistent than the V agents; the vapor pressures and acute toxicity of the G-series agents are sufficiently high for the vapors to be rapidly lethal (USACHPPM 1996). Within the G series, GB is considered a greater vapor hazard than agent GD (USACHPPM 1996). Agent GA represents a smaller vapor hazard and is

expected to present a relevant contact hazard (USACHPPM 1996). The vapor pressure of agent GF is intermediate between that of agents GA and GD.

Agent VX is an amber-colored liquid with a molecular weight of 267.38; it has a vapor density of 9.2 (air = 1) and a liquid density of 1.006 g/ml at 20 °C; its water solubility is 3 g per 100 g at 25 °C and 7.5 g per 100 g at 15 °C; and it has a low volatility (10.5 mg/m^3 at 25 °C) (DA 1990b). Agent VX is approximately 2,000 times less volatile than nerve agent GB (sarin) (DA 1990b). Because agent VX is considered odorless (Koon et al. 1959; DA 1990b), it possesses no olfactory warning properties. Chemical and physical data for agents GA, GB, GD, GF, and VX are presented in Tables 1-2 through 1-6.

2. HUMAN TOXICITY DATA

2.1. Acute Lethality

The acute lethal action of G agents and other anticholinesterase compounds results from their effects on the respiratory system at several levels: bronchoconstriction and excessive tracheobronchial secretion, paralysis of the diaphragm and other respiratory muscles, and depression of the CNS respiratory center (Mioduszewski et al. 1998).

G Agents

Based on extrapolations from historical animal data, the LCt_{50} for military personnel undergoing vapor exposures to GB has been estimated at 35 mg"min/m^3 for 2-10 min exposures at moderate temperatures (65-75 °F) for an individual with a respiratory minute volume of 15 liters (Reutter and Wade 1994). Reutter and Wade (1994) also estimated LCt_{50} values for military personnel undergoing vapor exposures to agents GA, GD, and GF; the estimates are 70 mg"min/m^3 for GA, 35 mg"min/m^3 for GD, and 35 mg"min/m^3 for GF. This Army report remains classified except for a summary table cited here that contains information on median exposure levels. The recommended LCt_{50} estimate for vapor exposure given in Reutter and Wade (1994) was calculated for 2-min exposure periods and then proposed

TABLE 1-2 Chemical and Physical Data for Nerve Agent GA

Parameter	Value	Reference
Chemical name	Dimethylamidocyanethylophosphate	Clark 1989; DA 1974, 1988, 1990a,b, 1992; Britton and Grant 1988; Small 1984; Windholz et al. 1983
Synonyms	Tabun; ethyl N,N-dimethyl phosphoro-amidocyanidate; N,N-dimethyl phosphoroamidocyanidate, ethyl ester.	
Chemical formula	$C_5H_{11}N_2O_2P$	
Chemical structure		DA 1990b
Molecular weight	162.13	DA 1990b
CAS Registry Number	77-81-6	DA 1974, 1990a,b, 1992
Physical state	Colorless to brown liquid	DA 1990b
Solubility in water (g/L)	98 (25 °C); 72 (20 °C)	DA 1990b
Vapor pressure (mm Hg, 20° C)	0.037	DA 1990b
Vapor density (air = 1)	5.63	DA 1990b
Liquid density (g/mL, 25° C)	1.073	DA 1990b
Melting point	-50 °C	DA 1974,1992
Boiling point	245 °C	DA 1974,1992
Flash point	78 °C	DA 1974,1992
Conversion factors in air	ppm = (0.15) × mg/m^3 (calculated) mg/m^3 = (6.6) × ppm (calculated)	Calculated from procedure outlined in ACGIH 2002 using molecular weight
$logK_{ow}$	1.18	Britton and Grant 1988
Bioconcentration factor (BCF)	Not available	
Henrys' law constant (atm m^3/mol)	1.52×10^{-7}	Opresko et al. 1998

TABLE 1-3 Chemical and Physical Data for Nerve Agent GB

Parameter	Value	Reference
Chemical Name	Isopropyl methylphosphonofluoridate	Clark 1989; DA 1974, 1988, 1990a,b, 1992; Britton and Grant 1988; Small 1984; Windholz et al. 1983
Synonyms	Sarin; methyl phosphonofluoridate, isopropyl ester	
Chemical formula	$C_4H_{10}FO_2P$	
Chemical structure		DA 1990b
Molecular weight	140.10	DA 1990b
CAS Registry Number	107-44-8	DA 1974, 1990a,b, 1992
Physical state	Colorless liquid	DA 1990b
Solubility in water (g/L)	Miscible with water	DA 1990b
Vapor pressure (mm Hg at 20 °C)	2.10	DA 1990b
Vapor density (air = 1)	4.86	DA 1990b
Liquid density (g/mL, 20 °C)	1.102	DA 1990b
Melting point	-56 °C	Clark 1989; DA 1974, 1988, 1990a,b, 1992; Britton and Grant 1988; Small 1984; Windholz et al. 1983
Boiling point	158 °C	
Flash point	>138 °C	
Conversion factors in air	ppm = (0.17) × mg/m^3 (calculated) mg/m^3 = (5.7) × ppm (calculated)	Calculated from procedure outlined in ACGIH 2002 using molecular weight
$logK_{ow}$	0.15	Britton and Grant 1988
Bioconcentration factor (BCF)	Not available	
Henrys' law constant (atm m^3/mol)	5.34×10^{-7}	Clark 1989; DA 1974, 1988, 1990a,b, 1992; Britton and Grant 1988; Small 1984; Windholz et al. 1983

TABLE 1-4 Chemical and Physical Data for Nerve Agent GD

Parameter	Value	Reference
Chemical name	Pinacolyl methylphosphonofluoridate	Sidell 1997; Clark 1989; DA 1974, 1988, 1990a,b, 1992; Britton and Grant 1988; Small 1984; Windholz et al. 1983
Synonyms	Soman; phosphonofluoridic acid, methyl-1,2,2-trimethylpropyl ester	
Chemical formula	$C_7H_{16}FO_2P$	USACHPPM 1996
Chemical structure		DA 1990b
Molecular weight	182.178	DA 1990b
CAS Registry No.	96-64-0	USACHPPM 1996
Physical state	Colorless liquid	DA 1974
Solubility in water (g/L)	21 (20 °C)	DA 1990b
Vapor pressure (mm Hg at 25° C)	0.40	Clark 1989; DA 1974, 1988, 1990a,b, 1992; Britton and Grant 1988; Small 1984; Windholz et al. 1983
Vapor density (air = 1)	6.33	DA 1990b
Liquid density (g/mL, 25° C)	1.0222	DA 1990b
Melting point	-42 °C	DA 1990b
Boiling point	198 °C	DA 1990b
Flash point	121 °C	DA 1990b
Conversion factors in air	ppm = (0.13) × mg/m^3 (calculated) mg/m^3 = (7.5) × ppm (calculated)	Calculated from procedure outlined in ACGIH 2002 using molecular weight
logK_{ow}	1.02	Britton and Grant 1988
Bioconcentration factor (BCF)	Not available	
Henrys' law constant (atm m^3/mol)	4.56×10^{-6}	Opresko et al. 1998

TABLE 1-5 Chemical and Physical Data for Nerve Agent GF

Parameter	Value	Reference
Chemical name	O-cyclohexyl-methylfluorophosphonate	DA 1990b
Synonyms	Cyclohexyl methylphosphonofluoridate (CMPF)	
Chemical formula	$C_7H_{14}FO_2P$	DA 1990b
Chemical structure		DA 1990b
Molecular weight	180.2	DA 1990b
CAS Registry Number	329-99-7	DA 1990b
Physical state	Liquid	DA 1990b
Solubility in water	0.37% (20 °C); almost entirely insoluble in water	DA 1990b
Vapor pressure (mm Hg, 25° C)	0.044	DA 1990b
Vapor density (air = 1)	6.2	DA 1990b
Liquid Density (g/mL, 20° C)	1.1327	DA 1990b
Melting point	-30 °C	DA 1990b
Boiling point	239 °C	DA 1990b
Flash point	94 °C	DA 1990b
Conversion factors in air	ppm = (0.14) × mg/m^3 (calculated) mg/m^3 = (7.4) × ppm (calculated)	Calculated from procedure outlined in ACGIH 2002, using molecular weight
$logK_{ow}$	Not available	
Bioconcentration factor (BCF)	Not available	
Henrys' law constant (atm m^3/mol)	Not available	

as a 2-10 min exposure estimate in the summary table. Thus, the LCt_{50} of 35 mg"min/m^3 assumes only short-term exposures of 2-10 min.

TABLE 1-6 Chemical and Physical Data for Nerve Agent VX

Parameter	Value	Reference
Chemical name	O-ethyl-S-(diisopropylamino-ethyl)methyl phosphonothiolate	Munro et al. 1999; DA 1990b
Synonyms	Agent VX; S-(2-diisopropyl-aminoethyl) O-ethyl methyl phosphonothiolate; ethyl-S-dimethylaminoethyl methylphosphonothiolate	
Chemical formula	$C_{11}H_{25}NO_2PS$	
Chemical structure		DA 1990b
Molecular weight	267.38	DA 1990b
CAS Registry Number	50782-69-9	DA 1990b
Physical state	Oily, amber-colored liquid	DA 1990b
Solubility in water (g/L)	3 g per 100 g at 25 °C 7.5 g per 100 g at 15 °C	DA 1974
Vapor pressure (mm Hg, 20° C)	0.0007 mm Hg at 20 °C	DA 1990b
Vapor density (air = 1)	9.2	DA 1990b
Liquid density	1.006 g/cc at 20 °C	DA 1990b
Melting point	-39 °C (calculated)	DA 1990b
Boiling point	298 °C	DA 1990b
Flash point	159 °C	DA 1990b
Conversion factors in air	$mg/m^3 = (10.936) \times ppm$ $ppm = (0.0914) \times mg/m^3$	Calculated from procedure outlined in ACGIH 2002 using molecular weight
$\log K_{ow}$	Not available	
Bioconcentration factor (BCF)	Not available	

A subcommittee of the National Research Council's Committee on Toxicology (COT) has examined the Reutter and Wade (1994) analysis and recommends that the proposed LCt_{50} estimates for agents GA, GB, GD, and GF for estimating vapor inhalation and percutaneous exposure effects in exposed military populations "should be lowered" in light of the need for additional data characterizing vapor inhalation and percutaneous vapor toxicity. Furthermore, the subcommittee considered the estimates of Reutter and Wade (1994) inappropriate for civilian applications (NRC 1997).

Agent VX

From animal data, Reutter and Wade (1994) estimated a LCt_{50} for military personnel of 15 mg"min/m^3 for 2-10 min vapor exposures at moderate temperatures (65-75 °F) for an individual with a respiratory minute volume of 15 L. As in the case for agent GB, this LCt_{50} estimate was calculated for 2-min exposure periods and then proposed as a 2-10 min exposure estimate. Thus, the LCt_{50} for VX at 15 mg"min/m^3 assumes only short-term exposures of 2-10 min.

The subcommittee recommends that the Reutter and Wade (1994) proposed LCt_{50} estimate of 15 mg"min/m^3 for military personnel "should be lowered" because of the low to moderate degree of confidence in the estimation, which considered effects from vapor inhalation and percutaneous vapor exposures. Further, the subcommittee considered the estimates of Reutter and Wade (1994) inappropriate for civilian applications (NRC 1997).

Bide and Risk (2000) estimated the human 10-min LCt_{50} value for a VX aerosol based on lethality data for several animal species (see Section 3.1). The human LCt_{50} value was an estimated 7 mg"min/m^3 for a 70 kg man breathing 15 L/min for 10 min.

2.1.1. Case Reports

Agent GB

In 1994 and 1995, two incidents of chemical terrorism involving nerve agent GB (sarin) occurred in Japan; in both incidents, civilian populations were deliberately exposed to lethal concentrations by followers of a cult originally local to Japan (Lillibridge 1995; Morita et al. 1995; Okumura et

al. 1996; Sidell 1996). Because of the state of emergency at the time of release and the initial unknown nature of the source, exposures and dose-response could not be quantified.

The first incident occurred in June of 1994 in the central highland city of Matsumoto, Japan, where seven people died shortly after exposure to an unknown vapor later determined to be agent GB (Morita et al. 1995) released into a residential area during the night. The Matsumoto incident resulted in 56 hospital admissions as well as 253 cases in which the affected individuals sought medical consultation. Reports of "mild symptoms" were presented by eight out of 53 rescue personnel and one attending physician (Morita et al. 1995). Prompt deaths ($N = 3$) and those who died before arriving at the hospital ($N = 4$) appear to have been the result of respiratory insufficiency. At the time of the Morita et al. (1995) report, one patient remained "in a vegetative state because of anoxic encephalopathy"; a report on the outcome of that case has not yet been found.

The second occurrence, widely known as the Tokyo Subway Incident, took place on March 20, 1995. The same terrorist group responsible for the Matsumoto incident employed sources of passive, evaporative release of nerve agent GB in five individual subway cars serving three separate subway lines during morning commuter rush hours (Lillibridge 1995; Okumura et al. 1996; Sidell 1996). Of the 5,510 persons known to have been given medical attention, there were eight prompt deaths; four more died later (hours to days). The "later" group included individuals who had initially presented with "critical" respiratory effects requiring mechanical ventilation and intensive care (Lillibridge 1995). The 12 fatalities included commuters and subway transport employees, and death appeared to be the result of respiratory insufficiency. On hospital day 28, an additional death occurred as a consequence of "severe hypoxic brain damage" sustained during the release incident (Okumura et al. 1996). This delayed fatality was a previously healthy woman, 21 years of age, who presented without heartbeat or spontaneous respiration at the hospital but was revived with CPR and treated with agent antidotes. Plasma and RBC cholinesterase returned to normal within a period of days, but the patient eventually succumbed to hypoxic brain damage (Okumura et al. 1996).

Neuropathological examination of one individual who died 15 mo after being severely exposed to agent GB during the Tokyo subway terrorist attack indicated that the victim suffered marked nerve-fiber decrease in the sural nerve and moderate nerve-fiber loss in the sciatic nerve, with no changes in the dorsal root ganglion, dorsal roots, or posterior column of the spinal cord (Himuro et al. 1998). The victim's CNS showed severe hypoxic-ischemic changes, which made it difficult to assess the specific

effects of agent GB. Himuro et al. (1998) concluded that the observations were consistent with the "dying back" of the peripheral nervous system and might have been indicative of delayed neuropathy associated with inhibition of neuropathy target esterase (NTE). Himuro et al. (1998) cite as additional evidence of sarin-induced distal axonopathy an earlier study (Ishiyama 1996) in which degeneration of intramuscular nerve fascicles with preservation of the anterior horn cells was observed in a patient who died 1 mo after the subway attack.

2.2. Nonlethal Toxicity

Exposure to acutely toxic concentrations of nerve agents can result in excessive bronchial, salivary, ocular, and intestinal secretion, sweating, miosis, bronchospasm, intestinal hypermotility, bradycardia, muscle fasciculations, twitching, weakness, paralysis, loss of consciousness, convulsions, and depression of the central respiratory drive (Dunn and Sidell 1989). Minimal effects seen at very low exposure levels include miosis and rhinorrhea. The effects of exposures to very low concentrations of the nerve agents are evaluated in the literature, which includes clinical case reports as well as several studies using human volunteers. Key to acceptance of human subject data for use in the AEGL process is evidence that subjects provided informed consent and that the studies were performed under appropriate clinical supervision (NRC 2001). These criteria were met by the nonlethal studies summarized in Section 2.2.2.

A number of investigators consider *both* miosis and rhinorrhea to be early signs of exposure to cholinesterase inhibitors (Sidell 1997; Mioduszewski et al. 2002b; H. van Helden, Pulmonary and CNS Pharmacology Lab, TNO, the Netherlands, personal communtication; S. Tattersall, Biomedical Sciences Division, Porton Down, United Kingdom, personal communication). The presence of rhinorrhea can be indicative of inhalation exposure and/or development of systemic effects, while miosis in the absence of other signs or symptoms is a local effect to the pupillary muscles of the eye. In consequence, the presence of miosis is considered an appropriately sensitive indicator of direct vapor exposure and has the additional advantage of being readily recognized and quantifiable.

Recent nerve agent releases by terrorist groups have exposed civilian populations. Survivors of the incidents have been examined, and the resulting evaluations are summarized in Section 2.2.1.

2.2.1. Case Reports

Agent GB

Clinical case reports exist for the survivor population of the 1994 agent GB (sarin) release in Matsumoto, Japan, and the 1995 sarin release in Tokyo; no estimates of exposure concentrations could be found in the literature for either of these incidents. In the Matsumoto incident detailed above (see Section 2.1.1), Morita and his colleagues (Morita et al. 1995) published the clinical and laboratory findings of 264 people who sought treatment and the results of health examinations performed on 155 Matsumoto residents at 3 weeks (wk) postexposure. During initial treatment, severely poisoned individuals exhibited severe miosis, tachycardia followed by bradycardia, salivation, rhinorrhea, muscle fasciculations, and abnormal epileptiform EEGs. Other reported acute exposure signs and symptoms included headache, vision disturbances, fatigue, dizziness, nausea, dyspnea, ocular pain, and dysesthesia of the extremities. Clinical findings for the same group at the time of examination included decreases in serum cholinesterase, erythrocyte acetylcholinesterase, and serum triglycerides as well as serum potassium and chloride and increases in serum creatine kinase, leucocytes, and ketones in urine. For a period of up to 30 d following the incident, some of the severely exposed population exhibited slight continuous fever and some epileptiform EEG abnormalities (N = 2 out of nine "severely affected people"). Nevertheless, follow-up examination revealed no persistent abnormal physical findings in any individual; acetylcholinesterase activity in erythrocytes and serum cholinesterase returned to normal within 3 mo in the examined population. Among some severe or moderately affected persons, subclinical miosis and some neuropathy were present 30 d after exposure. Morita et al. (1995) state that, in most people, "almost all symptoms of sarin exposure disappeared rapidly and left no sequelae."

In the hours following the Tokyo subway release of agent GB, the emergency department of St. Luke's International Hospital (located near the affected subway stations) received 640 patients (Okumura et al. 1996). Additional details of the incident are provided above (see Section 2.1.1). Of the 640 admissions, 528 (82.5%) were diagnosed by Okumura and his colleagues (1996) as "mild" and exhibited "only eye signs or symptoms" such as miosis, eye pain, dim vision, and decreased visual acuity. Of the remaining 112 patients, one died in the emergency department, 107 were

admitted as "moderate" cases (exhibiting "systemic signs and symptoms" such as weakness, fasciculations, convulsions, difficult breathing), and four were admitted as "severe" cases "requiring emergency respiratory support" such as intubation. Of the four severe cases, two patients experienced cardiac arrest but were revived, treated with agent antidotes and anticonvulsants, and eventually recovered fully (discharged on hospital day 3 and 5). Of the remaining two, both of whom required cardiopulmonary resuscitation, one recovered after vigorous treatment and was discharged on day 6. The remaining severely affected patient originally presented with no pulse and died on hospital day 28. For the three severe cases discharged, RBC-cholinesterase remained below normal activity levels for 51-72 d.

In the early 1970s, three men (ages 27, 50, and 52 y) working at Edgewood Arsenal (now Aberdeen Proving Ground in Edgewood, Maryland) in a chemical agent area containing stored containers of agent GB (sarin) were brought to an emergency room after sudden onset of rhinorrhea and respiratory discomfort approximately 20 min prior to arrival at the emergency room (Sidell 1974). It was determined later that one of the agent GB (sarin) containers in the work area had developed a leak and that the three individuals exhibiting signs had been working in the general area of the room where the leaking container was located. Examination indicated the presence of "mild respiratory distress, marked miosis with slight eye pain, rhinorrhea, a moderate increase in salivation, and scattered wheezes and rhonchi throughout all lung fields" (Sidell 1974). The men received no therapy but were observed for 6 h after emergency room arrival and were asymptomatic upon discharge except for eye irritation and "decreased vision in dim light." Blood cholinesterases were monitored and pupil diameter was recorded photographically for a period of 4 mo following exposure. Although 60-70% recovery of the ability to dark-adapt occurred within 2 wk, complete recovery of the ability to dark-adapt required 2 mo. Sidell (1974) did not report any estimates of the GB agent concentrations the men were exposed to.

Also in the early 1970s, a 52-y-old man in full protective gear employed in cleaning an agent GB-contaminated area at Edgewood Arsenal (now Aberdeen Proving Ground in Edgewood, Maryland) experienced breathing difficulty and increased oral and nasal secretions (Sidell 1974). It was later determined that there was a crack in the man's voicemitter diaphragm through which exposure most likely had occurred. Upon arrival at the emergency room 5-10 min after the first symptom, he was convulsing and cyanotic. Other evident signs included labored breathing, muscular

fasciculations, miosis, salivation, and rhinorrhea. He was treated aggressively with agent antidotes and provided assisted ventilation, and he recovered sufficiently to be able to walk through the ward by 9 h postadmission. Red blood cell cholinesterase (RBC-ChE) was monitored, as were EKGs. "While ChE activity in his blood was undetectable," the individual was conscious and alert (Sidell 1997). By 18 h postadmission, miosis was still evident. On day 4 and thereafter, the patient was asymptomatic; upon discharge 4 wk postexposure, he was "fully ambulatory and doing well." A 4-mo-postexposure EKG "was entirely within normal limit" (Sidell 1974). Sidell (1974) did not report any estimate of GB agent concentrations to which this individual was exposed.

In another incident of accidental exposure to GB vapors (0.09 mg/m^3 for an undefined duration resulting from a faulty ventilation hood), two men (ages 46 and 53 y) exhibited significantly lowered RBC-ChE for 80-90 d (one showed depression to 19% of baseline activity, the other to 84% of baseline activity) and extreme miosis that persisted for 30-45 d (Rengstorff 1985). These men exhibited no other signs or symptoms of nerve agent poisoning and required no treatment with antidotes.

2.2.2. Acute Studies

Agent GB

Vapor Exposures

Fairley and Mumford (1948) exposed 16 male volunteers to GB at 0.3 mg/m^3 for 0.5 min. Nine of the test subjects reported that they could detect the agent by smell; seven reported tightness of the chest, and 16 reported rhinorrhea.

McKee and Woolcott (1949) evaluated the effects of low concentrations of agent GB on 14 male volunteers. A single exposure to GB at 0.6 mg/m^3 for 1 min or at 0.06 mg/m^3 for 40 min resulted in miosis and slight tightness of the chest (4/4 subjects exhibited those signs and symptoms in both the 1-min and 40-min tests; within 24 h, signs and symptoms resolved in subjects receiving 1-min exposures, although more than 48 h were required for resolution in subjects receiving 40-min exposures). Exposure of five individuals to GB at 0.06 mg/m^3 for 20 min/d resulted in miosis, but only after the fourth day of exposure. When the subjects were exposed to GB at

0.06 mg/m^3 for 40 min/d, miosis occurred on the first or second day and additional symptoms (headache, blurred vision, eye pain) appeared on the second, third, and fourth day of exposure.

In summarizing the toxicity studies conducted at Porton Down, United Kingdom, Mumford (1950) concluded that the threshold for ocular effects is 1.5-5.0 mg"min/m^3 (exposure times of 5-6 min) and that exposures to GB at 6-12 mg"min /m^3 (exposure times of 5-8 min) would result in moderate to severe discomfort due to miosis and frontal headaches.

In a study reported by Johns (1952) and Harvey (1952), 128 adult males volunteered to be exposed to GB concentrations ranging from 0.05 mg/m^3 to 3.0 mg/m^3 for 2-20 min in a chamber. The corresponding Cts ranged from 1.0 mg"min/m^3 to 6.0 mg"min/m^3. The analytical methods used to measure the chamber concentrations of GB were not reported. Regression analysis of 150 observations, including 55 controls, indicated that the point at which a 50% decrease in pupil diameter would be attained was approximately 4.1 mg"min/m^3, with 90% confidence limits of about 2.7 and 5.7 mg"min/m^3 (Johns 1952). At the lowest test exposure level (0.05 mg/m^3 for 20 min), there were mean maximum decreases in pupil diameter of 0.82 mm (right eye) and 1.00 mm (left eye) (total of eight observations) compared with 0.36 mm (right eye) and 0.33 mm (left eye) in controls (55 observations). Johns (1952) defines “mild miosis” as a “decrease of 1 to 2 mm” in pupil diameter that usually disappears within 24 h. Although mild miosis, as defined by the author, was observed in some subjects at the lowest Ct tested (Ct = 1.0 mg"min/m^3), other subjects exhibited mean maximal pupil decreases of <1 mm. This indicates that a likely response threshold was attained at this level of cumulative exposure. The results of the Johns (1952) study are presented in Table 1-7. It should be noted that untreated controls exhibited a pupil diameter decrease of $0.33 mm. Johns (1952) attributes this difference to observer bias and points out that there is still a relative difference between the control group and the exposure groups.

From the same overall study, Harvey (1952) reported signs and symptoms resulting from the GB exposures; those results are presented in Table 1-8.

TABLE 1-7 Decrease in Pupil Diameter (mm) Following GB Vapor Exposures

	Exposure Duration										
	20 min				4 min		2 min				
Concentration (mg/m^3)	0	0.05	0.2	0.3	1.0	1.3	0.5	1.0	2.0	2.3	3.0
Number of observations	55	8	11	11	12	4	15	9	8	7	10
Right eye; mean maximal decrease in pupil diameter (mm)	0.36	0.82	2.18	2.91	2.75	2.00	0.51	1.72	2.50	2.36	3.00
Left eye; mean maximal decrease in pupil diameter (mm)	0.33	1.00	2.18	3.00	2.59	2.22	0.60	1.67	2.92	2.07	3.00

Source: Johns 1952.

TABLE 1-8 Number of Test Subjects Showing Effects from GB Vapor Exposures

	Exposure Duration									
	20 min					2 min				
Concentration (mg/m^3)	0	0.05	0.1	0.2	0.3	0	0.5	1.0	2.0	3.0
Number of test subjects	4	14	34	11	12	4	15	9	15	10
Headache	1	2	1	1	8		4	1		4
Eye pain		2			6	1	3			6
Dimness of vision					7				4	7
Twitching of lids					2			2	2	
Rhinorrhea		3	20	11	12		2	9	15	10
Salivation					2					
Throat irritation					5		1		3	
Tightness in chest		1	12	2	9			6	11	4
Sweating					4					
Cramps		1			6				1	2
Nausea		1			3	1			1	
Vomiting					1				1	
Giddiness					5					
Concentration difficulty					8					
Malaise (“Grippe”)		2			6		1		7	7

Source: Harvey 1952.

In tests on human volunteers, Sim (1956) found that pupil constriction occurs more slowly and is less severe following exposure to GB at 5 mg/m^3 than at 10 or 15 mg/m^3 (1-3 min exposures). Some of the test subjects (number not given) reported restricted vision and eye pain.

Rubin et al. (1957) evaluated the effects of agent GB on the visual threshold of three adult volunteers. The test subjects were exposed to GB at 2 mg/m^3 for 2 min with the eyes protected or unprotected. With the eyes unprotected, the exposure resulted in moderate miosis and no other obvious signs of cholinesterase inhibition, but there was a significant elevation of the absolute visual threshold in the dark-adapted eye.

Oberst et al. (1968) conducted inhalation studies in which 125 volunteers were exposed to low concentrations of GB vapors in order to measure levels of GB retention and changes in RBC-ChE activity. In one series of tests in which resting subjects ($N = 90$; minute volumes 5.6-8.4 L) were exposed to GB (concentrations in the supply chamber were 16.2 to 22.7 mg/m^3, average 20.7 mg/m^3) for 2 min, the calculated retained dose was 3.4-3.8 µg/kg and the percent inhibition of RBC-ChE activity was 39-63% (average 49%). In a second series of tests, in which exercising men ($N = 35$; minute volumes 41.5-64.9 L) were exposed to GB (supply chamber concentrations were 3.9 to 4.53 mg/m^3, average 4.19 mg/m^3) for 2 min, the calculated retained dose was 3.2-4.0 µg/kg and the percent inhibition of RBC-ChE activity was 29-58% (average 47%). The reported 2-min ChE_{50} dose for all 125 subjects (grouped data) was 3.95 µg/kg. From these data, the 2-min EC_{50} for cholinesterase inhibition can be estimated as approximately 21 mg/m^3 for resting men breathing about 7 L/min and about 4 mg/m^3 for exercising men breathing about 50 L/min. In these studies the subjects inhaled GB through a nosepiece or a mouthpiece; therefore, the potential effects of the agent on the eyes (i.e., miosis) could not be determined.

McNamara and Leitnaker (1971) applied mathematical and conceptual models to human and animal data and estimated that the threshold for neuromuscular effects and the ECt_{50} of GB for miosis in humans would be 4.0 $mg"min/m^3$ (0.2 mg/m^3 for 20 min). They further suggested that miosis would not occur at a Ct of 0.5 $mg"min/m^3$ (0.016 mg/m^3 for 30 min). McNamara and Leitnaker (1971) also estimated the Ct at which 50% inhibition of blood cholinesterase would occur; it was reported to be 20 $mg"min/m^3$ (0.67 mg/m^3 for 30 min). Blood cholinesterase activity was not expected to be affected at a Ct of 0.5 $mg"min/m^3$ (0.016 mg/m^3 for 30 min).

Callaway and Dirnhuber (1971) evaluated the "miotogenic potency" of GB vapor in humans exposed to GB "under goggles" (62 miosis responses

in 26 human volunteers). The "goggle" experiments were designed to deliver GB vapor directly to the air volume around the eye and enclose the vapor as a means of controlling the exposure (no inhalation or percutaneous exposure) and delivering the vapor directly to the surface of the eye (thereby reducing variability). An airstream of GB vapor (flow rate 0.1 L/min) was delivered to the space enclosed by each goggle. The unexposed pupil area of each eye was the baseline for pupil area decrement determinations for each eye. Exposure time periods ranged from 10 min to 5 h. Callaway and Dirnhuber (1971) reported a 50% loss of pupil area in the human dark-adapted eye at a Ct of 3.13 mg"min/m^3 (95% confidence interval [CI] = 2.15-4.57 mg"min/m^3). A 90% loss of pupil area occurred at a Ct of 13.85 mg"min/m^3 (95% CI = 6.00-32.02 mg"min/m^3).

Baker and Sedgwick (1996) exposed eight human volunteers to GB at 0.5 mg/m^3 for 30 min in an exposure chamber. During the exposure, test subjects walked at a rate of 96 paces per minute and breathed normally. It was reported that the test Ct of 15 mg"min/m^3 caused an inhibition of RBC-AChE activity to approximately 60% of individual baseline (reduction of 40%) at both 3 h and 3 d postexposure. Subjects exhibited miosis and, in some cases, photophobia and mild dyspnea following exposure. Respiratory symptoms resolved within minutes, and ocular effects resolved within 48 h. There were no clinical neuromuscular signs or symptoms; however, small changes in single fibre electromyography (SFEMG) of the forearm were measured at 3 h and 3 d postexposure and were still detectable at the first follow-up examination 4 to 15 mo postexposure. These changes were not detectable at the second follow-up examination 15 to 30 mo after exposure. Baker and Sedgwick (1996) suggested that these electrophysiological changes "may indicate subclinical onset of a non-depolarising type of neuromuscular block" that is fully reversible and has no clinical significance.

Oral Exposures

In clinical studies conducted by Grob and Harvey (1958), GB was administered orally in aqueous solution to eight normal subjects. Doses of 0.002 to 0.022 mg/kg resulted in 15-75% reduction in plasma and RBC-ChE activity. Grob and Harvey (1958) reported that the oral dose producing 50% depression of RBC-ChE was 0.01 mg/kg.

Intra-arterial Exposures

In clinical studies conducted by Grob and Harvey (1958), GB was administered by intra-arterial injection to eight normal subjects. Grob and Harvey (1958) reported that the intra-arterial dose of GB producing 50% depression of RBC-ChE was 0.003 mg/kg.

Agent GD

Fairley and Mumford (1948) exposed 15 male volunteers to GD at 0.3 mg/m^3 for 0.5 min. Fourteen men reported that they could detect the agent by smell, seven reported tightness in the chest, and 11 reported rhinorrhea.

Agent GA

Uhde and Moore (1945, as cited in Mioduszewski et al. 1998) reported that four men exposed to T2104 (agent GA) at a concentration of 0.35 mg/m^3 for 2 min were able to detect the agent by smell, and all reported slight, transient tightness of the chest, but none exhibited miosis. Ten men exposed to GA at 1.6 mg/m^3 for 2 min were able to detect the agent by smell, reported tightness of the chest, and exhibited miosis.

Agent VX

Local effects occurring at points of contact in the eyes and respiratory tract following exposure to low concentrations of VX vapor include miosis, rhinorrhea, and slight bronchoconstriction (Sidell 1992). These effects may occur without a significant decrease in activity of blood cholinesterases and without any signs of systemic toxicity (Sidell 1992). The ECt_{50} for mild effects (ocular effects, accompanied perhaps by chest tightness and rhinorrhea) resulting from vapor exposures has been estimated at 0.09 $mg"min/m^3$ for 2-10 min exposures at moderate temperatures (65-75 °F) for a respiratory minute volume of 15 L (Reutter and Wade 1994). Exposures sufficiently high to result in systemic uptake can result in muscular weakness, tremors, difficulty breathing, convulsions, paralysis, and death. The ECt_{50} for severe effects resulting from vapor exposures has been estimated at10 $mg"min/m^3$ for 2-10 min exposures at moderate temperatures

(65-75 °F) for a respiratory minute volume of 15 L (Reutter and Wade 1994).

According to an unclassified NRC report (NRC 1997), the Reutter and Wade (1994) estimated ECt_{50} of 0.09 mg"min/m^3 for mild effects (ocular effects and rhinorrhea in humans) is based on the study by Bramwell et al. (1963) (percutaneous and direct ocular exposure to humans). The Bramwell et al. (1963) study is not considered credible for reasons that are discussed below under "Inhalation Exposures." This conclusion also is supported by the evaluation of a U.S. Surgeon General's review panel in an August 2000 public hearing (67 Fed. Reg. 894 [2002]; DHHS 2002).

Because agent VX is considered odorless, it possesses no olfactory warning properties.

Vapor Exposures

Sixteen volunteers participated in an odor detection study of stabilized and unstabilized VX (Koon et al. 1959). The agent was inhaled through an osmoscope attached to a chamber containing freshly generated agent vapor. The osmoscope permitted dilutions of the agent vapor with room air to yield concentrations down to one-sixty-fourth that in the chamber (0.05-3.34 mg/m^3). Each subject sniffed the agent in the morning and in the afternoon on two successive days (presumably only one sniff at each time point). The estimated total doses for the four exposures ranged from 0.01 to 0.13 µg/kg. No significant changes in RBC- or plasma-ChE activity were demonstrated. Three subjects reported headaches the evening of the last test, and three other subjects reported slight chest tightness, dryness of the mouth, and nasal irritation for 30 min following the test. There was no agreement as to description of the odor. The median detectable concentration for VX vapor was estimated to be 3.6 mg/m^3 (95% CI = 0.8-16.4 mg/m^3).

One of the few experimental attempts to evaluate human exposure to VX vapor for time durations greater than a few minutes is the historically important study of Bramwell et al. (1963) in which eight individuals were exposed for time periods ranging from 2.25 seconds (s) to 24 min to VX vapor concentrations ranging from 0.23 mg/m^3 to 5 mg/m^3 (Cts = 0.7 to 25.6 mg"min/m^3). The Bramwell et al. (1963) study is not considered credible because of its seriously flawed exposure protocol but is presented here for completeness and context. The test subjects were exposed while

TABLE 1-9 ChE Inhibition in Humans Following Exposure to VX Vapors

		Exposure Conditions			Max ChE Inhibition
Trial	Subject	Time (min)	Concentration (mg/m^3)	Ct ($mg \cdot min/m^3$)	(% depression)
R1	SHE	3	0.2	0.6	20
R2	BIS	3	0.35	0.9	18
R3	LAD	3	0.31	0.9	22
R4	BUR	3	0.37	1.1	17
R5	BRA	3	0.4	1.2	14
R6	HOP	3	0.48	1.4	10
R7	CRO	3	0.57	1.7	12
R8	SHE	1.5	1.6	2.4	26
R9	BRA	1.5	1.73	2.6	25
R10	BUR	1.5	1.73	2.6	21
R11	BIS	1.5	1.93	2.8	28
R12	LAD	1.5	2.0	3.0	41
R13	HOP	1.5	2.07	3.1	18
R14	HOL	1.5	2.07	3.1	28
R15	CRO	1.5	2.4	3.6	20
R16	CRO	6	0.8	4.8	44
R17	LAD	7	0.79	5.5	70

(Continued)

TABLE 1-9 *Continued*

Trial	Subject	Exposure Conditions			Max ChE Inhibition (% depression)
		Time (min)	Concentration (mg/m^3)	Ct (mg·min/m^3)	
R18	SHE	6	1.02	6.1	47
R19	BUR	6	1.06	6.4	46

Note: These data are not considered credible for use in deriving AEGLs (see text).
Source: Bramwell et al. 1963, as cited in Reutter et al. 2000.

standing or seated at the mouth of a tunnel from which VX vapor was flowing in an airstream at 1 m/min at a temperature of 32 °C. Only the head and neck of the test subjects were exposed. A total of 19 exposures were conducted without respiratory protection (see Table 1-9). All but one of the tests were conducted with eyes closed without the use of eye protection in the form of goggles or face mask. The only symptoms noted during the exposures were slight tightness in the throat and upper respiratory tract; these symptoms were not reported by all subjects. In the individual exposed with eyes open (0.31 mg/m^3 for 3 min), miosis developed suddenly 20 to 30 min postexposure and was maximal at 1.5 h postexposure. In the individuals exposed with eyes closed, some miosis usually developed 1 to 3 h postexposure. The degree of miosis was quite variable among the individuals and appeared to be concentration-dependent. The miosis was often accompanied by a fluttering or twitching of the eyelids. Although the muscle effects were clearly reported by the subjects, they were not always obvious to the observers. Rhinorrhea occurred within 30 min of exposure in 14 of 19 trials. In four other trials, it developed more slowly; in one, it did not develop at all. Excessive salivation, lasting for about an hour, was reported in one subject after a 6-min exposure to a concentration of 1.06 mg/m^3. Two hours postexposure, one individual experienced some nausea and sweating; RBC-ChE activity was 60% depressed at that time. These effects abated somewhat and then recurred later when ChE inhibition had reached 70%. Several individuals also experienced malaise and lethargy. Based on all 19 trials, the inhaled dose estimated to produce inhibition of 50% of the RBC-ChE activity (ChE_{50}) was 13 µg/kg. However, the authors thought that apprehension had increased the subjects' minute volume during initial exposures. That would have effectively increased the dose to which the individuals were exposed and was thought to account for a relatively shallow probit slope. When those data were excluded, the estimated ChE_{50} was about 8 µg/kg, which was thought to compare favorably with intravenous data.

The Bramwell et al. (1963) study is not considered credible for use in deriving AEGLs for agent VX. Reutter et al. (2000) examined the Bramwell et al. study as a potential critical study for the estimation of worker population levels (WPLs) and general population levels (GPLs) for chronic exposure to VX vapor (8-h time-weighted average for WPL; 24-h continuous exposure for GPL). Reutter et al. (2000) rejected the Bramwell et al. study because of multiple deficiencies; the concentration of VX to which the subjects were exposed could not be determined (subjects were seated in front of a "tunnel" down which generated VX vapor flowed in an airstream of known velocity), both *C* and *t* were varied (resulting in no

replicate cumulative exposures), and the organic solvent benzene was used to help disperse the agent in the airstream to which subjects were exposed (Bramwell et al. did not address the potential effect of the carrier solvent on agent absorption by the subject). The majority of a U.S. Surgeon General's review panel concurred with the appraisal of Bramwell et al. (1963) at a public hearing convened by the CDC to examine the Reutter et al. (2000) report (67 Fed. Reg. 894 [2002]; DHHS 2002).

Oral Exposures

In clinical studies conducted by Sidell and Groff (1974), single oral doses of VX at 2-4.5 µg/kg (stock solution in absolute ethanol diluted in a solution of saline and dextrose and swallowed by each subject under supervision) produced gastrointestinal symptoms in 5 of 32 test subjects (more specific dose-response data not reported). Regression analysis of the dose-response data indicated that the RBC-ChE_{50} was 2.3 µg/kg. Sidell and Groff (1974) reported that the oral dose of VX needed to produce 70% ChE inhibition (4 µg/kg) was 3 times greater than that needed to produce the same effect after intravenous administration.

Sim et al. (1964) reported no signs of toxicity in seven human volunteers receiving VX at 1.43 µg/kg/d for 7 d (in four daily doses of 500 mL drinking water); however, average RBC-ChE activity was reduced 60% (to 40% of baseline values). The Sim et al. (1964) study resulted in a lower RBC-ChE_{50} value than the Sidell and Groff (1974) oral study, probably because of the cumulative effects of VX given over the 7 d in the Sim et al. study. The total dose in the Sim et al. (1964) study was about twice that used in the Sidell and Groff (1974) oral study.

Intravenous Exposures

Several studies have been conducted in which human volunteers were injected intravenously with VX. The experiment of Kimura et al. (1960) was performed with the informed consent of the participants, under full clinical supervision and in a hospital setting considered suitable at the time (resuscitation team at bedside "to administer atropine, oximes, oxygen, artificial resuscitation, and tracheotomy if indicated"). Kimura et al. (1960) reported that a 30-s intravenous injection of 0.04 µg/kg in one adult test subject caused frontal and retrobulbar headaches starting 45 min after the injection. The subject reported being tired and appeared irritable to

observers, but no change in RBC or whole blood cholinesterase activity was observed. A subsequent 30-s intravenous injection of 0.08 µg/kg 3.5 h later resulted in a 2-fold increase in airway resistance, a 25-30% decrease in respiratory rate, and a 15% drop in pulse rate 15 min after the exposure, but no change in RBC-ChE. Headaches began 20 min postexposure, and minute volume increased from 15 L to 32 L 30-45 min postexposure. Peak effects (increased sweating, lightheadedness, and abdominal cramping) appeared about 45 min after the dose was administered. A single 30-s intravenous dose of 0.225 µg/kg in one test subject resulted in a 27% decrease in baseline RBC-ChE activity within 15 min as well as retrobulbar headaches. Many of these observed effects are for the single subject participating in the dose-response range-finding study—Dr. Van Sim, MD, a principal investigator of the reported study. Six additional subjects (volunteers identified by subject code) received VX at 1 µg/kg by intravenous infusion over 1.75 to 4 h periods and exhibited 50-60% depression in cholinesterase activity but no signs of toxicity (except for one 84-kg individual who reported headaches).

The Kimura et al. (1960) study meets the criteria for acceptance of human subject data for use by the AEGL process (e.g., evidence that subjects provided informed consent and that the studies were performed under appropriate clinical supervision).

In clinical studies conducted by Sidell and Groff (1974), 34 test subjects were given VX by intravenous injection. The administered dose ranged from 1.2 to 1.7 µg/kg. An intrvenous dose of 1.5 µg/kg administered to18 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%). The test subjects exhibited a significant decrement in performance on a number facility test within 1 h after treatment. Regression analysis of the dose response data indicated that the RBC-ChE_{50} was 1.1 µg/kg (three individuals tested at 1.2 µg/kg, 1.3 µg/kg, 1.4 µg/kg, and 1.7 µg/kg; four at 1.6 µg/kg; and 18 at 1.5 µg/kg; estimated from graphic presentation of the data) (Sidell and Groff 1974).

Percutaneous Exposures

Dermal vapor absorption is a low priority for this compound, although there are certain release events that generate a dermal vapor threat. It is generally acknowledged that a specific toxicological end point for vapor exposure to nerve agent VX would be achieved at a lower concentration exposure for the inhalation route than for other routes (e.g., the estimated

human LCt_{50} for percutaneous vapor exposure to agent VX is 150 mg"min/m^3, while the estimated human LCt_{50} for inhalation vapor exposure to agent VX is <15 mg"min/m^3) (NRC 1997). Thus, AEGL estimates based on inhalation exposures are considered protective for both inhalation and dermal routes.

In studies conducted by Bramwell et al. (1963, as cited in Reutter et al. 2000) eight individuals were exposed for time periods ranging from 2.25 s to 24 min to VX vapor concentrations ranging from 0.23 mg/m^3 to 5 mg/m^3 (Cts = 0.7 to 25.6 mg"min/m^3). The test subjects were exposed while standing or seated at the mouth of a tunnel from which VX vapor was flowing in an airstream at 1 m/min at a temperature of 32 °C. Only the head and neck of the test subjects were exposed. Thirty-five of the exposures were performed with eyes closed (but without the use of eye protection in the form of goggles or face mask) and with respiratory protection (a nose clip was used and the subjects were breathing through a spirometer connected to a respirator canister). ChE inhibition was measurable within an hour of exposure and was greatest at 8-12 h postexposure. No signs or symptoms were noted during the exposure periods; however, 30 min or more after the initial exposure, miosis appeared in nearly all subjects and became maximal several hours later. It was usually accompanied or followed by fluttering and twitching of the eyelids and was more pronounced at the higher concentrations. Flushing of the skin of the head and neck was observed in five of the eight subjects, and all eight individuals reported local sweating in one or more tests. Although some subjects had the perception that they were experiencing "tunnel vision" postexposure, visual perimetry studies following three of the exposures were not confirmatory. Nor were there any changes in visual acuity or color vision. Five hours postexposure, one subject developed flatulence and abdominal discomfort. An hour later he did not feel well and was experiencing waves of nausea. Eight hours postexposure, he deteriorated rapidly and experienced severe nausea and vomiting. At that time, his RBC-ChE activity was only 30% of baseline; no further inhibition occurred. Bouts of vomiting and malaise continued, and he experienced cold sweating, pallor, and a feeling of motion sickness—minus the vertigo. At 12 h postexposure, he was able to sleep, but experienced a nightmare shortly after falling asleep. By the next morning no signs or symptoms remained. The Bramwell et al. (1963) study is not considered credible for deriving AEGL values.

Lubash and Clark (1960) reported that percutaneous doses of undiluted VX (20 µg/kg or 35 µg/kg) applied to the volar forearm of male volunteers

resulted in significant decreases in blood ChE as well as signs and symptoms of toxicity (lightheadedness, nausea, vomiting, diarrhea, hyperactive bowel sounds, epigastric discomfort, insomnia, and nightmares) in two of four subjects dosed with 20 µg/kg and in two of four subjects dosed with 35 µg/kg (eight total subjects).

Sim (1962) reported that head and neck areas were the most sensitive to percutaneously applied VX. A dose of VX at 5 µg/kg applied to these areas resulted in signs and symptoms of systemic toxicity (nausea, vomiting, and weakness) in 54% (28 of 40) of the tested individuals. Whole blood ChE was 50% of normal in 5.8 h and 33.5% of normal in 8.5 h. It was estimated that a VX dose of 5.1 µg/kg would be necessary to result in RBC-ChE_{30} (this end point was chosen because median ChE depression of 30% was associated with the onset of gastrointestinal signs and symptoms of nausea and vomiting).

Cresthull et al. (1963) studied the effects of percutaneous absorption of VX vapors on whole blood ChE activity in 29 male volunteers. Exposures were to the arm or forearm. The VX concentrations ranged from 1.2 to 12.2 mg/m^3 and the exposure times were from 2 to 75 min (Cts ranged from 6 to 765 mg"min/m^3). Two men were exposed at 1.2-1.5 mg/m^3 for 5 min (500 cm^2 surface area exposed); six to 2.5-4.9 mg/m^3 for 5-10 min (500 cm^2 surface area exposed); four to 4.8-7.3 mg/m^3 for 12-20 min (500 cm^2 surface area exposed); ten to 4.5-8.0 mg/m^3 for 20-60 min (1,000 cm^2 surface area exposed); and seven to 8.5-12.2 mg/m^3 for 60-75 min (1,000 cm^2 surface area exposed). The median decrease in whole blood ChE in those groups was 5%, 3%, 8%, 18%, and 43%, respectively. Although whole blood ChE was inhibited as much as 76% at 20 h after exposure, none of the test subjects exhibited any toxic signs. Cresthull et al. (1963) estimated that the whole blood ChE_{50} vapor concentration for percutaneous exposures would be 141 mg"min/m^3. The value was reported to be not statistically meaningful because of the wide confidence limits (lower 95% CI = 35 mg/m^3); however, by comparison with data for exposures to VX aerosols, Cresthull et al. concluded that the estimated ChE_{50} of 141 mg-min/m^3 was acceptable. Cresthull et al. (1963) also estimated that 1 to 1.25 h whole-body percutaneous exposure to a Ct of 38 mg"min/m^3 (0.51 mg/m^3 for 75 min) would not cause any signs of toxicity other than "partial" lowering of whole blood ChE (activity inhibition between 0% and 31% from baseline). Bowers et al. (1964) evaluated behavioral changes in 93 volunteers who were exposed percutaneously to small amounts of liquid EA-1701 (agent VX). The actual amounts of VX applied were not reported.

The test subjects were divided into three postexposure groups depending on the level of reduction in their whole blood ChE (there was no control group). Of 32 individuals whose whole blood ChE was 81-100% of control values (not explicitly stated but presumed to be individual preexposure values) following exposure, 6% showed symptomatology of intellectual impairment (impairment of ability to perform simple arithmetic tasks, inability to perform serial sevens, impairment of performance in reading or standard games of concentration, and other subjective symptoms such as "impairment in orientation"), and 3% reported unusual dreams. Of the 24 whose whole blood ChE was 40-80% of control values, 4% showed symptomatology of intellectual impairment (by the measures reported above), 33% reported unusual dreams, 8% exhibited anxiety (determined by the appearance of palpitations coupled with other, subjective symptoms such as "restlessness"), and 4% exhibited psychomotor depression (determined by the appearance of reply latency, slowed speech, and evidence of fatigue in addition to other, subjective symptoms such as reported feelings of being "slowed down"). Of the 37 whose ChE was 10-40% of control values, 57% showed symptomatology of intellectual impairment (by the measures reported above), 38% reported unusual dreams, 30% exhibited anxiety, and 57% exhibited psychomotor depression. The more severely affected cases exhibited mood alterations as determined by Clyde mood card sort before and after exposure, and some developed nausea and vomiting. Miosis, bronchoconstriction, hypermotility of the lower bowel, and muscle fasciculations were not observed in any of the test subjects. Bowers et al. (1964) concluded that, with the exception of excessive dreaming, psychological symptomatology did not develop in the exposed individuals unless whole blood ChE fell to 40% or less of control values. Very few of the test subjects whose blood ChE was 80% or more of control values exhibited any signs.

Data compiled by Sidell (1992) revealed that, for individuals exposed to VX percutaneously, gastrointestinal signs (vomiting) occurred in 0.6% (1/166) when RBC-ChE activity was at 50% of control values and in 8% (2/24) when RBC-ChE levels were 40-49% of controls. Thirty-three percent exhibited such signs when RBC-ChE levels were 30-39% of controls, and 45% (19/42) exhibited signs when RBC-ChE levels were 20-29% of controls. Sixty-seven percent (16/24) exhibited effects when RBC-ChE levels fell to less than 20% of control values.

2.2.3. Epidemiologic Studies

There are no human epidemiologic studies with dose-response data suitable for deriving AEGL estimates for the G agents.

Occupational exposures to agent GB have been associated with altered electroencephalograms (EEGs) (Duffy et al. 1979; Burchfiel and Duffy 1982). Burchfiel and Duffy (1982) evaluated the wake and sleep EEGs of 77 industrial workers who had been exposed at least once to agent GB (sarin); however, no exposures had occurred in the year preceding the study. Spectral analysis of the EEGs indicated significant increases in brain beta activity (12-30 Hz) in the exposed group compared with nonexposed controls, and sleep EEGs indicated significantly increased rapid eye movement in the exposed workers. Combinations of EEG components were subjected to computer analysis in an attempt to identify an exposed individual by EEG characteristics; however, the results were inconclusive. Burchfiel and Duffy (1982) concluded that there might be a threshold for this type of effect. In evaluating the data of Burchfiel and Duffy (1982), DHHS (1988) considered the EEG changes to be "of questionable significance—given the difficulty of demonstrating such changes and the absence of clinically significant effects even when EEG changes are present."

A retrospective analysis of possible chronic or delayed adverse health effects among servicemen who participated in chemical agent effects and therapy testing at Edgewood Arsenal during the years 1955-1975 was conducted by the Committee on Toxicology of the National Research Council (NRC 1985). The primary source of information was provided by participant response to a questionnaire, but there were no exposure data from which to derive a dose-response relationship. The chronic health effects of concern were "excess cancer risk, and adverse mental, neurologic, hepatic and reproductive effects."

Evaluation of questionnaire response indicated that data provided by subjects historically tested with anticholinesterase compounds did not significantly differ from that of control subjects or those tested with other compounds when self-evaluations of current health status were compared. The report candidly pointed out that the experimental design and comparison groups available were such that "only large effects were likely to be uncovered" because of the resulting large standard errors, self-reporting, and the potential for more than one exposure to eventually result in development of the same biological end point (NRC 1985).

A number of studies have been conducted on individuals exposed to agent GB as a result of terrorist attacks in Japan. Morita et al. (1995)

reported clinical findings for several hundred people who were exposed to agent GB in the city of Matsumoto in 1994. Subclinical miosis and neuropathy were still present in some individuals 30 d after exposure; however, most individuals exhibited no clinical signs of toxicity 6 mo after the exposure.

Several follow-up studies have examined the health of victims of the Tokyo subway terrorist attack that occurred in March of 1995. No clinical abnormalities were detected in 640 patients examined 3 mo after the incident (Okumura et al. 1996). Kato and Hamanaka (1996) examined 96 victims for ocular effects. The primary ocular signs and symptoms included miosis, conjunctival injection, and ocular pain. Some individuals had temporary blurring of vision, 36 patients complained of subjective accommodation impairment, and in 30 patients there were indications that agent GB (sarin) had caused a reduction in intraocular pressure (intraocular pressure was 11.6 ± 1.9 mm Hg within 2 h of exposure but increased to 14.6 ± 1.8 mm Hg when the pupil diameter returned to normal). These signs and symptoms spontaneously resolved within 3-21 d after exposure in most cases. Kato and Hamanaka (1996) note that none of the victims developed corneal injury, glaucoma attack, or retinal detachment, and although the ocular condition of the patients returned to normal, they suggest that exposure to agent GB may increase the risk of angle-closure glaucoma caused by anterior shift of the lens, retinal detachment, and vitreous hemorrhage caused by extensive contraction of the ciliary muscles. Murata et al. (1997) evaluated neurophysiological deficits in 18 victims of the subway attack who had exhibited signs and symptoms of agent GB poisoning (i.e., headache, miosis, increased lacrimation, dyspnea, nausea, diarrhea, para-esthesia, and decreased serum ChE activity). It was reported that 6 mo after the exposure, the exposed but no longer symptomatic individuals exhibited significantly prolonged latencies in event-evoked potentials and visual evoked potentials suggestive of persistent cognitive and visual dysfunction.

In another study, Yokoyama et al. (1998a,b) evaluated chronic neurobehavioral effects in nine male and nine female patients 6-8 mo after the incident. Although this study is for a very small number of those affected (only 18 out of approximately 5,500 people) and suffers from low recruitment, the results will be presented here for completeness. The neurobehavioral tests included (1) digit symbol (psychomotor performance), (2) picture completion (visual perception), (3) digit span (attention and memory), (4) finger tapping (psychomotor performance),(5) reaction time (psychomotor performance), (6) continuous performance test

(sustained visual attention), (7) paired-associate learning (learning and memory), (8) General Health Questionnaire (GHQ) (psychiatric symptoms), and (9) Profile of Mood States (POMS) (mood). Fifteen controls were used in the tests. Analysis of covariance of the test results suggested to the investigators that "perhaps a chronic effect on psychomotor performance [digit symbol test only] was caused directly by acute agent GB (sarin) poisoning; on the other hand, the effects of psychiatric symptoms (GHQ) and fatigue (POMS) appeared to result from post-traumatic stress disorder induced by exposure to sarin." Yokoyama et al. (1998c) have also reported ves-tibulocerebellar effects (increased postural sway) in 18 patients tested 6-8 mo after the incident. Postural sway was significantly greater than controls in exposed females but not in males. In both genders postural sway was correlated with the plasma cholinesterase activity measured immediately after the exposure.

The U.S. Department of Defense reported in 1997 that military personnel might have been exposed to nerve agents as a result of the demolition of Iraqi munition storage sites. Retrospective studies have evaluated the post-war health of soldiers who may have been exposed to nerve agents. Landrigan (1997) reviewed the principal epidemiologic studies published before 1997. Kang and Bullman (1996) reported a 0.8% higher death rate among Gulf War veterans (10.4%) compared with other veterans of the same time period (9.6%); this difference was largely due to accidents, and no excess deaths from suicides or specific diseases were observed. Gray et al. (1996, 1999) reported no consistent pattern for increased occurrence of any specific disease or hospitalization among Gulf War veterans. Further, Gray et al. (1999) indicate that "this data analysis does not support the hypothesis that Gulf War veterans are suffering postwar morbidity from subclinical nerve agent exposure."

Other epidemiologic studies have reported an increase in neurologic disorders among selected groups of Gulf War veterans but have not linked any reported signs, symptoms, or clinical effects with potential nerve agent exposure (Goldstein et al. 1996; Kotler-Cope et al. 1996; Haley et al. 1997a,b; Haley and Kurt 1997; Hom et al. 1997; Schwartz et al. 1997; Vasterling et al. 1998).

Epidemiologic studies regarding human exposure to agent VX were not found in the available literature.

2.3. Neurotoxicity

The G agents (GA [tabun], GB [sarin], GD [soman], and GF) and agent

VX are toxic organophosphate ester derivatives of phosphonic acid. They are commonly termed "nerve" agents as a consequence of their potent anticholinesterase properties and subsequent adverse effects on both smooth and skeletal muscle function as well as the central and peripheral nervous systems. These neurotoxic properties were discussed in detail in Sections 2.1 and 2.2.

Although the inhibition of cholinesterases within neuroeffector junctions or the effector itself is thought to be responsible for the major toxic effects of nerve agents, these compounds can affect nerve impulse transmission by more direct processes as well (e.g., direct effects on neurotransmitter receptors) (see Section 4.2).

2.4. Developmental and Reproductive Toxicity

The retrospective study of agent-exposed servicemen discussed in preceding sections (NRC 1985) requested self-reported information on fertility. Two comparison groups of men were used. One was a "no chemical test" (NCT) group who met the requirements for military service but did not meet the more rigorous requirements (physical and mental screening exams for contraindications) necessary for chemical exposure tests. Those individuals were exposed to placebos, equipment only, or "FDA approved drugs" not otherwise identified. A second comparison group comprised men tested with compounds other than those being evaluated in a particular test, the "other chemical test" (OCT) group. These individuals also met the requirements for military service. They were exposed to test chemicals other than the chemicals of interest. The OCT compounds appear to include cannabinoids, "approved drugs," and "innocuous chemicals and controls" not otherwise identified (NRC 1985). When the collected data were adjusted for volunteer age when the last test was performed (to accommodate national trends toward smaller and delayed families), there "was no difference between the observed fertility pattern of men exposed to anticholinergic chemicals and that expected on the basis of men who were exposed to other chemicals" (NRC 1985). Nevertheless, these data are not useful for application to the derivation of an AEGL given that no exposure data were collected.

Iranian soldiers and civilians were exposed to multiple chemical warfare agents during the Iran/Iraq conflict. Exposures may have been to the nerve agents GA and/or GB as well as to the vesicant sulfur mustard. Follow-up studies have been conducted on some of the individuals. It has been reported that the offspring of these chemical warfare victims born after

the Iran/Iraq conflict were more likely to have birth defects than those born before the war (Pour-Jafari 1994a). It was also reported that the offspring had an altered gender ratio (Pour-Jafari 1994b). Because of the possibility that exposures to multiple chemicals had occurred, it is impossible to determine if, or to what extent, exposure to any of the G agents contributed to the reported effects.

There have been several reports of potential increased incidence of birth defects among the offspring of military personnel who served in the Persian Gulf War. Araneta et al. (1997) reported a slight increase (relative risk 3.03, with 95% CI = 0.63-20.57) in Goldenhar syndrome among infants born in military hospitals to Gulf War veterans. Goldenhar syndrome is a craniofacial anomaly of unclear etiology. According to Araneta et al. (1997), suggested associations with its occurrence have included chromosomal abnormalities, a genetic pattern of inheritance, maternal diabetes, or prenatal exposure to several controlled or therapeutic drugs (e.g., cocaine, tamoxifen); the role of male-mediated effects is undefined. At least one mother of one case infant exhibited mild facial asymmetry upon examination, and the family of another case infant had a history of birth defects. In all five cases of confirmed Goldenhar syndrome among the 34,069 infants born to veterans of the Gulf War and included in this retrospective study, only the paternal parent served in the military. Among nondeployed veterans (two cases of a total 41,345 births examined), only the paternal parent served in the military.

Araneta and colleagues (1997) point out that differences in prevalence rates of Goldenhar syndrome among the offspring of Gulf War veterans (14.7, 95% CI = 5.4-36.4) are not significantly different from those of nondeployed veterans (4.8, 95% CI = 0.8-19.5) because of the small sample sizes and wide confidence intervals. Araneta et al. (1997) determined that, with the sample size maintained as a constant, "the risk would have had to be at least 5.75 times higher among the Gulf War veterans' infants in order to be statistically significant."

As a consequence of this finding of no significance, the Goldenhar syndrome issue was not further pursued in the AEGL analysis for chemical warfare agents. Its utility is further compromised by the fact that there is no confirmed report of veteran exposure to chemical warfare agents, and the Gulf War fathers in the Araneta et al. (1997) study all served in different units and were deployed in theater at different times.

No data are available regarding the potential reproductive and developmental toxicity of agent VX in humans.

2.5. Genotoxicity

There is no information available to evaluate the genotoxicity of G agents or agent VX in humans.

2.6. Carcinogenicity

There are no human data to suggest that G agents or agent VX are carcinogenic.

2.7. Summary

G-series Agents

Available information on the acute inhalation toxicity of agent GB (sarin) to humans is summarized in Table 1-10. Minimal effects observed at low concentrations include miosis, tightness of the chest, rhinorrhea, and dyspnea. The threshold for minimal effects appears to fall in the range of 0.05 to 0.5 mg/m^3 for 10-30 min exposures. The results from different studies are not consistent in identifying the threshold, and that may be due to differences in individual sensitivities, breathing rates of the test subjects, experimental protocols, or analytical methods.

A number of investigators consider *both* miosis and rhinorrhea to be early signs of exposure to cholinesterase inhibitors (Sidell 1997; Mioduszewski et al. 2002b; H. van Helden, Pulmonary and CNS Pharmacology Lab, TNO, the Netherlands, personal communication; S. Tattersall, Biomedical Sciences Division, Porton Down, United Kingdom, personal communication). The presence of rhinorrhea can be indicative of inhalation exposure and/or development of systemic effects, while miosis alone in the absence of other signs or symptoms is a local effect to the pupillary muscles of the eye. As a consequence, the presence of miosis is considered an appropriately sensitive indicator of direct vapor exposure and has the additional advantage of being readily recognized and quantifiable.

TABLE 1-10 Human Experimental Data For GB Vapor (Single Exposures)

Study	GB Concentration (mg/m^3)	Duration	Ct ($mg \cdot min/m^3$)	Signs and Symptoms
Harvey 1952	0.05	20 min	1	Headache, eye pain, rhinorrhea, tightness in chest, cramps, nausea, malaise (N = 14)
Johns 1952	0.05	20 min	1	Mild miosis (mean maximum decrease in pupil diameter 1-2 mm) in some of the test subjects (150 observations)
McKee and Woolcott 1949	0.06	20 min	1.2	No reported effects (N = 5)
McKee and Woolcott 1949	0.06	40 min	2.0	"Threshold" for miosis; no other signs or symptoms (N = 4)
Fairley and Mumford 1948	0.3	0.5 min	0.15	Rhinorrhea in 16 and tightness in chest in 7 (N = 16)
Baker and Sedgwick 1996	0.5	30 min	1.5	Miosis, dyspnea, photophobia, 40% inhibition of RBC-ChE, changes in SFEMG (N = 8)
McKee and Woolcott 1949	0.6	1 min	0.6	Miosis and slight tightness in chest (N = 4)
Rubin et al. 1957	2	2 min	4	Miosis; no other signs of ChE inhibition (N = 3)
Callaway and Dirnhuber 1971		10 min to 5 h	3.13[a]	50% pupil area decrement

(Continued)

TABLE 1-10 *Continued*

Study	GB Concentration (mg/m^3)	Duration	Ct ($mg \cdot min/m^3$)	Signs and Symptoms
Callaway and Dirnhuber 1971		10 min to 5 h	13.85[b]	90% pupil area decrement
Oberst et al. 1968	4.19	2 min	8.38	47% inhibition of RBC-ChE; no other effects; eyes not exposed (breathing rate 5.6-8.4 L/min)
Oberst et al. 1968	20.7	2 min	41.4	49% inhibition of RBC-ChE; no other effects; eyes not exposed (breathing rate 47-65 L/min)

Note: Entries are from primary sources and known experimental data

[a]95% confidence limits 2.15-4.57 $mg \cdot min/m^3$.

[b]95% confidence limits 6.00-32.02 $mg \cdot min/m^3$.

There is no evidence that exposure to any of the G agents results in developmental or reproductive toxicity, nor are there any data available to evaluate potential genotoxicity in humans. The G agents have not been identified as human carcinogens.

Agent VX

Experimental data on the effects of acute VX exposures to humans are summarized in Tables 1-11 and 1-12; very few studies have been conducted using exposures to VX vapor, and available data are not of sufficient quality to be used directly in the development of AEGL estimates.

A comparison of the results of the intravenous studies, in terms of the estimated absorbed dose, allows for an evaluation of the dose-response relationship (Table 1-11).

Studies indicate that an intravenous dose of about 1 μg/kg can result in 50% ChE depression and some symptoms of toxicity (headaches); an intravenous dose of about 0.1 μg/kg is unlikely to affect RBC-ChE, but may cause mild effects (headache, chest tightness, dyspnea); and an intravenous dose of 0.01 μg/kg may be below the effects threshold. The estimated equivalent air concentrations for these dose levels, using standard default values for body weight (70 kg) and breathing rate (0.0138 m^3/min), are also listed in Table 1-11. They are highly derivative values and are only presented for comparative purposes.

Experimental data from the Bramwell et al. (1963) study are summarized in Table 1-12. Although the Bramwell et al. (1963) data are considered suspect, they provide a means of comparison with the equivalent concentrations estimated from the intravenous data. Both sets of data suggest that a 10-min exposure to VX at 0.5 mg/m^3 or higher may produce substantial depression of RBC-ChE activity and some clinical signs of toxicity.

Available data summarized suggest that a 10-min exposure to VX at 0.5 mg/m^3 or higher may produce depression of RBC-AChE activity and some clinical signs of toxicity.

TABLE 1-11 Human Experimental Data for VX

Dose (μg/kg)	Exposure Route	Estimated Equivalent Concentration (mg/m^3)[a]	End Point	Reference
0.01-0.13 (estimated)	Inhalation (sniff test)	0.05-3.34	No ChE change; headache, chest tightness, dryness of the mouth	Koon et al. 1959
0.04	Intravenous (30 s injection)	0.41 (0.02 for 10 min)[b]	No ChE change; headache, tiredness, irritability	Kimura et al. 1960
0.12	Intravenous (2 doses over 3.5 h)	0.003 (0.06 for 10 min)[b]	No ChE change; headache, light headedness, abdominal cramps, decrease in respiration and pulse rates, increase in airway resistance and minute volume	Kimura et al. 1960
1.0	Intravenous (over 1.75-4 h)	0.021(0.5 for 10 min)[b]	50-60% depression in ChE activity; headaches in 1/6 individuals	Kimura et al. 1960
1.0	Intravenous (1 dose)	10 (0.51 for 10 min)[b]	50% inhibition of RBC-ChE	Sidell and Groff 1974
1.5	Intravenous (1 dose)	15(0.76 for 10 min)[b]	75% depression in ChE: dizziness (11/18), nausea (4/18), vomiting (6/18)	Sidell and Groff 1974
1.43	Oral (1 dose/d for 7 d)	NA	60% inhibition of RBC-ChE; no signs or symptoms of toxicity	Sim et al. 1964
2.3	Oral	NA	50% inhibition of RBC-ChE	Sidell and Groff 1974

2-4.5	Oral	NA	Gastrointestinal symptoms in 5/32	Sidell and Groff 1974

Entries are from primary sources and known experimental data

[a]Equivalent concentration estimated from intravenous dose, using as default values 70 kg body weight, and breathing rate of 0.0138 m^3/min (13.8 L/min), and the maximum infusion time listed for the intravenous dose; for single intravenous doses, an estimated time of 30 s was used.

[b]Values in parentheses are for inhalation exposures, standardized to 10 min, using linear extrapolation. For a breathing rate of 0.055 m^3/min (55 L/min) corresponding to heavy activity, the estimated 10-min equivalent concentrations would be approximately one-fourth of the values listed.

TABLE 1-12 Human Experimental Data For VX Vapor from Bramwell et al. (1963)[a]

VX Concentration (mg/m^3)	Duration	Estimated Ct ($mg \cdot min/m^3$)	Signs and Symptoms
0.2-0.57	3 min	0.6-1.7	10-22% ChE inhibition; slight tightness in throat and upper respiratory tract, some miosis ($N = 7$); eyes closed
0.31	3 min	0.9	Sudden miosis in one individual with eyes open
1.6-2.4	1.5 min	2.4-3.6	18-41% ChE inhibition: slight tightness in throat and upper respiratory tract; some miosis ($N = 8$); eyes closed
0.8-1.06	6-7 min	4.8-6.4	44-70% ChE inhibition; slight tightness in throat and upper respiratory tract; some miosis ($N = 4$); eyes closed

[a]The majority of a Surgeon General's review panel convened by the CDC in Atlanta in August 2000 considered the Bramwell data to be "suspect" and recommended that they not be used in deriving exposure estimates (67 Fed. Reg. 894 [2002]; DHHS 2002). They are presented here for completeness.

3. ANIMAL TOXICITY DATA

3.1. Acute Lethality

Acute inhalation lethality data for agents GB, GA, GD, GF, and VX for several laboratory species are summarized in Tables 1-13 through 1-17. Additional lethality information is presented in the following subsections.

3.1.1. Nonhuman Primates

Agent GB

The National Defense Research Committee (NDRC) (1946) reported an LCt_{50} value of 150 mg"min/m^3 for 10-min exposure in five monkeys. Cresthull et al. (1959) reported that inhalation doses of GB at 19.2-48.4 µg/kg (0.55-min exposures) resulted in 33-100% mortality in monkeys.

Johnson et al. (1988) conducted a series of lethality studies in which nonhuman primates (baboons) were exposed to GB by the inhalation pathway. Baboons were considered to be a more appropriate test species than rodents or dogs because of the similarities between baboon and human lungs in both biochemical and functional characteristics. Young male baboons were exposed to GB vapor at 1.25-1.3 LD_{50} ($N = 6$) or GD vapor at 2 LD_{50} ($N = 5$). As a result of these tests, Johnson et al. (1988) (see also Anzueto et al. [1990]) reported that inhalation exposures were in close agreement with the reported intravenous LD_{50} values (approximately 13 µg/kg). Woodard et al. (1994) reported an intravenous LD_{50} of 14.7 µg/kg for GB in rhesus monkeys.

Vapor inhalation studies were conducted by Oberst (1961) on monkeys (species not identified) fitted with masks through which GB concentrations were administered. The eyes were protected by eyepieces and were not exposed. The resulting 2-min LCt_{50} value of 42 mg"min/m^3 is reported in Table 1-9.

Agent GA

DA (1974) (secondary source) reported LCt_{50} values of 187 and 135 mg"min/m^3 for monkeys.

TABLE 1-13 Acute Inhalation Lethality Values for Agent GB in Animals (Toxicity Value, LCt_{50})

Species	Duration (min)	Ct (mg"min/m^3)	Reference
Monkey	10	74	DA 1974
Monkey	2	42	DA 1974
Monkey	0.167	27	DA 1974
Monkey	10	150	NDRC 1946[a]
Monkey	2	42	Oberst 1961[a]
Dog	10	60	DA 1974
Dog	2	56	Oberst 1961[a]
Rabbit	10	120	DA 1974
Guinea pig	1	140	Oberst 1961[a]
Guinea pig	10	180	DA 1974
Rat	5	164 (f)	Mioduszewski et al. 2000, 2001[a]
Rat	10	181 (f)	Mioduszewski et al. 2000, 2001[a]
Rat	30	255 (f)	Mioduszewski et al. 2000, 2001[a]
Rat	60	383 (f)	Mioduszewski et al. 2000, 2001[a]
Rat	90	401 (f)	Mioduszewski et al. 2000, 2001[a]
Rat	240	727 (f)	Mioduszewski et al. 2000, 2001[a]
Rat	360	947 (f)	Mioduszewski et al. 2000, 2001[a]
Rat	5	230 (m)	Mioduszewski et al. 2000, 2001[a]
Rat	10	226 (m)	Mioduszewski et al. 2000, 2001[a]
Rat	30	265 (m)	Mioduszewski et al. 2000, 2001[a]
Rat	60	453 (m)	Mioduszewski et al. 2000, 2001[a]

(continued)

TABLE 1-13 *Continued*

Species	Duration (min)	Ct (mg"min/m^3)	Reference
Rat	90	433 (m)	Mioduszewski et al. 2000, 2001[a]
Rat	240	982 (m)	Mioduszewski et al. 2000, 2001[a]
Rat	360	1040 (m)	Mioduszewski et al. 2000, 2001[a]
Rat	10	220	DA 1974
Rat	10	300	Cohen et al. 1954[a]
Rat	1	220 (m), 118 (f)	Callaway and Blackburn 1954[a]
Rat	5.0-6.7	191 (f)	Schoene et al. 1985[a]
Mouse	30	501	Bide et al. 1999
Mouse	30	600	Husain et al. 1993[a]
Mouse (active)	10	240	DA 1974
Mouse (resting)	10	310	DA 1974

[a]Italic entries are from primary sources and known experimental data.
Abbreviations: f, female; m, male.

Agent GD

In tests conducted on baboons, Johnson et al. (1988) found that the effects of inhalation exposures were in close agreement with the reported intravenous LD_{50} values of 6.6 µg/kg. Adams et al. (1975) reported a 15-d intramuscular LD_{50} of 6.57 µg/kg.

3.1.2. Dogs

Agent GB

NDRC reported LCt_{50} values of 100-150 mg"min/m^3 for 10-min exposures. Cresthull et al. (1959) reported that inhalation doses of GB at

TABLE 1-14 Acute Inhalation Lethality Values for Agent GA in Animals (Toxicity Value, LCt_{50})

Species	Duration (min)	Ct (mg"min/m^3)	Reference
Monkey	10	187	DA 1974[a]
Monkey	10	135	DA 1974[a]
Dog	2	320	DA 1974[a]
Rabbit	10	960	DA 1974[a]
Rat	10	450	DA 1974[a]

[a]DA (1974) is a secondary source.

25.1 and 26.0 µg/kg (0.6- and 2.23-min exposures) were not lethal to dogs, but inhalation doses of GB at 32.5 µg/kg or higher caused 40% or more mortality. Bide et al. (1999) and Yee et al. (1999) developed a three dimensional probit model to calculate lethality values (LC_{05}, $LC_{50,}$ LC_{95}) from historic laboratory data and to estimate equivalent human values. Using the species-specific constants for inhalation rates, body mass, et cetera, provided by these authors, the 30-min LC_{50} value for dogs was calculated to be 4.3 mg/m^3.

Inhalation studies were conducted by Oberst (1961) on dogs (breed not identified) fitted with masks through which GB vapor flowed. The eyes were protected by eyepieces and were not exposed. The resulting 2-min LCt_{50} value of 56 mg"min/m^3 is reported in Table 1-9.

Agent GA

DA (1974) (secondary source) reported an LCt_{50} value of 320 mg"min/m^3 for dogs.

Agent VX

Bide and Risk (2000) cite several earlier studies in which the LCt_{50} values for VX aerosols were reported to be 15 mg"min/m^3 (whole body) (Krackow 1956) and 15.1 mg"min/m^3 (Punte and Atkinson 1960).

TABLE 1-15 Acute Inhalation Lethality Values for Agent GD in Animals (Toxicity Value, LCt_{50})

Species	Duration (min)	Ct (mg"min/m^3)	Reference
Rabbit	10	160	DA 1974
Rat	1	196 (m) 135 (f)	Callaway and Blackburn 1954[a]
Rat	5.3-8.5	211 (f)	Schoene et al. 1985[a]
Rat	10	279	DA 1974
Rat	10	230	DA 1974
Rat	< 30 (threshold at 16)	400 (threshold at 335)	Aas et al. 1985[a]
Guinea pig	8	480	Langenberg et al. 1998[a]

[a]Entries are from primary sources and known experimental data.
Abbreviations: f, female; m, male.

3.1.3. Rats

Agent GB

NDRC (1946) reported LCt_{50} values of 150-300 mg"min/m^3 for 10-min exposures. Bide et al. (1999) and Yee et al. (1999) developed a three dimensional probit model to calculate lethality values (LC_{05}, $LC_{50,}$ LC_{95}) from historic laboratory data and to estimate equivalent human values. Using the species-specific constants provided, the 30-min LC_{50} value for rats was calculated to be 8.2 mg/m^3.

In studies conducted by Mioduszewski et al. (2000, 2001, 2002a), the acute lethal toxicity of GB to male and female Sprague-Dawley rats was evaluated for time periods of 10, 30, 60, 90, 240 and 360 min in a whole-body dynamic chamber. The final report of this study (Mioduszewski et al. 2001, 2002a) is further documentation of the findings presented below. Ten males and 10 females were used for each concentration-time (Ct) combination, and 50 males and 50 females were used for each time point. GB concentrations ranged from about 2 mg/m^3 to 56 mg/m^3. Agent concentrations were confirmed in the exposure chamber by three procedures ("Edgewood" bubblers, solid sorbent tubes, and a phosphorous monitor) to

TABLE 1-16 Acute Inhalation Lethality Values for Agent GF in Animals (Toxicity Value, LCt_{50})[a]

Species	Duration (min)	Ct (mg"min/m^3)	Reference
Rat	10	368 (m) 253 (f)	Anthony et al. 2002
Rat	60	396 (m) 334 (f)	Anthony et al. 2002
Rat	240	595 (m) 533 (f)	Anthony et al. 2002

[a]24-h postexposure lethality.
Abbreviations: f, female; m, male.

allow point and continuous determinations (Mioduszewski et al. 2000, 2001, 2002a). Lethality was assessed at 24 h and at 14 d. Female rats were reported to be more sensitive to GB vapor toxicity than males over the range of exposure concentrations and durations studied (Mioduszewski et al. 2000, 2001, 2002a). Gender differences were reported to be significant at $p < 0.01$. Probit analysis (MINITAB, version 13) presented in Mioduszewski et al. (2000) gave the following 14-d LC_{50} values for female rats exposed to GB vapor: 18.1 mg/m^3 for 10 min; 8.51 mg/m^3 for 30 min; 6.39 mg/m^3 for 60 min; 3.03 mg/m^3 for 4 h; and 2.63 mg/m^3 for 6 h.

Probit analysis presented in Mioduszewski et al. (2000) gave the following 14-d LC_{50} values for male rats:22.6 mg/m^3 for 10 min; 8.84 mg/m^3 for 30 min; 7.55 mg/m^3 for 60 min; 4.09 mg/m^3 for 240 min; 2.89 mg/m^3 for 360 min.

Based on a probit analysis of the data (Mioduszewski et al. 2000), the estimated 14-d LC_{01} values for the females are as follows: 11.54 mg/m^3 for 10 min; 5.84 mg/m^3 for 30 min; 4.01 mg/m^3 for 60 min; 2.09 mg/m^3 for 4 h; 1.76 mg/m^3 for 6 h.

This is the critical study and data set (females) for determination of AEGL-3 values.

GB vapor exposure significantly inhibited rat blood cholinesterase activity in the Mioduszewski et al. (2000, 2001, 2002a) study. However, no correlation between severity of clinical signs and cholinesterase inhibition was reported.

Cohen et al. (1954) reported that exposure of rats to GB at 30 mg/m^3 for 10 min (Ct = 300 mg"min/m^3) resulted in mortality rates of close to 50%

TABLE 1-17 Lethality in Laboratory Species Following Inhalation Exposure to Agent VX Vapor (End Point, LCt_{50})

Species	Duration (min)	Ct (mg"min/m^3)	Reference
Mouse (head only)	10 min	13.6	Koon et al. 1960, as cited in NRC 1997
Mouse (whole body)	10 min	4.0	Koon et al. 1960, as cited in NRC 1997
Mouse (nose only)	_	71	Carroll et al. 1957
Mouse (whole body)	_	16.1	Carroll et al. 1957
Goat	10 min	9.2	Koon et al. 1960, as cited in NRC 1997
Mouse	6 h/d, 5 d/wk, 2 wk	0.9	Crook et al. 1983 (noncredible data)
Rat	6 h/d, 5 d/wk, 2 wk	24.9	Crook et al. 1983 (noncredible data)
Guinea pig	6 h/d, 5 d/wk, 2 wk	238.6	Crook et al. 1983 (noncredible data)
Rabbit	6 h/d, 5 d/wk, 2 wk	No deaths	Crook et al. 1983 (noncredible data)

and a decrease in brain cholinesterase activity levels to less than 5% of normal.

Schoene et al. (1985) reported an LCt_{50} of 191 mg"min/m^3 (95% CI = 178-204 mg"min/m^3) for exposure times of 5.0-6.7 min for female Wistar rats. The corresponding 5-min LC_{50} is 38 mg/m^3. Callaway and Blackburn (1954) reported LCt_{50} values of 220 mg"min/m^3 for male albino rats and 118 mg"min/m^3 for female albino rats (1-min exposures).

Agent GA

DA (1974) (secondary source) reported an LCt_{50} value of 450 mg"min/m^3 for the rat.

Agent GD

In a study designed to secondarily examine agent GD toxicity, Aas et al. (1985) reported that the LCt_{50} for GD in rats (six animals tested at each of three exposure levels for periods of time <30 min) was 400 mg"min/m^3. Aas et al. (1985) graphically present their data as an LCt-versus-mortality curve. The lethality threshold estimated from the curve is about 335 mg"min/m^3. Because the reported GD concentration was fixed at 21 mg/m^3 for the duration of the study, the exposure time corresponding to the lethal threshold is 16 min (see Table 1-11).

The principal objective of the Aas et al. (1985) study was to test an experimental dynamic flow system that would allow study of highly toxic vapors. In consequence, it was necessary to continually generate small amounts of the toxic material in question. Agent GD (soman) was the compound selected to best test the system. Secondary objectives of the study were to determine the (short-term) inhalation toxicity of agent GD (soman) and to study inhibition of acetylcholinesterase, cholinesterase, and carboxylesterase activity in the respiratory tract (relative to other tissues).

Schoene et al. (1985) reported an LCt_{50} of 211 mg"min/m^3 (95% CI = 195-229 mg"min/m^3) for exposure times of 5.3-8.5 min for female Wistar rats. Callaway and Blackburn (1954) reported LCt_{50} values of 196 mg"min/m^3 for male albino rats and 135 mg"min/m^3 for female albino rats (1-min exposures) (Table 1-11).

Agent GF

Callaway and Blackburn (1954) reported LCt_{50} values of 181 mg"min/m^3 for male albino rats and 110 mg"min/m^3 for female albino rats (1-min exposures). Kassa and Cabal (1999) reported an intramuscular LD_{50} of 80 µg/kg for rats.

A recent study of GF vapor inhalation toxicity in male and female SD rats reported 24-h postexposure LC_{50} and LCt_{50} values for three exposure periods (10, 60, and 240 min) (Anthony et al. 2002). Young adult rats were exposed whole-body in a dynamic 750-L chamber under protocols similar to those previously published by Mioduszewski et al. (2001, 2002a) but with additional accommodation for the lesser volatility of agent GF. For female rats, Anthony et al. (2002) report 24-h LC_{50} values as follows: 10 min, 25.3 mg/m^3; 60 min, 5.56 mg/m^3; 240 min, 2.22 mg/m^3. For male rats,

24-h LC_{50} values are as follows: 10 min, 36.8 mg/m^3; 60 min, 6.60 mg/m^3; 240 min, 2.48 mg/m^3. These results are summarized as LCt_{50} values in Table 1-16.

Agent VX

Crook et al. (1983) reported an LCt_{50} of 24.9 mg"min/m^3 for animals exposed 6 h/d, 5 d/wk, for 2 wk; however, the results of the study are not considered credible (see Section 3.2).

Bide and Risk (2000) exposed outbred male CD1(SD)BR rats to NaCl aerosols containing entrained VX. The animals (five per test group) were tested with a nose-only inhalation system and for an exposure time of 12 min. Test concentrations were not reported. The observed LCt_{50} was 67 mg"min/m^3.

In studies conducted by Maxwell (1992) on Sprague-Dawley rats, the subcutaneous LD_{50} for VX was reported to be 0.027 µmol/kg.

DA (1974) (secondary source) reports intragastric LD_{50} values of 0.1 mg/kg and 0.077-0.1280 mg/kg for rats.

3.1.4. Mice

Agent GB

NDRC (1946) reported LCt_{50} values of 150-300 mg"min/m^3 for 10-min exposures, 360 mg"min/m^3 for a 20-min exposure, and 420 mg"min/m^3 for a 30-min exposure (14 mg/m^3 for 30 min). Clement (1992) reported a subcutaneous LD_{50} value of 170 µg/kg for male CD-1 mice with body weight ranging from 30-40 g (five animals per test group). A subcutaneous LD_{50} of 0.212 mg/kg was reported for male and female Shanghai mice (18-22 g body weight, eight mice per group, five exposure groups) (Luo and Liang 1997). Bide et al. (1999) and Yee et al. (1999) developed a three dimensional probit model to calculate lethality values (LC_{05}, $LC_{50,}$ LC_{95}) from recently conducted laboratory experiments on mice and historic toxicity data for other laboratory species and to estimate equivalent human values. Using the species-specific constants provided by Bide et al. (1999), the 30-min LC_{50} value for the mouse was calculated to be 16.7 mg/m^3.

The LCt_{50} for agent GB in female Swiss albino mice has been reported

to be 600 mg"min/m^3 (Husain et al. 1993), equivalent to a 30-min exposure at 20 mg/m^3. Lohs (1960) reported a 30-min inhalation lethality value of value of 5 mg/m^3.

Agent GD

Lohs (1960) reported a 30-min inhalation lethality value of 1 mg/m^3.

Agent GF

Inhalation lethality data for agent GF were not found in the available literature. Luo and Liang (1997) reported an LD_{50} of 0.346 mg/kg in mice injected with the agent subcutaneously. Clement (1992) reported a subcutaneous LD_{50} value of 243 µg/kg for male CD-1 mice with body weights ranging from 30-40 g (five animals per test group).

Agent VX

Ten-minute LCt_{50} values of 4.0 mg"min/m^3 (whole body) and 13.6 mg"min/m^3 (head only) have been reported for mice exposed to VX vapors (Table 1-6) (Koon et al. 1960, as cited in NRC 1997). LCt_{50} values of 71 mg"min/m^3 for female mice for nose-only exposures and 16.1 mg"min/m^3 for whole-body exposures were reported by Carroll et al. (1957) for female mice; however, in this study it was reported that the concentration of the agent in the exposure chamber was not measured directly but was estimated from the mortality level, which was correlated with the LD_{50} for an intravenous injection (estimated to be 17 µg/kg).

Crook et al. (1983) reported an LCt_{50} of 0.9 mg"min/m^3 for animals exposed 6 h/d, 5 d/wk, for 2 wk to VX vapors; however, the results of this study are not considered credible.

Koplovitz et al. (1992) exposed Swiss ICR mice intramuscularly to GB and VX. The resulting acute (24-h) LD_{50} are as follows: LD_{50} for GB of 204.81 µg/kg, LD_{50} of VX of 13.07 µg/kg.

Bide and Risk (2000) exposed outbred male CD1(ICR)BR mice to NaCl aerosols containing entrained VX. The animals (five per test group) were tested with a nose-only inhalation system and for a exposure time of

12 min. Test concentrations were not reported. The observed LCt_{50} was 72 mg"min/m^3. Bide and Risk (2000) also cite several earlier studies in which the LCt_{50} values for VX aerosols were reported to be 7 mg"min/m^3 (Krackow 1956) and 6.1 mg"min/m^3 (Punte and Atkinson 1960).

3.1.5. Guinea pigs

Agent GB

NDRC (1946) reported LCt_{50} values of 150-250 mg"min/m^3 for 10-min exposures. Bide et al. (1999) and Yee et al. (1999) developed a three dimensional probit model to calculate lethality values (LC_{05}, $LC_{50,}$ LC_{95}) from historic laboratory data and to estimate equivalent human values. Using the species-specific constants provided, the 30-min LC_{50} value for guinea pigs was calculated to be 7.5 mg/m^3.

Oberst (1961) conducted inhalation studies on guinea pigs administered GB vapor by means of face masks "which filled the face without leakage." The author does not mention eye protection for guinea pigs. The resulting 1-min LCt_{50} of 140 mg"min/m^3 is reported in Table 1-9.

Atchison et al. (2001) reported that subcutaneous injections of 0.6 LD_{50} GB once per day, 5 d/wk, in young male Hartley guinea pigs (600 g) resulted in 50% mortality (two of four) after 2 wk of exposure and 100% mortality after 3 wk. The subcutaneous LD_{50} for guinea pigs was reported to be 42 µg/kg.

Agent GD

Allon et al. (1998) reported an inhalation LD_{50} of 101 µg/kg for guinea pigs, considerably higher than the reported intravenous LD_{50} values of 22 µg/kg (Sterri et al. 1982) and 3.5 µg/kg (Due et al. 1993). For guinea pigs weighing 0.84 kg and breathing 0.4 m^3/d (EPA defaults), a dose of 101 µg/kg would be equivalent to an exposure to GD at 0.009 mg/m^3 for 24 h, 0.22 mg/m^3 for 1 h, or 0.44 mg/m^3 for 30 min. Langenberg et al. (1998) reported an 8-min LCt_{50} of 480 mg"min/m^3 for guinea pigs. This value is equivalent to an LC_{50} of 60 mg/m^3 for 8 min or 16 mg/m^3 for 30 min (assuming linear scaling).

Atchison et al. (2001) reported that subcutaneous injections of 0.6 LD_{50}

GD once per day, 5 d/wk, in young male Hartley guinea pigs (600 g) resulted in 50% mortality (two of four) after 2 wk of exposure and 100% mortality after 9 wk. The subcutaneous LD_{50} for guinea pigs was reported to be 28 µg/kg.

Agent VX

Crook et al. (1983) reported an LCt_{50} of 238 mg"min/m^3 for animals exposed 6 h/d, 5 d/wk, for 2 wk; however, the results of this study are not considered credible.

Koplovitz et al. (1992) exposed Hartley albino guinea pigs (subcutaneous) to GB and VX. The resulting acute (24-h) LD_{50} are as follows: LD_{50} for GB of 41.26 µg/kg, LD_{50} for VX of 6.89 µg/kg.

Bide and Risk (2000) exposed outbred male (HA)BR guinea pigs to NaCl aerosols containing entrained VX. The animals (five per test group) were tested with a nose-only inhalation system and for an exposure time of 12 min. Test concentrations were not reported. The observed LCt_{50} was 30 mg"min/m^3. Bide and Risk (2000) also cite several earlier studies in which the LCt_{50} values for VX aerosols were reported to be 30 mg"min/m^3 (whole body) (Krackow 1956) and 29.5 mg"min/m^3 (Punte and Atkinson 1960).

Atchison et al. (2001) reported that subcutaneous injections of 0.6 LD_{50} VX once per day, 5 d/wk, in young male Hartley guinea pigs (600 g) resulted in 33% mortality (two of six) after 10 wk of exposure and 83% mortality (five of six) after 9 wk. One animal survived the full 13 wk of treatment. The subcutaneous LD_{50} for guinea pigs was reported to be 9 µg/kg.

3.1. 6. Rabbits

Agent GB

NDRC (1946) reported LCt_{50} values of 120-250 mg"min/m^3 for 10-min exposures. Bide et al. (1999) and Yee et al. (1999) developed a three dimensional probit model to calculate lethality values (LC_{05}, $LC_{50,}$ LC_{95}) from historic laboratory data and to estimate equivalent human values. Using the species-specific constants provided, the 30-min LC_{50} value for rabbits was calculated to be 5.6 mg/m^3.