Agent GA

DA (1974) (secondary source) reported an LCt_{50} of 960 mg"min/m^3 for a 10-min exposure.

Agent VX

Bide and Risk (2000) cite an earlier study in which the LCt_{50} values for a VX aerosol was reported to be 22.1 mg"min/m^3 (Punte and Atkinson 1960).

3.1.7. Cats

NDRC (1946) reported an LCt_{50} value of 100 mg"min/m^3 for a 10-min exposure. 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 by Bide et al. (1999), the 30-min LC_{50} value for cats was calculated to be 3.9 mg/m^3.

3.1.8. Goats

Agent VX

A single 10-min LCt_{50} of 9.2 mg"min/m^3 has been reported for goats (Table 1-6) (Koon et al. 1960, as cited in NRC 1997).

3.1.9. Hamsters

Agent VX

Bide and Risk (2000) cite several earlier studies in which the LCt_{50} values for VX aerosols were reported to be 17 mg"min/m^3 (whole body) (Krackow 1956) and 14.7 mg"min/m^3 (Punte and Atkinson 1960).

3.1.10. Summary of Acute Lethality Data in Animals

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.

In addition to the data presented in Sections 3.1 through 3.9, Tables 1-13 through 1-17 contain data obtained from several historical reference handbooks (NDRC 1946; DA 1974). The DA (1974) reference source also contains LCt_{50} values for exposure times less than 10 min (i.e., from 2 s to 2 min). Christensen et al. (1958) used data sets for agent GB to develop LCt_{50}-exposure time curves for each species. The original sources of the lethality data are cited by Christensen et al. (1958). A similar data set (exposures from 2 s to 12 min) was also used by Yee (1996) (see also Yee et al. [1999] and Bide et al. [1999]) who evaluated the relationship between lethal concentrations and exposure times.

3.2. Nonlethal Toxicity

3.2.1. Nonhuman Primates

Agent GB

Increases in high frequency beta activity were observed by Burchfiel and Duffy (1982) in the EEGs of rhesus monkeys who had been injected with agent GB (sarin) 1 y earlier (with either a single 5 µg/kg intravenous dose or with a series of intramuscular injections of 1 µg/kg, given once per week for 10 wk). Control animals did not show any changes in EEG. Neurobehavioral tests were not conducted on the exposed animals. In a similar series of tests in which marmosets were injected intramuscularly with 3 µg/kg, a slight but nonsignificant increase in beta activity was observed 15 mo after the exposure (Pearce et al. 1999). Behavioral tests indicated no deleterious effects on cognitive performance. RBC-ChE activity was reduced by 51.3% in the dosed animals.

Christensen et al. (1958) cite several earlier studies (Cresthull et al. 1957; Callaway and Crichton 1954) in which the incapacitation Ct_{50} for GB for monkeys was reported to be 67-75% of the LCt_{50} value. The ICt_{50} value is estimated to be 47 mg"min/m^3 for a 2-min exposure (derived from the graphic presentation of the data given by Christensen et al. [1958]). Incapacitation was defined as convulsions, collapse, or death. Anzueto et al. (1990) reported that inhalation of 30 µg/kg (2 times the LD_{50}) by four baboons resulted in cardiac arrhythmias, apnea, and a significant decrease

in mean blood pressure. Single intramuscular injections of 6 µg/kg to marmosets resulted in adverse behavioral effects when the animals were tested for hand-eye coordination, but no adverse effects were seen in a visual discrimination test (Wolthuis 1992). A dose of 3 µg/kg had no adverse effects on behavior, and hand-eye coordination was improved (versus each individual animal's baseline performance prior to exposure) in three of six animals (Wolthuis 1992).

Ashwick and deCandole (1961) reported that subcutaneous GB doses of greater than 0.04 mg/kg would result in convulsions in monkeys.

van Helden et al. (2001, 2002) exposed nearly equal numbers of male and female marmosets (*Callithrix jacchus*, Harlan, United Kingdom) (whole-body) to GB vapor concentrations at 0.05 to 150 µg/m^3 for 5 h. The lowest cumulative exposure at which the internal dose became measurable (based on fluoride-regenerated GB from blood BuChE) was 0.04 ± 0.01 mg"min/m^3 (N = 5). The LOAELs for miosis, EEG effects, and visual evoked response (VER) were determined by testing one animal at each of the following concentrations: 7.5, 15, 25, 50, and 150 µg/m^3. Controls (N = 5) were exposed to air for 5 h. For miosis, the LOAEL (10% decrease in pupil size compared with controls; estimated at approximately 20% decrement in pupil area; $p < 0.05$) was reported to be 2.5 ± 0.8 mg"min/m^3. The LOAEL ($p < 0.05$) for changes in EEG parameters was 0.2 mg"min/m^3 (indicative value), and the LOAEL for VER was 25 mg"min/m^3 (indicative value). The blood AChE activity for marmosets was significantly ($p < 0.05$) inhibited at all GB vapor exposure concentrations and exhibited dose dependence. Although van Helden et al. (2001, 2002) reported that the EEG signal appeared to be more sensitive to GB than the eye, they noted that the EEG effects are more complex and concluded that "the miotic response, showing a clear dose-relationship, might therefore be considered at this moment as the most reliable biomarker of exposure to low levels of GB."

Agent GD

Anzueto et al. (1990) reported that inhalation of GD at 13.14 µg/kg (2 times the LD_{50}) by five baboons resulted in cardiac arrhythmias, apnea, and a significant decrease in mean blood pressure. Lipp and Dola (1980) reported that intramuscular injections of 30-75 µg/kg would result in seizure activity and convulsions in female rhesus monkey.

3.2.2. Dogs

Agent GB

Harris et al. (1953) exposed four mongrel dogs in a chamber to an average Ct of 10.5 mg"min/m^3 for an exposure duration of 20 min/d (equivalent to an average concentration of 0.53 mg/m^3), 5 d/wk for 2 mo. The only reported clinical sign was miosis, which appeared with each exposure but disappeared prior to the next exposure. However, when each daily exposure was increased to 15 mg"min/m^3, toxic signs (body tremors, dyspnea, loss of muscle control, convulsions) occurred within 7-10 d and several dogs died. When the Ct was again reduced to 10 mg"min/m^3, all signs but miosis disappeared and RBC-AChE stabilized at a level between zero and 20% of normal in the surviving dogs. Sixty-one percent of the total blood ChE activity in dogs is found in the RBC (Osweiler et al. 1985).

Fogleman et al. (1954) exposed three beagle dogs (average body weight 11.4 kg) to agent GB (sarin) vapors (face-only) for three successive exposure periods (4, 6, and 6 wk) with intervening time periods to allow for complete recovery of RBC-AChE (recovery times not reported). During each test period, the animals were exposed for time periods ranging from 8-24 min/d for 5 d/wk. In the first exposure period (series I), a concentration of 0.24-0.26 mg/m^3 for 8, 16, or 24 min/d for a total of 17-20 exposures produced only mild salivation and rhinorrhea. These effects were thought to be due to the type of mask used on the animals (the Snell dog mask). RBC-AChE activity was not recorded. In series II, in which the Saunders-Fogleman dog mask was used, 39 exposures over 6 wk at a concentration of 0.73-0.75 µg/L (0.73-0.75 mg/m^3) for 8, 16, or 24 min/d produced dyspnea when the daily total amount of agent GB (sarin) retained exceeded 2 µg/kg. One of the three dogs in series II exhibited gluteal muscle fasciculations when the total retained dose reached 64.5 µg/kg (after about 23 exposures). In series III (exposures at 2.38-2.43 mg/m^3 for 8, 12, or 16 min/d and a total of 30 exposures over 6 wk), dyspnea and fasciculations in the region of the gluteal muscle occurred when the daily retained dose exceeded 2 µg/kg. RBC-AChE activity of all three dogs dropped to approximately 65% of normal (percent of preexposure value) after the first exposure. After the fourth exposure, RBC-AChE activity in the dog exposed for 16 min/d dropped to zero while the RBC-AChE activity in the other two dogs was about 35% of normal (range of 32-38% for the 12-min and 8-min exposure animals, respectively). Analysis of expired air allowed

for the estimation of the total amount of agent GB (sarin) retained in the body; average measured retention rates ranged from 78.9% to 84.3% in the series III tests.

Jacobson et al. (1959) exposed male beagle dogs (three per group) at 0.04 mg/m^3, 4 h/d, 5 or 7 d/wk for 6 mo or at 0.50 mg/m^3, 20 min/d, 5 or 7 d/wk for 6 mo. The lowest test concentration of 0.04 mg/m^3 for 4 h/d resulted in decreased RBC-AChE activity (to less than 30% of the baseline values), miosis, dyspnea, increased salivation, and rhinorrhea. The effects were more severe in animals exposed 7 d/wk rather than 5 d/wk and in animals exposed to the higher concentration for 20 min/d rather than the lower concentration for 4 h/d. Miosis persisted throughout the entire 6-mo test period. Jacobson et al. (1959) autopsied two dogs in each exposure group at the end of the 6-mo period and found "some thickening of the musculature of the bronchioles and alveolar ducts ... dilation of the mucous glands in the bronchial trees and some flattening of the epithelium ... (and) ... some emphysematous areas and interstitial pneumonitis." Histopathology of other organs was not reported. Brain ChE activity (measured in one dog per exposure group and in one control at autopsy, by the manometric method of Ammon [1933] as modified by Cohen et al. [1954]) was not significantly affected by the GB exposure except possibly in the dog exposed at 0.5 mg/m^3, 20 min/d, 7 d/wk. In the latter case, brain ChE activity was 45% of the control value. Weekly electrocardiograms (EKGs) did not show any changes in the exposed animals except those that might be associated with hypoxia. Hematological counts showed no significant changes; clinical chemistry was not reported.

Weimer et al. (1979) exposed purebred beagle dogs at 0, 0.0001, or 0.001 mg/m^3 for 6 h/d, 5 d/wk, for up to 52 wk. Four male and eight female beagles were exposed to each test concentration; however, only two females per exposure group were exposed for the full 52-wk period. In the exposed animals, statistically significant changes in RBC-AChE activity occurred occasionally (blood samples drawn after 1 and 2 wk of exposure, and thereafter on a monthly basis). However, these changes did not follow a clear dose-response or duration-response pattern. Two of 12 dogs exposed at 0.0001 mg/m^3 and three of 12 exposed at 0.001 mg/m^3 exhibited abnormal EKGs at the time of sacrifice (one each at 4, 12, and 52 wk and two at 24 wk); elevated P waves were suggestive of right atrial hypertrophy. However, there was no evidence of enlargement or physical abnormalities of the heart. Weimer et al. (1979) noted that the anomalies could have been preexisting conditions. Baseline EKGs, which were available only for four dogs exposed for 2 mo and for four dogs exposed for 36 wk (and

surgically modified to allow for periodic physiological measurements), did not reveal any evidence of EKG abnormalities. The absence of baseline data for the other test animals precludes identifying the reported EKG changes as being caused by the GB exposure, and statistical analysis of the data is not possible because of the small number of test animals (only two animals per exposure group were tested for each exposure duration).

3.2.3. Rats

Agent GB

Kassa et al. (2001) exposed male albino Wistar rats for 60 min in an inhalation chamber once or repeatedly to GB concentrations at 0.8, 1.25, or 2.5 mg/m^3. The lowest concentration (level 1) was reported to be asymptomatic based on clinical and laboratory measurements. The second concentration (level 2) was reported to be asymptomatic based on clinical signs but produced a significant inhibition of RBC-AChE (by 30%). The level 2 concentration was tested using a single exposure or three exposures during 1 wk. The highest test concentration (level 3) was reported to be a nonconvulsive symptomatic exposure. Controls were exposed to pure air only. Three months following the exposure, the control and exposed animals (10 per test group) were evaluated for GB-induced effects using biochemical, hematological, neurophysiological, behavioral, and immunotoxicological methods. None of the exposed animals showed any clinical signs of intoxication 3 mo after exposure; their body weights did not differ significantly from control values, and there were no changes in hematological or biochemical parameters, including blood and brain cholinesterase. Test animals exposed at 0.8 mg/m^3 (level 1) for 60 min showed no alterations in immune function, as measured by in vitro spontaneous or lipopolysaccharides-stimulated proliferation of spleen cells, or by in vitro evaluation of the production of reactive nitrogen intermediates (N-oxides), indicative of bactericidal efficacy of peritoneal macrophages. Level 1 test animals also showed no neurotoxic effects after 3 mo when monitored using a functional observatory battery (FOB) and a test of excitability of the CNS on the basis of the observation of convulsive activity after intraperitoneal administration of pentamethylenetetrazol. The only significant effect ($p < 0.05$) observed in rats exposed once to 1.25 mg/m^3 was an increase in stereotyped behavior. Effects observed in rats exposed three times to 1.25 mg/m^3 included a significant increase ($p < 0.05$)

in the excitability of the CNS, significant alterations of mobility score ($p < 0.01$) and gait disorder ($p < 0.001$) characterized by ataxia, and a significant increase in stereotyped behavior ($p < 0.001$). Animals exposed once to 2.5 mg/m^3 exhibited significant changes in some immune functions ($p < 0.05$), mobility score ($p < 0.01$), activity ($p < 0.01$), gait score ($p < 0.01$), gait disorder ($p < 0.001$), and stereotyped behavior ($p < 0.01$).

Henderson et al. (2000, 2001, 2002), Conn et al. (2002), and Kalra et al. (2002) exposed male F344 rats at 0.0, 0.2, or 0.4 mg/m^3 (nose-only) for 1 h/d for 1 d, 5 d, or 10 d, with sacrifices at 1 d after exposure and at 1 mo after exposure. Tests were conducted under normal temperatures (25 °C) and under heat stress (32 °C) conditions (core body temperature raised 1 °C). Study parameters included overt symptoms of toxicity, body temperature and activity, body weight, breathing patterns, cytokine levels in brain, Con-A-stimulated mitogenesis in splenic lymphocytes, number of cholinergic receptor sites in brain, and apoptosis in brain cells. It is reported that no overt symptoms of neurotoxicity (tremors) occurred at either exposure level after a single, 1-d exposure. Single exposures were associated with little inhibition of RBC cholinesterase activity (inhibition of "7 and 11% for the low- and high-exposure groups, respectively") (Henderson et al. 2002); after the 10-d exposure, RBC-ChE activity was reduced 60% for the high-exposure group. Inhibition of plasma cholinesterase activity in heat-stressed animals was greater (approximately 20%) following a single exposure than after repeated exposures (no significant activity changes after 10 d; p # 0.05) (Henderson et al. 2002). Cholinesterase changes measured after single exposures were not associated with clinical signs. Repeated exposures induced some signs of suppression of the immune system in terms of reduced ability to maintain body temperature, and a dose-dependent reduction in the response of splenic lymphocytes to mitogens was recorded. In addition, dose-dependent induction of cytokine expression (IL-1$, IL-6, and TNF-") was observed in the brain. No signs of increased apoptosis were seen in any of the rats exposed for 1, 5, or 10 d. Further, heat stress in combination with sarin exposure led to an increase in the number of M3 receptor sites in olfactory and adjacent areas of the rat brain. Repetitive heat stress alone reduced body weight gain; sarin exposure did not affect body weights (Henderson et al. 2002).

To better characterize the relationship between miosis and GB vapor exposure concentration and duration, Mioduszewski et al. (2002b) exposed young adult (8-10 wk) male and female Sprague-Dawley rats to GB vapor at a range of concentrations (0.01-0.48 mg/m^3) and three time durations (10,

60, and 240 min). A total of 283 rats (142 female, 141 male) were exposed whole-body "to GB vapor in a 750-L dynamic airflow inhalation chamber" following published protocols (Mioduszewski et al. 2001, 2002a). Including range-finding experiments and controls ($N = 130$), a total of 423 rats were employed in this well-conducted study. Approximately 30 min postexposure, rat pupil diameters were assessed by means of individual examination with a simple microscope fitted with a reticule eyepiece. Blood samples were also collected (24 h preexposure, 60 min postexposure, and 7 d postexposure at sacrifice) from tail vein and heart (postmortem only) for RBC and plasma carboxylesterase (CaE) and cholinesterase activity determinations using a modified Ellman method (Ellman et al. 1961). Animals were also observed for development of clinical signs during 7 d postexposure. The miosis data were used to generate EC_{50} and ECt_{50} values for both genders for each of the three exposure durations (female EC_{50} was 0.068 mg/m^3 for 10 min, 0.020 mg/m^3 for 60 min, and 0.012 for 240 min or 0.68 mg"min/m^3 for 10 min, 1.20 mg"min/m^3 for 60 min, and 2.88 mg"min/m^3 for 240 min) (male EC_{50} was 0.087 mg/m^3 for 10 min, 0.030 mg/m^3 for 60 min, and 0.024 mg/m^3 at 240 min or 0.87 mg"min/m^3 for 10 min, 1.80 mg"min/m^3 for 60 min, and 5.76 mg"min/m^3 at 240 min). The Mioduszewski et al. (2002b) study defined the EC_{50} and ECt_{50} points as the statistical concentration (or cumulative exposure [Ct]) required for postexposure pupil diameters of 50% or less of the pre-exposure pupil diameter in 50% of the exposed population. 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 ($p = 0.054$). Whole-body exposure to GB vapor did not result in significant activity inhibition for any blood enzyme monitored—RBC-AChE, plasma BuChE, or CaE for any GB vapor concentrations and duration tested. The authors conclude that "observable clinical signs associated with whole-body GB vapor exposure can be limited to miosis, even in the absence of significant changes in AChE, BuChE, or CaE activity" (Mioduszewski et al. 2002b, p. 21).

This is the critical study and data set (female Sprague-Dawley rats) for determination of AEGL-1 values for agent GB.

In tests conducted by Cohen et al. (1954), hypertonicity and hyperactivity of the musculature, increased response to stimuli, rigidity, and convulsions were seen in some test animals exposed at 50 mg"min/m^3 (1 mg/m^3 for 50 min, daily). Brain cholinesterase activity became depressed only after erythrocyte cholinesterase activity had dropped to about 30% of the normal levels (after approximately 58 d exposure at a Ct of 75

mg"min/m^3). All rats exposed to an LCt_{50} at 300 mg"min/m^3 for 10 min had brain ChE values below 5% of normal.

Noninhalation studies have demonstrated that single exposures to GB can result in neurobehavioral changes. An intraperitoneal dose of 50 µg/kg resulted in decreases in rearing and grooming behavior and locomotive activity in male Wistar rats (Nieminen et al. 1990). A subcutaneous injection of 61 µg/kg increased spontaneous motor activity in male Sprague-Dawley rats; a dose of 71 µg/kg produced conditioned flavor aversions; 84 and 115 µg/kg caused significant decreases in spontaneous locomotive activity; and doses of 98 and 115 µg/kg resulted in significant decrements in rotorod performance. At exposures #84 µg/kg, no significant effects in rotorod performance were observed (Landauer and Romano 1984). Male Sprague-Dawley rats exposed to a single 100 µg/kg intramuscular dose of GB (LD_{50}) showed significant inhibition of cholinesterase in brain and blood plasma and an increase in choline acetyl transferase activity in cortex and brain stem, but not in the mid-brain (Khan et al. 2000a, b). Olson et al. (2000) reported that subcutaneous doses of GB (once per day for 4 d) sufficient to lower whole blood cholinesterase by 20-30% caused no neurobehavioral or neuropathologic effects in rats. That finding is consistent with what Cohen et al. (1954) reported above.

Young et al. (2001) evaluated the correlation of blood cholinesterase levels with sarin-induced toxicity in female, nonpregnant CD rats (Crl:COBS CD [SD BR Rat Outbred]) treated by gavage with type I sarin at 380 µg/kg once per day for 10 d. Based on the results of previous studies, a dose of 380 µg/kg was expected to result in 30% mortality. Baseline blood cholinesterase values were determined before treatment. After the first dose, there was a drop in plasma cholinesterase which remained low throughout the 10-d test period. A statistically significant correlation ($p < 0.0001$) was found between body weight loss and plasma cholinesterase levels during the period of dosing. However, RBC-cholinesterase levels were not different between control and treated animals. Neither plasma nor RBC-AChE baseline cholinesterase activity levels nor the relative or absolute decline in cholinesterase values could be used as predictors of mortality in the treated animals.

Abu-Qare and Abou-Donia (2001) examined the ability of a single intramuscular dose of agent GB (80 µg/kg) alone, or in combination with a single oral dose of pyridostigmine bromide, to induce markers of oxidative stress. Urine samples of treated and control adult SD rats were collected at various times post-treatment (16-96 h). No increase in the

concentrations of stress markers 3-nitrotyrosine and 8-hydroxy-2'-deoxyguanosine was detected following a single dose of sarin.

Jones et al. (2000) investigated potential subchronic neurotoxic effects of GB concentrations administered in fractions of the LD_{50} dose (intramuscular at 0.01, 0.1, 0.5 , and 1 × LD_{50}) to male Sprague-Dawley rats, after which the rats were maintained for 90 d. Potential changes in blood-brain barrier (BBB) permeability were monitored in the cortex, brainstem, midbrain, and cerebellum; other parameters monitored included plasma butyrylcholinesterase activity as well as m2-selective muscarinic acetylcholine receptor (m2-mAChR) and nicotinic acetylcholine receptor (nAChR) ligand binding. Plasma butyrylcholinesterase activity recovers rapidly and cannot, therefore, serve as a reliable biomarker for potential long-term toxicity of sarin exposure. Ninety days after single sarin exposure, changes in brain regional binding densities of the two receptors were noted; the clinical significance of those changes was not reported.

In a subchronic inhalation study conducted on Fischer 344 rats, no signs of toxicity were observed in animals exposed to GB at 0.0001 or 0.001 mg/m^3 6 h/d, 5 d/wk (excluding holidays), for up to 24 wk (Weimer et al. 1979). In a continuation of these studies, Sprague-Dawley/Wistar (colony) and Fischer 344 rats were exposed at 0, 0.0001, or 0.001 mg/m^3 6 h/d, 5 d/wk, for up to 52 wk (Weimer et al. 1979). Fifty animals of each gender of each strain were exposed to each test concentration, and blood samples were drawn for RBC-ChE determination at the time of sacrifice. During a 3-wk period, the test animals exhibited heat stress due to loss of chamber temperature control (temperatures exceeded 90 °F) and many of the rats died (16 in the low exposure group and 12 in the high exposure group). Fluctuations in blood chemistries (including RBC-AChE) for the exposed animals were no greater than controls, and although statistically significant changes in RBC-AChE occurred occasionally, the changes did not follow a clear dose-response or duration-response pattern. Atrophy of the seminiferous tubules was observed in Fischer 344 rats exposed to GB; however, Weimer et al. (1979) noted that this inbred strain of rat is susceptible to numerous genetically based defects that may appear under experimental conditions of stress. The tests were repeated using the same experimental protocol for 12 and 24 wk, and none of the exposed rats in the second assay exhibited testicular atrophy. A high incidence of tracheitis occurred in both the Fischer rats and in the colony rats exposed to GB. The most severe cases reportedly occurred in the high-exposure group. The incidence of tracheitis in colony rats is summarized in Table 1-18; the results were

analyzed statistically using the Fisher Exact Test (statistical analysis was not provided by Weimer et al. [1979]). Although there were statistically significant differences between exposed and control groups after 4, 8, and 12 wk of exposure, the differences were not significant for longer exposure durations. A similar response was seen in Fischer rats (i.e., a few cases of tracheitis early in the study, but none in animals exposed for 52 wk).

Tracheitis was often common in animal colonies during the time of the Weimer et al. (1979) study and is now considered reflective of incomplete infectious-disease control in the colony. This evidence of disease, coupled with the loss of chamber temperature control and subsequent heat-stress deaths of test animals, compromise the results and disqualify Weimer et al. (1979) from use as a critical study for AEGL estimation.

Agent GD

Walday et al. (1991) exposed male Wistar rats to GD at 0.05 or 0.2 mg/m^3 for a single 40-h period. No clinical signs of toxicity were seen during the exposures. Acetylcholinesterase and butyrylcholinesterase were significantly inhibited in all tissues except the brain.

Agent GF

A recent study of lethal GF vapor exposure toxicity in male and female SD rats also reported sublethal clinical signs of tremors, convulsions, salivation, and miosis following whole-body dynamic chamber exposures (Anthony et al. 2002). Blood samples were also drawn for BuChE activity determinations. A range of near-lethal vapor concentrations were employed for three exposure durations (10, 60, and 240 min). Because the experimental protocol was designed for LC_{50} lethality-effects determination, effective concentration determinations (EC_{50}, ECt_{50}) for nonlethal effects were not estimated by Anthony et al. (2002). Miosis was observed in all exposed rats during the first hour postexposure; the effect was reversible

TABLE 1-18 Incidence of Tracheitis in Colony Rats Exposed to Agent GB[a]

	Exposure Group		
Exposure Period	Control	0.0001 mg/m^3	0.001 mg/m^3
4 wk	0/10	5/10[b]	0/10
8 wk	0/10	4/10[b]	9/10[c]
12 wk	0/10	5/8[c]	5/7[c]
16 wk	0/9	0/10	1/10
20 wk	0/10	0/5	2/6
24 wk	1/10	1/5	0/6
36 wk	0/9	2/5	2/7
52 wk	2/10	1/10	6/10
6 mo	0/19	7/19[c]	9/28[c]

[a]Statistical analysis using the Fisher Exact Test.
[b]Significantly different from control, $p < 0.05$.
[c]Significantly different from control, $p < 0.01$; the postexposure population was made up of groups of each rodent strain held for 6-mo observation after the experimental exposure period ended.
Source: Weimer et al. 1979.

and pupil sizes were normal at 14 d postexposure. Preliminary analysis of BuChE activity indicates statistically significant depression "immediately after exposure" and statistically significant elevations at 14 d postexposure to near-lethal vapor concentrations; at neither time period is the BuChE delta correlated with cumulative exposure (Ct) (Anthony et al. 2002).

Agent VX

Crook et al. (1983) conducted VX vapor exposure studies in male and female Sprague Dawley rats. Crook and his colleagues consider their results to be nonverifiable and suspect for the reasons outlined earlier. These data are thus considered too unreliable for any application to development of AEGL estimates for agent VX.

3.2.4. Mice

Agent GB

Signs suggestive of delayed neuropathy have been observed in female Swiss albino mice ($N = 6$) exposed to GB at 5 mg/m^3 for 20 min daily for 10 d (Husain et al. 1993). Muscular weakness of the limbs and slight ataxia occurred on the day 14 after the start of the exposures (the number of animals showing these effects was not specified). Significant ($p < 0.001$) inhibition of NTE activity in the brain (59.2%), spinal cord (47.4%), and platelets (55.4%) was observed in the test animals ($N = 6$). Histological examination of the spinal cord revealed focal axonal degeneration that was reported to be moderate in two animals and light in four. The same exposure inhibited blood AChE by 27.3% and brain AChE by 19.2% but was not associated with any anti-AChE symptoms. The LCt_{50} for this strain of mice was reported to be 600 mg"min/m^3 (Husain et al. 1993), presumably for a 1-min exposure.

Agent VX

Crook et al. (1983) conducted VX vapor exposure studies in male and female ICR mice. Crook and his colleagues consider their results to be nonverifiable and suspect for the reasons outlined earlier. The data are thus considered too unreliable for any application to development of AEGL estimates for agent VX.

3.2.5. Guinea pigs

Agent GB

Van Helden et al. (2001, 2002) exposed male Dunkin-Hartley albino (HSD-Harlan [Harlan]) guinea pigs (whole-body) to GB vapor concentrations at 0.05 to 150 µg/m^3 for 5 h. The lowest cumulative exposure at which the internal dose became measurable (based on fluoride-regenerated GB from blood BuChE) was 0.010 ± 0.002 mg"min/m^3 ($N =$ 12). The LOAELs for miosis, EEG effects, and visual evoked response (VER) were evaluated at 7.5, 15, 25, 50, and 150 µg/m^3 ($N = 2$ per group).

Controls ($N = 6$) were exposed to air for 5 h. For miosis, the LOAEL (5% decrement in pupil size compared with controls; estimated to be equivalent to approximately 10% decrement in pupil area; $p < 0.05$) was reported to be 1.8 ± 0.3 mg"min/m^3. The LOAEL ($p < 0.05$) for changes in EEG parameters and VER was 0.8 mg"min/m^3 (indicative value). There was no significant decrease in blood AChE activity at any GB vapor exposure concentration tested.

Atchison et al. (2001) reported that subcutaneous injections of 0.4 LD_{50} GB once per day, 5 d/wk, for 13 wk in young male Hartley guinea pigs (600 g) resulted in no clinical signs of acute toxicity and no changes in body weight, body temperature, complete blood counts, or blood chemistry; however, RBC-ChE activity was decreased by about 90%. The subcutaneous LD_{50} for guinea pigs was reported to be 42 µg/kg.

Agent GD

Benschop et al. (1998) evaluated the toxicokinetics and effects of single inhalation exposures of the four stereoisomers of soman to guinea pigs. The test animals (male albino outbred guinea pigs of the Dunkin-Hartley type, 450-620 g body weight) were anesthetized and atropinized and then exposed, nose-only, for 5 h to each of the four stereoisomers, at a concentration of 20 ppb (160 ± 16 µg/m^3). During the exposure there was a gradual increase in the inhibition of RBC-AChE, which correlated well with the increase in the concentration of the toxic stereoisomers (C(±)P(-) soman) in the blood. Inhibition of AChE in the brain and diaphragm was not significant at the end of the exposure period.

Atchison et al. (2001) reported that subcutaneous injections of 0.4 LD_{50} GD once per day, 5 d/wk, for 13k w in young male Hartley guinea pigs (600 g) resulted in no clinical signs of acute toxicity, no agent related pathology, and no change in blood chemistry other than a 91% inhibition of RBC-ChE. The subcutaneous LD_{50} for guinea pigs was reported to be 28 µg/kg.

Agent VX

Atchison et al. (2001) reported that subcutaneous injections of 0.2 LD_{50} VX once per day, 5 d/wk, for 13 wk in young male Hartley guinea pigs (600 g) resulted in no clinical signs of acute toxicity and no changes in body weight, blood count, blood chemistry of gross, or histopathology; however,

RBC-ChE activity was inhibited about 90%. The subcutaneous LD_{50} for guinea pigs was reported to be 9 μg/kg.

Crook et al. (1983) conducted VX vapor exposure studies in male and female Hartley guinea pigs. Crook and his colleagues consider their results to be nonverifiable and suspect for the reasons outlined earlier. These data are thus considered too unreliable for application to development of AEGL estimates for agent VX.

3.2.6. Rabbits

Callaway and Dirnhuber (1971) performed a study in which pupil area decrement was measured from electronic flash photographs of dark-adapted eyes for which baseline pupil area had been previously determined. The nominal cumulative exposure (Cts) necessary to produce 50% and 90% decrement in total pupil area were determined and compared for GB vapor (46 eye measurements from 14 rabbits), GD vapor (153 eye measurements from 48 rabbits), and T-2715 (GF analog) vapor (85 measurements on 19 rabbits). The cumulative exposure needed to produce miosis sufficient to generate 90% pupil area decrement was 2.71 mg"min/m^3 (95% CI = 1.84-4.00 mg"min/m^3) for agent GB, 2.19 mg"min/m^3 (95% CI = 1.45-3.29 mg"min/m^3) for agent GD, and 1.79 mg"min/m^3 (95% CI = 1.40-2.29 mg"min/ m^3) for agent GF. The cumulative exposure needed to produce miosis sufficient to generate 50% pupil area decrement was 1.32 mg"min/m^3 (95% CI = 1.05-1.67 mg-min/m^3) for agent GB, 0.59 mg-min/m^3 (95% CI = 0.49-0.70 mg"min/m^3) for agent GD, and 0.75 mg"min/m^3 (95% CI = 0.65-0.87 mg"min/m^3) for agent GF.

Callaway and Dirnhuber (1971) also evaluated the "miotogenic potency" of GB vapor in rabbits exposed to GB "under goggles" (43 miosis responses in 10 albino rabbits). 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 was delivered to the space enclosed by each goggle. The unexposed pupil area of each eye was considered to be the baseline for pupil area decrement determinations for each eye. Exposure periods ranged from 10 min to 5 h. Callaway and Dirnhuber (1971) reported a 50% decrement of pupil area in the rabbit dark-adapted eye (goggles) at a Ct of 2.33 mg"min/m^3 (95% CI

= 1.65-3.31 mg"min/m^3). A 90% decrement of pupil area occurred at a Ct of 7.68 mg"min/m^3 (95% CI = 4.90-19.50 mg"min/m^3).

Agent VX

Crook et al. (1983) conducted VX vapor exposure studies in male and female New Zealand white rabbits. Crook and his colleagues consider their results to be nonverifiable and suspect for the reasons outlined earlier. These data are thus considered too unreliable for any application to development of AEGL estimates for agent VX.

In tests conducted by Goldman et al. (1988), blood cholinesterase levels were monitored in female rabbits (three per dose group) injected subcutaneously with VX at 0, 0.25, 1.0, 4.0, or 8.0 µg/kg once per day for 7 d. The 8.0 µg/kg dose was severely toxic (1/3 died, 2/3 ataxic). RBC-ChE activity was inhibited to 0.71 of the control value in the 0.25-µg/kg group, to 0.36 of the control value in the 1-µg/kg group, and to 0.24 of the control value in the 4.0-µg/kg group.

In a study of miotogenic potency, Callaway and Dirnhuber (1971) exposed the eyes of male and female "albino" rabbits (N = 45; no strain identified; 94 observations) to concentrations of VX agent vapor ranging from approximately 0.5 µg/m^3 to 25 µg/m^3 for varying time periods (approximately 2-400 min). Pupil diameters were recorded only after attaining maximal decrease, and decrease in pupil area per Ct was expressed as a percentage of the original area of the same eye. Maximal pupil diameter decrease usually occurred at times >30 min postexposure. The "percentage decrease" data underwent probit transformation to derive Cts necessary to produce 50% and 90% decrease in pupil area in the dark-adapted eye. For comparison, experimental exposures to nerve agents GB and GD under a similar protocol were also performed by the authors (Callaway and Dirnhuber 1971). Their results are reported in Table 1-19 below.

3.2.7. Summary of Nonlethal Toxicity in Animals

The summary of animal toxicity data has focused on short-term, subchronic, or chronic exposures to agent GB (Table 1-20). Results of inhalation exposure studies are emphasized; however, some pertinent data for other exposure pathways are included.

TABLE 1-19 Miosis in Rabbits Following Vapor Exposure to Agents VX, GB, and GD

Agent	50% Pupil Area Decrease ($mg \cdot min/m^3$)	95% CI	90% Pupil Area Decrease ($mg \cdot min/m^3$)	95% CI	Slope (*b*)
VX	0.04	0.03-0.05	0.23	0.12-0.45	1.70
GB	1.32	1.05-1.67	2.71	1.84-4.00	4.11
GD	0.59	0.49-0.70	2.19	1.45-3.29	2.24

Source: Callaway and Dirnhuber 1971.

The miotogenesis studies of GB vapor exposure recently published by van Helden et al. (2001, 2002) (male and female marmosets and male guinea pigs) and Mioduszewski et al. (2002b) (male and female SD rats) were well conducted, employed modern protocols, and examined a range of exposure durations significant to the AEGL process. Mioduszewski et al. (2002b) is the critical study for deriving AEGL-1 values for agent GB; van Helden et al. (2001, 2002) is a secondary and supportive study.

3.3. 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 system. 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 apparently affect nerve impulse transmission by more direct processes as well (for example, direct effects on muscarinic receptors) (see Section 4.2).

As described in Section 3.2.3, Kassa et al. (2001) evaluated the neurotoxic effects of agent GB in male albino Wistar rats exposed for 60 min, once or repeatedly, to concentrations at 0.8, 1.25, or 2.5 mg/m^3. The lowest concentration was determined asymptomatic based on clinical and laboratory measurements. The second concentration was determined asymptomatic based on clinical signs, but produced a significant inhibition of RBC-AChE (30%). The highest test concentration was a nonconvulsive

TABLE 1-20 Nonlethal Toxicity of Agent GB Vapor to Animals[a]

Species	Exposure	Duration	End Point	Comments	Reference
Dog	10.5 mg·min/m^3 20 min/d	2 mo	LOAEL	Miosis	Harris et al. 1953
Dog	15 mg·min/m^3 20 min/d	7-10 d[b]	LOAEL	Body tremors, dyspnea, loss of muscle control, convulsions	Harris et al. 1953
Dog	0.24-0.26 mg/m^3; 8, 16, 24 min/d	17-21 times over 4 wk	NOAEL	Nose only exposures; no reported toxic signs; ChE was not monitored	Fogleman et al. 1954
Dog	0.73-0.75 mg/m^3; 8, 16, 24 min/d	30 times over 6 wk	LOAEL	Dyspnea, gluteal muscle fasciculations in one of three test animals	Fogleman et al. 1954
Dog	2.38-2.43 mg/m^3; 8, 12, 16 min/d	30 times over 6 wk	LOAEL	Dyspnea; gluteal muscle fasciculations; RBC-ChE levels 0, 35%, and 35% of normal after 4 d	Fogleman et al. 1954
Dog	0.04 mg/m^3 4 h/d, 5 d/wk	6 mo	LOAEL	Decreased RBC-ACHE; dyspnea, salivation, rhinorrhea, miosis	Jacobson et al. 1959
Dog	0.001 mg/m^3 6 h/d, 5 d/wk	52 wk	NOAEL	Abnormal EKGs in some dogs; however, baseline measurements were not available for all the test animals	Weimer et al. 1979
Rabbit	1.32 mg·min/m^3	10 min to 5 h	ECt_{50}	50% miosis	Callaway and Dirnhuber 1971

Rabbit	2.71 mg·min/m^3	10 min to 5 h	ECt_{50}	90% miosis	Callaway and Dirnhuber 1971
Guinea pig	0.8 mg·min/m^3	5 h	LOAEL	EEG changes and visual evoked response	van Helden et al. 2001, 2002
Guinea pig	1.8 mg·min/m^3	5 h	LOAEL	Miosis	van Helden et al. 2001
Marmoset	0.2 mg·min/m^3	5 h	LOAEL	EEG changes	van Helden et al. 2001, 2002
Marmoset	2.5 mg·min/m^3	5 h	LOAEL	Miosis	van Helden et al. 2001, 2002
Marmoset	25 mg·min/m^3	5 h	LOAEL	Visual evoked responses	van Helden et al. 2001, 2002
Mouse	5 mg/m^3, 20 min/d	10 d	LOAEL	Muscular weakness of the limbs and slight ataxia; inhibition ($p <$ 0.001) of NTE activity in the brain (59.2%), spinal cord (47.4%), and platelets (55.4%); focal axonal degeneration of spinal cord; blood AChE inhibited by 27.3% and brain AChE by 19.2%	Husain et al. 1993
Rat	0.068 mg/m^3 (female)	10 min	EC_{50}	Miosis	Mioduszewski et al. 2002b

(Continued)

TABLE 1-20 *Continued*

Species	Exposure	Duration	End Point	Comments	Reference
Rat	0.020 mg/m^3 (female)	60 min	EC_{50}	Miosis	Mioduszewski et al. 2002b
Rat	0.012 mg/m^3 (female)	240 min	EC_{50}	Miosis	Mioduszewski et al. 2002b
Rat	0.087 mg/m^3 (male)	10 min	EC_{50}	Miosis	Mioduszewski et al. 2002b
Rat	0.030 mg/m^3 (male)	60 min	EC_{50}	Miosis	Mioduszewski et al. 2002b
Rat	0.024 mg/m^3 (male)	240 min	EC_{50}	Miosis	Mioduszewski et al. 2002b
Rat	0.8 mg/m^3	60 min	NOAEL	Asymptomatic	Kassa et al. 2001
Rat	1.25 mg/m^3	60 min	NOAEL	Asymptomatic but with significant inhibition of RBC-ChE	Kassa et al. 2001
Rat	2.5 mg/m^3	60 min	LOAEL	Changes in immune system and neurobehavioral effects	Kassa et al. 2001
Rat	0.4 mg/m^3 1 h/d	1 d	NOEL	No overt neurotoxicity (tremors) observed	Henderson et al. 2000, 2001, 2002
Rat	0.001 mg/m^3 6 h/d, 5 d/wk	24 wk	NOAEL	No observed inhibition of blood ChE	Weimer et al. 1979

Rat	0.001 mg/m^3 6 h/d, 5 d/wk	52 wk	NOAEL	No observed inhibition of blood ChE; tracheitis occurred in some animals (see text); atrophy of the seminiferous tubules was not considered to be agent-related	Weimer et al. 1979

[a]Experimental data.

[b]Following a 2-mo exposure to a Ct of 10.5 mg·min/m^3.

symptomatic exposure. Controls were exposed to pure air only. Three months following the exposure, the control and exposed animals (10 per test group) were evaluated for GB-induced effects using biochemical, hematological, neurophysiological, behavioral, and immunotoxicological methods. None of the exposed animals showed any clinical signs of intoxication 3 mo after exposure. Test animals exposed to GB at 0.8 mg/m^3 exhibited no neurotoxic effects after 3 mo, when monitored using a functional observatory battery (FOB) and a test of excitability of the CNS, on the basis of observation of convulsive activity after intraperitoneal administration of pentamethylenetetrazol. The only significant effect ($p < 0.05$) observed in rats exposed once to GB at 1.25 mg/m^3 was an increase in stereotyped behavior. Effects observed in rats exposed three times at 1.25 mg/m^3 included a significant increase ($p < 0.05$) in the excitability of the CNS, significant alterations of mobility score ($p < 0.01$), gait disorder ($p < 0.001$) characterized by ataxia, and a significant increase in stereotyped behavior ($p < 0.001$). Animals exposed once at 2.5 mg/m^3 exhibited significant changes in mobility score ($p < 0.01$), activity ($p < 0.01$), gait score ($p < 0.01$), gait disorder ($p < 0.001$), and stereotyped behavior ($p < 0.01$).

3.4. Developmental and Reproductive Effects

Due to the limited database for evaluating developmental or reproductive effects of nerve agent vapor inhalation exposure, other exposure routes were also examined.

3.4.1. Rats

Agent GB

The reproductive and developmental toxicity of GB was evaluated in a pilot study in which Sprague-Dawley rats were exposed to GB vapors (Denk 1975). In one series of inhalation tests, male rats were exposed to GB at 0.1 or 1 μg/m^3 for 6 h/d, 5 d/wk, for 1, 2, 8, or 12 wk or 6, 9, or 12 mo and then mated to unexposed females. Nineteen days after mating, the females were sacrificed and examined for number of corpora lutea, deciduomata, number of fetal deaths, and number of live fetuses. Mated pairs of rats were also exposed to the same GB concentrations for 1, 2, or

3 wk or until the pups were whelped. The incidence of intrauterine deaths was recorded and all fetuses were examined for abnormalities. In a third series of tests, males and females were exposed to agent GB (sarin) for 10 mo and then mated. The F_1 generation was mated at 12 wk of age, as was the F_2 generation. The number and gender of offspring, number of preweaning deaths, number weaned, and pup weights at various ages were recorded. Denk (1975) reported reduced rates of whelping in the F_0 generation, but reduced whelping rates were also seen in the controls, and this effect was thought to be due to the age of the animals at mating (12 mo old). No other adverse effects with respect to dominant lethal mutations, reproductive performance, fetal toxicity, and teratogenesis were observed.

Oral exposure studies in laboratory animals indicate that developmental or reproductive effects are not likely, even at dose levels that are maternally toxic. LaBorde and Bates (1986) (see also LaBorde et al. [1996]) conducted developmental toxicity studies on agent GB type I and GB type II using CD rats. The test animals were dosed with 0, 100, 240, or 380 µg/kg orally on days 6-15 of gestation. Females were weighed on gestational day 0, gestational days 6-16, and before death on gestational day 20. The test animals were observed for clinical signs of toxicity. At sacrifice, gravid uteri were weighed and examined for number and status of implants (alive, resorbed, or dead). Individual fetal body weight and internal or external malformations were recorded. Maternal toxicity (evidenced by excessive salivation, ataxia, lacrimation) and mortality (8/29 for GB type I and 13/29 for GB type II) occurred in the high-dose group. There were no significant differences among treatment groups in the incidence of resorptions or in the average body weight of live fetuses per litter. The only fetal morphological anomaly was fetal hydroureter, which occurred at a rate of 5.2%, 1.9%, 5.3%, and 2.1% with GB type I; and 4%, 5%, 3.2%, and 0.5% with GB type II in the 0-, 100-, 240-, and 300-µg/kg dose groups, respectively. The observed effect was not dose related and was therefore considered a spontaneous variant. Skeletal and cartilage variants occurred between dose groups, but they were not statistically significant.

Agent GA

There are intraperitoneal and subcutaneous exposure studies of agent GA in which developmental and reproductive toxicity were studied in maternal CD rats (Bucci et al. 1993). In both studies, the LOAEL for ma-

ternal toxicity (salivation, lacrimation, nasal discharge, diarrhea) was attained in the absence of fetal malformations or adverse effects on fetal implantations or fetal weight.

Agent GD

Developmental studies in maternal rats orally exposed to agent GD (soman) were reported by Bates et al. (1990); the protocol was the same as that employed in the GB oral exposure studies of LaBorde and Bates (1986) and LaBorde et al. (1996) reported earlier. At doses that produced significant maternal toxicity and mortality, there was no evidence of fetal toxicity or prenatal mortality as evidenced by postimplantation loss, average body weight of live fetuses per litter, or malformations (Bates et al. 1990).

Agent VX

In studies conducted by Schreider et al. (1984), pregnant rats were dosed with VX at 0.25, 1.0, or 4.0 µg/kg by subcutaneous injection on days 6-15 of gestation (doses higher than 4.0 µg/kg were expected to cause excessive deaths). The animals were sacrificed on day 20 of gestation. The examined fetuses showed no evidence of malformations. Fetal body weight, litter size, and gender ratio were within normal limits.

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

In a modified dominant lethal study, Goldman et al. (1988) administered VX by subcutaneous injection to male and/or female Sprague-Dawley rats and observed the effects on various parameters including terminal body weight, testes weight, testicular histopathology, maternal weight, implantation sites, resorptions, and total corpora lutea. The test animals were dosed with VX at 0 (saline control), 0.25, 1.0, or 4 µg/kg/d for 10 wk. Triethylenemelamine was used as a positive control. Exposure to VX produced no significant changes in body or organ weights. VX had no adverse effects on pre-implantation losses as evaluated by number of im-

plants, live fetuses, dead fetuses, and resorptions. Microscopic examination of the testes did not reveal any abnormalities that could be attributed to VX exposure.

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

sion of the investigators was that there were no organ-weight or microscopic changes that could be attributed specifically to the action of VX.

3.4.2. Guinea Pigs

Pregnant guinea pigs were administered GD orally (7 µg/kg/d) on gestation days 42, 43, and 44 (Mehl et al. 1994). It had been determined prior to the study that a dose of 13 µg/kg, while tolerated by nonpregnant females, was "highly toxic" to pregnant animals. The administered dose of GD caused no significant change in brain weight of neonates, the end point of concern, or in total body weight.

3.4.3. Rabbits

Agent GB

LaBorde and Bates (1986) (see also LaBorde et al. [1996]) conducted developmental toxicity studies on agent GB type I and GB type II using New Zealand rabbits. The same protocol as previously outlined for the rat oral studies by these same investigators (see Section 3.4.1) was employed in the rabbit study. The test animals were dosed with GB at 0, 5, 10, or 15 µg/kg orally on days 6-19 of gestation. No fetal toxicity or teratogenicity was observed. The only observed fetal anomaly was retinal folding, which occurred at a rate of 6.8%, 3.9 %, 4.3 %, and 7.4% for GB type I and 17%, 18%, 25%, and 19% for GB type II in the 0-, 5-, 10-, and 15-µg/kg dose groups, respectively. The frequency of the anomaly was not dose-related and was, therefore, considered to be a spontaneously occurring malformation. Maternal toxicity, evidenced by excessive salivation, ataxia, and lacrimation, occurred at the highest dose.

Agent GA

The developmental and reproductive toxicity of GA was studied in maternal New Zealand white rabbits dosed intraperitoneally or subcutaneously (Bucci et al. 1993). In both studies, the LOAEL for maternal toxicity

(salivation, lacrimation, nasal discharge, diarrhea) was attained in the absence of fetal malformations or adverse effects on fetal implantations or fetal weight.

Agent GD

Developmental studies in maternal rabbits orally exposed to agent GD (soman) were reported by Bates et al. (1990); the protocol was the same as that employed in the GB oral exposure studies of LaBorde and Bates (1986) and LaBorde et al. (1996) reported earlier. At doses that produced significant maternal toxicity and mortality, there was no evidence of fetal toxicity or prenatal mortality as evidenced by post-implantation loss, average body weight of live fetuses per litter, or malformations (Bates et al. 1990).

Agent VX

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

3.4.4. Sheep

Agent VX

The effects of VX on the development and reproduction of sheep were evaluated by Van Kampen et al. (1970) following an accidental release of VX in Skull Valley, Utah. Of some 6,300 affected animals, about 4,500

died or were killed (Van Kampen et al. 1970). Seventy-nine surviving animals pregnant at the time of exposure, and their lambs, were evaluated for changes in RBC-AChE activity and for signs of toxicity over a 6-mo postexposure period. RBC-AChE activity in the ewes remained significantly depressed for about 4 mo and then returned to normal. Ewes that were sacrificed at 2-wk intervals had no gross or microscopic evidence of damage to the central nervous system. Torticollis (wryneck) developed in one ewe 1 wk following exposure and persisted for 9 mo. (Van Kampen et al. [1970] reported that a similar effect was seen in one of 38 ewes dosed in the laboratory with an undisclosed amount of VX.) Of the lambs born 2-3 mo after the exposure of the ewes, only one (total number examined not reported) exhibited a deformity (extra oral opening below the right ear), but Van Kampen et al. believed the anomaly originated developmentally and before the poisoning episode. None of the lambs displayed neurotoxic signs or symptoms, and their whole blood cholinesterase activity was not reduced even when suckling from exposed and affected ewes. Five months after exposure, the ewes exposed in the field as well as ewes dosed with an undisclosed amount of VX 4 mo prior were mated to unexposed males. Examination 4 mo later indicated that fetal growth and development were normal except for one fetus that appeared stunted (total number examined not reported). The investigators concluded that VX had little or no effect on fetal growth or development.

3.4.5. Summary

Animal data from vapor and oral exposure studies for agent GB suggest that agent GB does not induce reproductive or developmental effects in mammals. Oral exposure studies of agents GA and GD in laboratory animals as well as injection exposure studies of agent GA suggest the lack of reproductive or developmental effects for these agents. Available data indicate that agent VX does not cause reproductive or developmental effects.

3.5. Genotoxicity

Agent GB

In bioassays using bacteria and mammalian cell cultures, agent GB was

not genotoxic or mutagenic when tested with or without metabolic activation (Goldman et al. 1987). GB did not induce biologically significant increases in mutations (e.g., highest concentration tested failed to exceed a doubling of the spontaneous rate) when tested in the Ames *Salmonella* assay using five revertant strains (TA135, TA100, TA98, TA1537, and TA1538) (Goldman et al. 1987). GB type I and GB type II did not induce significant increases in forward mutations when tested on mouse L5178Y lymphoma cells at concentrations of 50, 100, or 200 µg/mL (Goldman et al. 1987). An increase in sister chromatid exchanges (SCE) was not observed in Chinese hamster ovary cells exposed in vitro to GB at 200 µg/mL (Goldman et al. 1987). Mice treated in vivo with a maximally tolerated intraperitoneal dose of GB at 360 µg/kg did not exhibit a significant increase in SCE in splenic lymphocytes (Goldman et al. 1987). Exposure of rat hepatocytes to GB concentrations as high as 2.4×10^{-3} M resulted in a decrease in DNA repair synthesis, leading Goldman et al. (1987) to conclude that GB probably did not damage DNA directly but that it might inhibit DNA synthesis after non-agent-induced DNA damage had occurred.

Agent GA

Genotoxicity and mutagenicity data for agent GA are available from microbial assays and in vitro and in vivo tests on laboratory animals (Wilson et al. 1994). GA was found to be weakly mutagenic in eight of 11 Ames *Salmonella* assays using the revertant strains TA98, TA100, TA1535, and TA1538 and S-9 activation. GA also induced dose-related increases in mutation rates when tested on mouse L5178Y lymphoma cells without metabolic activation; the increase observed at a test concentration of 100 µg/mL was nearly 3 times that of the control. An increase in sister chromatid exchanges (SCE) was observed in Chinese hamster ovary cells exposed in vitro to GA concentrations at 25-200 µg/mL. Dose-responses were linear and highly statistically significant; however, the number of SCEs did not exceed twice the control value at any of the concentrations tested. C57B1/6 mice treated in vivo with a maximally tolerated intraperitoneal dose of GA at 700 µg/kg did not exhibit a significant increase in SCE in splenic lymphocytes. Exposure of rat hepatocytes to GA concentrations as high as 200 µg/mL resulted in inhibition of unscheduled DNA synthesis. From the results of these studies (i.e., three positive responses in five assays), Wilson et al. (1994) concluded that GA was a weakly acting mutagen.

Agent VX

In tests on microorganisms and mammalian cell cultures, VX was not found to be mutagenic or was only weakly mutagenic (Crook et al. 1983; Goldman et al. 1988). Crook et al. (1983) reported that VX gave negative results when tested in the mouse micronucleus assay (exposures for 6 h/d for 9 d to VX at 0.002 mg/m^3) and when tested in the Ames assay with five strains of *Salmonella typhimurium* (compared with positive controls; no other data reported). VX did not induce biologically significant increases in mutations when tested in the Ames *Salmonella* assay using five revertant strains (TA135, TA100, TA98, TA1537, and TA1538) with and without metabolic activation (Goldman et al. 1988). In tests using the yeast *Saccharomyces cerevisiae*, VX did not induce recombinants following exposures to concentrations as high as 100 µg/mL (Goldman et al. 1988). VX also failed to induce forward mutations when tested on mouse L5178Y lymphoma cells at concentrations less than 50 µg/mL (Goldman et al. 1988). Although doses of VX at 50 and 100 µg/mL resulted in increased numbers of mutations; these were not more than 1.5 times the control level. (A 2-fold increase was considered the minimum required to establish a positive result.)

Crook et al. (1983) reported that VX gave negative results for mutagenicity when tested in the sex-linked recessive lethal assay using *Drosophila melanogaster*.

Summary

Agents GB and VX were not found to be genotoxic in a series of microbial, cellular and mammalian assays. Agent GA was reported to be weakly mutagenic in some microbial assays.

3.6. Carcinogenicity

Agent GB

As part of the chronic inhalation studies conducted by Weimer et al. (1979), the tissues of animals exposed to GB for up to 1 y were examined for microscopic lesions including tumors. The test species included ICR

Swiss mice, strain-A mice, Sprague-Dawley/Wistar rats, Fischer 344 rats, and purebred beagle dogs. The exposures were to GB at 0.0001 or 0.001 mg/m^3 6 h/d, 5 d/wk. Weimer et al. (1979) reported that agent-related tumors did not occur in any of the exposed species. Pulmonary tumors did occur in strain-A mice; after 52 wk of exposure, pulmonary adenomas were present in 3/19 animals exposed to GB at 0.0001 mg/m^3, in 3/20 animals exposed to GB at 0.001 mg/m^3, and in 0/20 controls. For animals maintained for 6 mo postexposure, the incidence rates for pulmonary adenocarcinomas were 5/19, 6/18, and 9/29, respectively. However, these lesions were not considered to be agent-related. Strain-A mice have a high natural propensity to form pulmonary tumors; the incidence of spontaneous pulmonary tumors being about 53% in animals 12 mo of age and 90% in animals 18 mo of age (Heston 1942). Overall, the studies of Weimer et al. (1979) indicate that agent GB is not carcinogenic.

Agent GA

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

Agent VX

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

Summary

There is no evidence that agents GB, GA, or VX are carcinogenic. It is noted that a 90-d study, such as that performed by Bucci et al. (1992) for agent GA, is of insufficient duration to fully evaluate tumor incidence rates.

3.7. Summary

G Agents

Acute lethality data for inhalation exposures to the G agents are available in the form of LCt_{50} values for exposure times of 10 min or less. In only one published study was information presented from which a lethality threshold could be estimated for agent GD (Aas et al. 1985). Acute inhalation studies on rats exposed to agent GB vapor for the time periods of 10, 30, 90, 240, and 360 min have been conducted by the U.S. Army's Edgewood Chemical Biological Center (ECBC) at Aberdeen Proving Ground, Maryland (Mioduszewski et al. 2000, 2001, 2002a). Mioduszewski et al. (2000, 2001, 2002a) is the critical study for deriving AEGL-3 values for agent GB. Nonlethal toxicity studies conducted primarily on dogs indicate that low concentrations of the G agents may cause 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.

Animal data from vapor and oral exposure studies suggest that agent GB does not induce reproductive or developmental effects in mammals. Oral exposure studies of agents GB and GD in lab animals as well as injection exposure studies of agent GA suggest the lack of reproductive or development effects for these agents. Agent GB was not 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 that agents GB and GA are carcinogenic.

Agent VX

Credible acute lethality data for vapor inhalation exposure to agent VX vapors are available for only two species (mice and goats) (Koon et al. 1960, as cited in NRC 1997). LCt_{50} values are 13.6 $mg \cdot min/m^3$ for mice and 9.2 $mg \cdot min/m^3$ for goats. In a short-term inhalation study, no signs of toxicity except miosis were seen in rats, mice, guinea pigs, or rabbits exposed to VX vapor concentrations at 0.0002 mg/m^3 or less (6 h/d, 5 d/wk, for 2 wk) (Crook et al. 1983).

The available data indicate that VX does not cause reproductive or developmental toxicity. There is no evidence suggesting that VX is genotoxic or carcinogenic.

4. SPECIAL CONSIDERATIONS

4.1. Metabolism, Toxicokinetics, and Disposition

4.1.1. Absorption

Although nerve agents may be absorbed through any body surface, the route through which absorption is most rapid and complete is the respiratory tract. It has been reported that as much as 70% of an inhaled dose of agent GB is retained in guinea pigs, dogs, and monkeys (Oberst 1961). In studies conducted on human volunteers, Oberst et al. (1968) found that mean percent retention of an inhaled dose of agent GB ranged from about 80% to 90%. Resting men (minute volume 6.9-7.9 L/min) retained a similar percent of the inhaled dose regardless of whether they were breathing exclusively through the mouth or nose; however, exercising men (minute volume 42.5 L/min) retained a significantly lower percentage (80%). Toxicity studies on nonhuman primates indicate that the intravenous and inhalation dose levels producing a similar level of effect are about the same, also suggesting that absorption through the respiratory tract may be close to 100% of the inhaled dose (Johnson et al. 1988; Anzueto et al. 1990). However, in species such as rodents that are nasal breathers, a proportionally greater amount of toxicant may be removed before reaching the lungs (the mechanisms of removal are thought to be hydrolysis or a reaction with epithelial tissues). In guinea pigs, Allon et al. (1998) found that approximately 29% of an inhaled dose of a racemic mixture of agent GD (soman) reached the blood.

4.1.2. Toxicokinetics

Spruit et al. (2000) conducted toxicokinetic studies of (±) sarin in anesthetized, atropinized, restrained guinea pigs. The test animals were exposed (nose-only) to doses corresponding to 0.4 and 0.8 LCt_{50} for 8-min exposure times. Toxicokinetics was also studied after an intravenous bolus corresponding to 0.8 LD_{50}. The LC_{50} for sarin was calculated by probit analysis to be 47 mg/m^3 (95% CL = 44-50 mg/m^3), and the LCt_{50} for an 8-min exposure was estimated to be 376 mg·min/m^3. In both the intravenous and inhalation studies the concentration of the nontoxic (+) isomer in the blood was below detection limits (<5 pg/mL blood). In the intravenous test, the toxicokinetics of the toxic (-) isomer followed a bi-exponential

equation. In the inhalation tests, the blood AChE activity decreased to about 70% of control values at 0.4 LCt_{50} and to about 15% of control values at 0.8 LCt_{50}; however, there were no effects on respiratory parameters (respiratory minute volume or respiratory frequency). The toxic (-) isomer appeared to be rapidly absorbed and the toxicokinetics followed a discontinuous process with a mono-exponential equation for the exposure period and a bi-exponential equation for the postexposure period.

Benschop et al. (2000) (see also Benschop [1999]) studied the toxicokinetics of several VX stereoisomers [(±)-] in hairless guinea pigs (intravenous and percutaneous exposures) and marmosets (intravenous exposures only). Following an intravenous dose of 28 µg/kg (marmosets) or 56 µg/kg (guinea pigs), VX was found in the blood at toxicologically relevant levels even after 6 h. Detoxification proceeded at a slower rate in marmosets than in guinea pigs. The VX metabolite, O-ethyl methyphosphonic acid (EMPA), was found in the blood of the exposed animals; however, the metabolite contributed only 5% to the recovery of the phosphonyl moieties related to the VX dose. Metabolites of VX were also evaluated in in vitro studies by treating liver homogenates and plasma from hairless guinea pigs, marmosets, and humans with the radio-labeled compounds, ^{35}S-VX and [$^{14}CH_3$-P]-VX. The potential toxic metabolite VX-N-oxide was not found. Desethyl-VX was found after incubation of VX in plasma of all three species; however, because of its slow rate of formation, Benschop et al. (2000) concluded that it would be unlikely that this compound would be present at toxicologically relevant levels after administration of VX in vivo. In vitro studies with ^{35}S-VX revealed that a significant part of the thiol-containing leaving group (S-2-(N, N-diisopropylamino)ethane thiol, DPAT) was bound to proteins such as albumin. It was found that the sulfur-containing leaving group was also transformed into a variety of oxidation products.

4.1.3. Disposition and Metabolism

There are a number of enzyme systems in mammalian blood and tissues capable of the binding with and/or metabolically detoxifying organophosphate nerve agents. A primary disposition pathway is the binding of the compounds with blood cholinesterases and carboxylesterases. Of the cholinesterases present in blood (RBC- and plasma-ChE), VX preferentially inhibits RBC-ChE (Sidell and Groff 1974). Plasma cholinesterase may likely serve as a buffer to offset the binding of nerve agents (and pref-

erential binding of agent VX) to RBC-AChE. It has been reported that pretreatment with human plasma cholinesterase protected laboratory rats (Ashani et al. 1993) and monkeys (Raveh et al. 1997) from lethal and other acute toxic effects of VX exposure.

The G-agents have a strong affinity for carboxylesterases (Jokanović 1989), in contrast to agent VX, which has a quaternary ammonium group that prevents it from being a substrate for carboxylesterases. In tests on male SD rats, Maxwell (1992) experimentally confirmed that endogenous carboxylesterases provide "significant protection" against in vivo toxicity of the organophosphorous (OP) agents GA, GB, and GD, but not VX. Maxwell (1992) goes on to conclude that "CaE [carboxylesterase] detoxification does not appear to be important" against exposures to lethal concentrations of agent VX in the laboratory rat.

Because of the lack of other reactive esterases, agent VX induces a toxic response at lower concentrations than the G agents.

While carboxylesterases were once widely considered to be absent from the blood plasma of humans, carboxylesterases are, indeed, present in human erythrocytes and monocytes as well as in human liver, kidney, lung, skin, and nasal tissue (Cashman et al. 1996). Additional literature documents the presence of carboxylesterases in many human tissues and fluids, including brain, milk, mammary gland, pancreas, small intestine, colon, stomach, placenta, and plasma and serum (Chanda et al. 2002; Kaliste-Korhonen et al. 1996). The lung carboxylesterases are associated with alveolar macrophages (Munger et al. 1991). Further, carboxylesterases are present in human tissues and organs where exposure to nerve agent vapors would likely first occur (nasal tissues and the lung), would be distributed (erythrocytes, monocytes, plasma), and would generate effects (brain, stomach, colon, etc.). Carboxylesterase is also present in human serum. Chanda et al. (2002) indicate that full characterization of the OP-protective capabilities of carboxylesterases requires assessment not only of the *amount* but also of the *affinity* exhibited by carboxylesterases for the inhibitor as well as the total carboxylesterase activity unlikely to be inhibited (inhibitor resistant esterase activity [IRE]). The detoxification potential of carboxylesterases is multifaceted and is an area requiring further experimental characterization.

It is acknowledged that the CaE profile in humans is not well known and that there are few data from which to characterize the contributions that CaE may make to human protection from anticholinesterase poisoning. Chanda et al. (2002) consider that full characterization of CaE amount, affinity, and IRE in human tissues will be necessary before accurate predic-

tions can be made regarding CaE detoxification potential following anticholinesterase exposures to humans.

Phosphorylphosphatases associated with the hydrolysis of GD (somanase), GA (tabunase), and GB (sarinase) have been reported. Sterri et al. (1980) reported that the liver of rats was capable of hydrolyzing GD at a rate of 743 µmol/g of liver per hour. At low substrate concentrations some phosphorylphosphatases have been shown to be stereospecific in their activity. Sarinase from the plasma of rats preferentially catalyzes the hydrolysis of the less toxic isomer of agent GB (Christen and van den Muysenberg 1965); however, tabunase targets the toxic stereoisomer of agent GA (reviewed by Gupta et al. [1987]). Somanase from rat liver (Wahllander and Szinicz 1990) or swine kidney (Nordgren et al. 1984; Benschop et al. 1981) preferentially inhibits the less toxic isomers of agent GD; however, another hepatic enzyme in rat liver has been reported to be capable of hydrolyzing all four isomers of GD (Little et al. 1989). The same hepatic enzyme also catalyzed the hydrolysis of agents GA and GB (Little et al. 1989). In studies conducted on rats dosed subcutaneously with agent GB, GD, or GF at 75 µg/kg, Shih et al. (1994) found that the major metabolite formed by a nonsaturable mechanism and excreted in the urine was an alkylmethyl phosphonic acid. Little et al. (1986) identified ^{3}H-labeled GB metabolites in the tissues of mice following intravenous administration of a sublethal dose (80 µg/kg). Most of the label was associated with free isopropyl methylphosphonic acid (IMPA), the hydrolytic metabolite of GB. In individuals allegedly exposed to GB, Noort et al. (1998) found O-isopropyl methylphosphonic acid in serum samples, and Nakajima et al. (1998) reported that methylphosphonic acid and isopropyl methylphosphonic acid were detected as urinary metabolites of GB. Distribution of the low-sarinase allele appears to be somewhat ethnically related. The Japanese population has a higher frequency of the low-sarinase isoform (allele frequency of 0.66) than other ethnic groups (0.24 to 0.31) (Yamasaki et al. 1997).

A-esterases (paraoxonase/arylesterase) present in the blood and liver are also capable of hydrolyzing phosphate esters (Cashman et al. 1996). Paraoxonase is one A-esterase from humans known to hydrolyze the phosphorus-fluorine bond of the nerve agents GB and GD (Davies et al. 1996). A-somanase isolated from human liver (Wang et al. 1998) is capable of hydrolyzing agent GD as well as agent GA with P-F or P-CN bonding, but cannot hydrolyze paraoxon or nerve agent VX with P-O or P-S bonding. Agent GB was not tested in the studies of Wang et al. (1998). A-

esterases are considered to provide protection against the adverse effects of some OP compounds (Pond et al. 1995).

Paraoxonase is polymorphic in human populations, and individual differences are wide (LaDu et al. 1986, as cited in Davies et al. 1996; Furlong et al. 1988, 1989). In one population tested, differences in paraoxonase activity among three genotypes was approximately 6-fold (Kujiraoka et al. 2000). Among a "caucasoid" population sampled in Seattle, Washington, a 40-fold variation in human serum paraoxonase activity was observed (Furlong et al. 1989) and was associated with three phenotypes: homozygotes for the low-activity allele, heterozygotes, and homozygotes for the high-activity allele.

Individuals expressing certain isomeric forms of the enzyme with low hydrolyzing activity are considered to be more susceptible to organophosphate anticholinesterase poisoning (Yamasaki et al. 1997). The polymorphic paraoxonase gene (PON1) has an important role in the detoxifying metabolism of nerve agents and OP insecticides. The $PON1_{R192}$ paraoxonase isoform hydrolyzes agents sarin (GB) and soman (GD) slowly when compared with the $PON1_{Q192}$ isoform (Furlong et al. 2002; Davies et al. 1996). The human population can be organized into three PON1*192 genotypes: $PON1_{Q192}$ homozygotes; heterozygotes; and $PON1_{R192}$ homozygotes (Furlong et al. 2002; Allebrandt et al. 2002). Frequency distributions of the PON1*192 variants have been examined in ethnically diverse populations (Allebrandt et al. 2002). The allele expressing low activity for agent GB and agent GD hydrolysis ($PON1_{R192}$) is significantly more frequent in African Americans (sampled in Brazil and North America) and Asians (sampled in China, Japan, and Canada) than in individuals of Indo-European descent (sampled in East India, Turkey, Canada, Russia, Germany, North America, England, France, the Netherlands, and Brazil). Nevertheless, Furlong et al. (2002) point out that "genotyping alone provides no information about PON1 levels, which can vary up to 13-fold between individuals" (homozygous for the low-activity allele) (see also Furlong et al. [1989] and Davies et al. [1996]).

The serum paraoxonase activity ranges observed by Furlong et al. (1989) and discussed in Davies et al. (1996) illustrate the presence of human genetic variability in one of several metabolic detoxification systems that can denature certain G agents. It is understood, however, that mere determination of serum paraoxonase activity alone is not sufficient to characterize whole-organism susceptibility to anticholinesterase exposure. There are many other metabolic detoxification mechanisms that are also

simultaneously active (e.g., RBC-ChE, tissue carboxylesterases). Further experimentation will be necessary before 13-time or 40-time differences in human serum paraoxonase activity can be translated into quantitative differences in whole-organism susceptibility to anticholinesterase compounds.

Some investigators have previously considered that low levels of paraoxonase in newborns may contribute to the observed sensitivity of newborn rats to organophosphate insecticides (Benke and Murphy 1975; Burnett and Chambers 1994, as cited in Davies et al. 1996). A recent investigation (Chanda et al. 2002) presents in vitro and in vivo evidence that carboxylesterases "are critical for explaining age-related sensitivity" of rat pups to the OP insecticide chlorpyrifos. The presence of low carboxylesterase activity, however, does not sufficiently characterize the greater susceptibility of rat pups to neurotoxic effects of some OP insecticides (Chanda et al. 2002).

A novel mouse-liver enzyme, unrelated to the paraoxonases, has been found to hydrolyze agents GB and GD (Billecke et al. 1999) in an in vitro assay of soluble fraction extracts from commercially available frozen mouse livers.

4.1.4. Distribution and Excretion

Several studies have examined the tissue distribution and excretion of G agents and their metabolites following parenteral administration to rodents. In studies conducted on rats dosed subcutaneously with agent GB (sarin), agent GD (soman), or GF at 75 µg/kg, Shih et al. (1994) found that the major route of elimination for all three agents was urinary excretion. For GD, the lung was the major organ of accumulation. McPhail and Adie (1960) dosed rabbits with radio-labeled (^{32}P) GB and found the highest levels of radioactivity in the lungs and kidney. Kadar et al. (1985) injected mice intravenously with a LD_{50} dose of ^{3}H-labeled agent GD. High levels of radioactivity were found in the lung and skin at 5 min to 24 h after the injection, with very small amounts in the CNS. Considerable accumulation of the label occurred in the urine, gall bladder, and intestinal lumen, suggesting that these were the main pathways of excretion. Little et al. (1986) measured the distribution of ^{3}H-labeled agent GB (sarin) and sarin metabolites in the tissues of mice following intravenous administration of a sublethal dose (80 µg/kg). Within 1 min all tissues contained large amounts

of the label, of which less than 10% represented agent GB (sarin). High concentrations of the metabolite were found in the kidneys and lungs, and only trace amounts of ^{3}H-labeled agent GB (sarin) were found in the brain within 15 min. In a continuation of these studies, Little et al. (1988) evaluated the distribution of ^{3}H-labeled agent GD (soman) and agent GB (sarin) in the brain of mice following sublethal intravenous doses (25 µg/kg and 80 µg/kg). The nerve agents were distributed evenly throughout the brain tissue with the exception of a 2- to 5-fold greater concentration in the hypothalamus.

4.2. Mechanism of Toxicity

The acute toxicity of the nerve agents is considered to be initiated by inhibition of acetylcholinesterase (AChE), an enzyme responsible for deactivating the neurotransmitter acetylcholine at neuronal synapses and myoneural junctions. Nerve agents phosphorylate the enzyme, thereby preventing deactivation of acetylcholine. Although the inhibited cholinesterase can be reactivated by the process of dephosphorylation, that is not possible once the nerve agent-cholinesterase complex undergoes "aging," which is thought to happen because of a loss of an alkyl or alkoxy group. Agent GD ages very rapidly, with a $t_{½}$ (time required for 50% of the enzyme to become resistant to reactivation) of 1.3 min (Harris et al. 1978). The aging half-time for agent GA is 46 h, as calculated from a rate constant of 2.5×10^{-4} per minute (de Jong and Wolring 1978), and the $t_{½}$ for agent GB has been reported to be 5 h (Sidell and Groff 1974). In the latter case, approximately 5% of the GB-enzyme complex reactivated spontaneously. In contrast to the results of these latter studies, Grob and Harvey (1958) had earlier reported that both GA and GB combined with ChE almost irreversibly within 1 h when tested in vitro. The complex formed between ChE and agent VX does not age significantly (half-life about 48 h), and the rate of spontaneous reactivation in humans has been reported to be as fast as 1% per hour (Sidell and Groff 1974).

Although nerve agents exert toxic effects on the central and peripheral nervous system indirectly through AChE inhibition (Koelle 1976, 1981), nerve agents may also affect nerve impulse transmission by additional mechanisms at neuromuscular junctions (Somani et al. 1992) and at neurotransmitter receptor sites in the CNS. Rao et al. (1987) reported that VX caused an increase in acetylcholine release at neuromuscular junctions in

the frog by an interaction with the nicotinic acetylcholine receptor-ion channel complex. Aas et al. (1987) reported alterations in muscarinic receptors in rat bronchi and lung tissue after subacute inhalation exposures to agent GD. In the CNS, nerve agents may act directly on muscarinic, nicotinic, and glutamate receptors. Bakry et al. (1988) reported that nanomolar concentrations of agent GD affected muscarinic ACh receptors that have a high affinity for [^{3}H]-*cis*-methyldioxalane binding. Rocha et al. (1998, 1999) reported that, in cultured rat hippocampal neurons, VX at 0.01 nM reduced the evoked release of the neurotransmitters γ-aminobutyric acid (GABA) and reduced the amplitude of evoked GABAergic postsynaptic currents. VX concentrations >1 nM decreased the amplitude of evoked glutamatergic currents. In the presence of a Na^+ channel blocker, VX increased the frequency of GABA- and glutamate-mediated miniature postsynaptic currents, a Ca^+ dependent effect reported to be unrelated to cholinesterase inhibition (Rocha et al. 1999). Chebabo et al. (1999) reported that 0.3-1 nM of agent GB reduced the amplitude of GABA-mediated postsynaptic currents but had no effect on the amplitude of glutamatergic-mediated postsynaptic currents. The observed effect was thought to be due to the direct interaction of GB with muscarinic acetylcholine receptors present on presynaptic GABAergic neurons. Chebabo et al. (1999) suggest that the selective reduction in the action-potential-dependent release of GABA in the hippocampus might account for GB-induced seizures. Lallement et al. (1991a,b) had earlier suggested that GD-induced overstimulation of glutamatergic receptors contributed to maintenance of seizures. Although these data indicate that nerve agents may have direct effects on the nervous system unrelated to AChE inhibition, the in vitro data do not provide a means of relating electrophysiological alterations in rat hippocampal neurons or determining a dose conversion to the integrative end point of whole-body lethality. Neither do they allow qualitative/quantitative comparisons directly relevant to lethality. The results were obtained largely from single cells in isolation from whole organisms and systems, and extrapolation from observations on individual cells is not presently possible. At present, nM-induced amplitude changes in postsynaptic currents in rat hippocampal neurons in vitro cannot be correlated to dose levels resulting in multisystem failure and death such as are needed for AEGL estimation.

It should be further noted that the effects of nerve agents on GABAergic transmission in the CNS may have profound implications for behavioral effects in laboratory animals and humans and may also contrib-

ute to the induction of convulsions at higher doses (Bakshi et al. 2000). Nevertheless, given the present undefined application of noncholinergic data to AEGL estimation, reliance on the primary assumption of anticholinesterase action is consistent with recognized opinion (Bakshi et al. 2000).

Recent studies with cholinesterase inhibitors such as galantamine, which affect neuronal nicotinic AChE receptors in a similar manner to that reported for VX, have shown that such compounds have therapeutic benefits for patients with mild to moderately severe Alzheimer's disease (Maelicke et al. 2001). As such, these compounds might be helpful in stabilizing behavior in such patients by improving memory and cognitive and daily function.

As pentavalent phosphorous-containing compounds, the G agents may also indirectly generate neurotoxic effects through a noncholinergic mechanism involving the kinase-mediated protein Ca^{2+}/calmodulin kinase II (Ca^{2+}/CaM kinase II) (de Wolff et al. 2002; Abou-Donia and Lapadula 1990). The Ca^{2+}/CaM kinase II protein becomes activated by OP-induced phosphorylation and reacts with the cytoskeletal proteins found in neurofilaments to produce axonal degeneration in the large-diameter tracts of the spinal cord.

It is also understood that OP compounds interact with detoxification enzymes such as the carboxylesterases and A-esterases and that the degree of such interaction may alter the magnitude and extent of the toxic cascade following AChE inhibition (Pope and Liu 2002). Recent studies indicate that full characterization of the OP-protective capabilities of carboxylesterases requires assessment not only of the *amount* but also of the *affinity* exhibited by carboxylesterases for the inhibitor as well as the total carboxylesterase activity unlikely to be inhibited (inhibitor resistant esterase activity [IRE]) (Chanda et al. 2002). The detoxification potential of carboxylesterases is multifaceted and is an area requiring further experimental characterization.

4.3. Relative Toxic Potency

Because of the sparse animal and human toxicity data for agents GA, GD, GF, and VX, AEGLs for those agents will necessarily be derived from the AEGLs for agent GB by a relative potency method. The database for the nerve agents as a group is considered reasonably complete in that there

exist (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 nerve agents with the 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 nerve agents, data uncertainty is reduced and target organ effects are expected to be identical and to differ only in magnitude. Thus, a comparative method of relative potency analysis from the more complete data set for agent GB is appropriate. This approach has been applied before, in the estimation of nerve agent exposure limits (Watson et al. 1992; Mioduszewski et al. 1998). The relative toxic potency of cholinesterase inhibitors can be expressed in several ways, based on in vitro or in vivo data.

4.3.1. In Vitro Potency

The in vitro potency can be measured by either the bimolecular rate constant (k_i, in M/min) for the reaction of the agent compound with the enzyme or by the molar concentration causing 50% inhibition of the enzyme (I_{50}) in vitro. The relationship between I_{50} and k_i for a fixed time (t) of incubation is expressed by the following equation (Eto 1974):

$$I_{50} = \frac{0.695}{t \times k_i}$$

As summarized by A.D. Little, Inc. (1985), k_i values for GB are in the range of 1×10^6 to 2×10^7 M^{-1}/min^{-1} for acetylcholinesterase in rat brain tissue, and 1×10^7 M^{-1}/min^{-1} for butyrylcholinesterase in human serum. Reported k_i values for agent GD are 3.7×10^7 M^{-1}/min^{-1} for acetylcholinesterase in rat brain tissue, and 1×10^7 M^{-1}/min^{-1} for butyrylcholinesterase in human serum (A.D. Little, Inc. 1985). More recently, Maxwell (1992) reported k_i values of 4.5 (± 0.7) $\times 10^6$ M^{-1}/min^{-1} for agent GA, 1.2 (± 0.3) $\times 10^7$ M^{-1}/min^{-1} for agent GB, and 3.6 (± 0.5) $\times 10^7$ M^{-1}/min^{-1} for agent GD in in vitro tests conducted on rat brain AChE.

I_{50} data for several G agents have been tabulated by Dacre (1984). The pI_{50} (negative log of the molar concentration causing 50% inhibition of

cholinesterase) was reported to be 8.4-8.6 for GA and 9.2 for GD (Dacre 1984; Holmstedt 1959). Grob and Harvey (1958) reported that the in vitro potency of GB ($I_{50} = 0.3 \times 10^{-8}$ mol/L) was 5 times that for GA ($I_{50} = 1.5 \times 10^{-8}$ mol/L).

The k_i values for agent VX have been reported to be $1.4 \pm 0.3 \times 10^8$ M/min, respectively, for in vitro tests conducted on rat brain AChE (Maxwell 1992). In comparison, Maxwell (1992) reported a k_i value of $1.2 \pm 0.3 \times 10^7$ M/min for agent GB. The corresponding I_{50} values are 5.8×10^{-8} M for agent GB and 5.0×10^{-9} M for agent VX. The GB:VX ratio for the I_{50} values is 11.7, indicating that VX is nearly 12 times more potent than GB in inhibiting rat brain acetylcholinesterase in vitro. This comparison is one way to express the relative potency of agent VX.

4.3.2. In Vivo Potency

Relative potency of nerve agents can also be expressed in terms of the in vivo dose necessary to produce the same toxic effect by a specific exposure route.

G Agents

A summary of the estimated inhalation and visual effects values for the G agents is given in Tables 1-21 and 1-22. The information presented on animal toxicity values is derived from Callaway and Dirnhuber (1971) and Mioduszewski et al. (2002b) for nonlethal visual effects; and Oberst (1961), Callaway and Blackburn (1954), Mioduszewski et al. (2001, 2002a), and Anthony et al. (2002) for lethality. Another source is the largely unpublished experimental data summarized by the NDRC in 1946.

Estimates of lethality and severe effect levels in humans are based on extrapolations from animal data and on modeling studies. Several of the estimates are presented in Table 1-21, together with the limited human data for miosis. For the end point of miosis, ratios for ECt_{50}, ECt_{90}, and threshold effects are summarized in Table 1-21 for both experimentally derived and estimated toxicity values. For miosis as a critical effect, comparison of effective doses to achieve 50% pupil area decrement in the eye of the albino rabbit (Callaway and Dirnhuber 1971) indicates that agents GD and GF are more miotogenic than GB at approximately 50% of the GB Ct (GB/GD of 2.24; GB/GF of 1.76; relative potency to agent GB of approximately

TABLE 1-21 Comparison of Visual Effects Values for G Agents

	Toxicity value (ECt [mg·min/m^3])				Ratios				
Species	GB	GA	GD	GF	GB:GA	GB:GD	GB:GF	GD:GF	References
Human (10 min to 5 h) (ECt_{90}, miosis)	13.85								Callaway and Dirnhuber 1971
Human (20 min) (ECt_{50}, miosis)	4								Johns 1952
Human (ECt_{50}, incapacitation)	2.5	7.5	0.4		0.33	6.25			Wells et al. 1993[a]
Human (10 min to 5 h) (ECt_{50}, miosis)	2.33								Callaway and Dirnhuber 1971
Human (20 min) (No effect, miosis)	1.2								McKee and Woolcott 1949
Human (2 min) (ECt_{50} , mild effects)	0.5	0.5	0.2	0.2	1.0	2.5	2.5	1.0	Reutter and Wade 1994[a] (unclassified summary table)
Human (2 min) (ECt_{50} , mild effects)	0.5	0.5	0.25	0.25	1.0	2.0	2.0	1.0	Mioduszewski et al. 1998[a]
Human (2-10 min) (ECt_{50} , mild effects)	<2								NRC 1997[a]
Human (1 min) (<ECt_{01} , miosis)	0.5								McNamara and Leitnaker 1971[a]

Rat (f, m) (10 min) (ECt_{50} miosis)	0.68, 0.87						Mioduszewski et al. 2002b
Rat (f, m) (60 min) (ECt_{50}, miosis)	1.20, 1.80						Mioduszewski et al. 2002b
Rat (f, m) (240 min) (ECt_{50}, miosis)	2.88, 5.76						Mioduszewski et al. 2002b
Guinea pig (5 h) (LOAEL, miosis)	1.8						van Helden et al. 2001, 2002
Marmoset (5 h) (LOAEL, miosis)	2.5						van Helden et al. 2001, 2002
Rabbit (10 min to 5 h) (ECt, 50% miosis)	1.32	0.59	0.75[b]	2.24	1.76	0.79	Callaway and Dirnhuber 1971
Rabbit (10 min to 5 h) (ECt, 90% miosis)	2.71	2.19	1.79[b]	1.23	1.51	1.22	Callaway and Dirnhuber 1971

[a]Secondary sources.
[b]Data for agent T2715, (2-methylcyclohexyl methylphosphonfluoridate), analog for agent GF.

TABLE 1-22 Acute Lethal Inhalation Toxicity Values for G-Agents

Species (Exposure Time)	Toxicity Value (LCt_{50} [mg·min/m^3])				Ratios				Reference
	GB	GA	GD	GF	GB:GA	GB:GD	GB:GF	GD:GF	
Monkey	74	187			0.40				DA 1974[a]
Monkey (2 min)	42	135			0.31				Oberst 1961; DA 1974
Monkey (10 min)	150	250 180			0.71[b]				NDRC 1946[a,c]
Geometric Mean (monkey data)					0.44				
Rat (female) (1-min)	118		135	110		0.87	1.07	1.23	Callaway and Blackburn 1954
Rat (male) (1-min)	220		196	181		1.12	1.22	1.08	Callaway and Blackburn 1954
Rat (10-min)	220	450	230 279		0.49	0.87[d]			DA 1974[a]
Rat (female) (10-min; 24-h lethality)	184			253			0.73		Mioduszewski et al. 2001; Anthony et al. 2002
Rat (female) (60-min; 24-h lethality)	387			334			1.16		Mioduszewski et al. 2001; Anthony et al. 2002

Rat (female) (240-min; 24-h lethality)	741	533			1.39	Mioduszewski et al. 2001; Anthony et al. 2002
Rat (male) (10-min; 24-h lethality)	231	368			0.63	Mioduszewski et al. 2001; Anthony et al. 2002
Rat (male) (60-min; 24-h lethality)	459	396			1.16	Mioduszewski et al. 2001; Anthony et al. 2002
Rat (male) (240-min; 24-h lethality)	1,040	595			1.75	Mioduszewski et al. 2001; Anthony et al. 2002
Geometric Mean (rat data)			0.49	0.95	1.09	1.15	
Overall Geometric Mean (rat and monkey)			0.47	0.95	1.09	1.15	

[a]Secondary sources.
[b]Based on geometric mean of 212 mg·min/m^3 for the two data points for GA.
[c]Summary of largely unpublished experimental data.
[d]Based on geometric mean of 253 mg·min/m^3 for the two data points for GD.

2) and that the GD:GF ratio approximates 1.0 (equal to 0.79). For 90% pupil area decrement in the rabbit, agents GD and GF are again more effective than agent GB for inducing this end point (GB/GD of 1.23, GB/GF of 1.51; relative potency range to agent GB of approximately 1.2 to 1.5) with a GD:GF ratio of 1.22. A protective determination of relative potency to agent GB is 2.0. Thus, agents GD and GF are considered equipotent and approximately twice as potent as agent GB for inducing miosis. For more severe effects, such as lethality, resulting from vapor exposures, the relative potency estimates presented in Table 1-22 indicate that agents GB, GD, and GF are equally potent and are twice as potent as agent GA.

At a public hearing in 2000 convened by the Chemical Demilitarization Branch of the Centers for Disease Control and Prevention, a U.S. Surgeon General's review panel concluded that because (1) the data base for GB is relatively robust, and (2) the data for the other G agents are limited, it is appropriate to utilize a relative potency approach for comparing G agents (67 Fed. Reg. 895 [2002]; DHHS 2002).

Agent VX

The in vivo doses of agents VX and GB required to produce the same level of blood cholinesterase inhibition in the same species by a specific exposure route are shown in Table 1-23. In humans, the experimentally determined RBC-AChE$_{50}$ for VX is 0.0023 mg/kg for an oral dose (Sidell and Groff 1974). In contrast, for agent GB, an oral dose of 0.010 mg/kg is required to produce about the same level of effect (Grob and Harvey 1958). The GB:VX ratio for this effect is approximately 4.3. In studies conducted by Gupta et al. (1991) in which rats were injected subcutaneously, VX was found to be 10 times more toxic than GB for ChE inhibition and myonecrosis end points. The relative potency of agents VX and GB are shown in Table 1-23.

In studies conducted by Maxwell (1992) on Sprague-Dawley rats, subcutaneous LD$_{50}$ values for GB and VX were 0.51 and 0.027 μmol/kg, respectively, indicating that VX is about 19 times more toxic than GB in rats for subcutaneous lethality, on a molar basis (if the micromoles of each compound are converted to grams using 140 as the molecular weight of GB and $\times 10^{-5}$ g/kg for GB and 8.022×10^{-5} g/kg for VX, resulting in a GB:VX ratio of 9.9). Analysis of parenteral data for Hartley albino guinea pigs (subcutaneous) and Swiss ICR mice (intramuscular) in studies by Koplovitz

TABLE 1-23 Relative Potency Estimates for Agents GB and VX Experimental Data

Species	Toxicity End Point	Units	GB	VX	GB:VX Ratio
Human	Inhalation Ct ChE_{50}[a]	mg-min/m^3	42	~6.5	~6.5
Human	Oral RBC-ChE_{50}[b]	µg/kg	10	2.4	4.3
Human	Intra-arterial/intravenous RBC-ChE_{50}[b]	µg/kg	3	1.1	2.7
Monkey[l]	Intravenous LD_{50}[c]	µg/kg	20	6-11.9	1.8-3.3
Rat[l]	Intravenous LD_{50}[c,d]	µg/kg	45-63	6.9-10	4.5-9.1
Mouse	Intramuscular LD_{50}[e]	µg/kg	204.81	13.07	15.7
Mouse[l]	Intravenous LD_{50}[c]	µg/kg	100	12-15	6.7-8.3
Mouse[l]	10-min LCt_{50}[c,d]	mg·min/m^3	240-310	4-13	18.5-77.5
Mouse[l]	Percutaneous LD_{50}[c,f]	µg/kg	1000	36 - 59	17 - 28
Guinea pig	Subcutaneous LD_{50}[e]	µg/kg	41.26	6.89	5.99
Rat	Subcutaneous LD_{50}[g]	µmol/kg	0.57	0.03	19 (9.9)[k]
Rat[l]	Oral LD_{50}[c]	µg/kg	870-1,060	77-128	6.8-13.8
Rabbit[l]	Percutaneous LCt_{50}[c,h]	mg·min/m^3	2,000	8.3-28	71-241
Rabbit	Vapor exposure; 50% pupil area decrement[i]	mg·min/m^3	1.32	0.04	33
Rabbit	Vapor exposure; 90% pupil area decrement[i]	mg·min/m^3	2.71	0.23	11.8

[a]GB, Oberst et al. (1968); VX, Bramwell et al. (1963) (estimated from tabulated data; not verifiable).

[b]GB, Grob and Harvey (1958); VX, Sidell and Groff (1974).
[c]DA (1974) (secondary source; not verifiable).
[d]Dacre (1984) (secondary source; not verifiable).
[e]Koplovitz et al. (1992)
[f]Liquid exposures.
[g]Maxwell (1992).
[h]Vapor exposures.
[i]Callaway and Dirnhuber (1971).
[k]Ratio shown in parenthesis based on grams per kilogram.
[l]Secondary source data.

et al. (1992) resulted in acute (24-h) LD_{50} estimates as follows: in the guinea pig, LD_{50} for GB of 41.26 µg/kg, LD_{50} for VX of 6.89 µg/kg; and in the mouse, LD_{50} for GB of 204.81 µg/kg, LD_{50} for VX of 13.07 µg/kg. Inhalation lethality data for mice include LCt_{50} values of GB at 240 mg·min/m^3 (forced activity); GB at 310 mg·min/m^3 (resting animals); VX at 4 mg·min/m^3 (total animal exposures); and VX at 13.6 mg·min/m^3 (head-only exposures) (DA 1974; Koon et al. 1960, as cited in NRC 1997).

The Cts necessary to generate 50% and 90% decrease in pupil area in the albino rabbit eye (Callaway and Dirnhuber 1971) were summarized in Table 1-19. The calculated Cts for 50% decrease are 1.32 mg·min/m^3 for GB and 0.04 mg·min/m^3 for VX (a GB:VX ratio of 33), while the Cts for 90% decrease are 2.71 mg·min/m^3 for GB and 0.23 mg·min/m^3 for VX (a GB:VX ratio of 11.8) (see Table 1-23). Callaway and Dirnhuber (1971) consider the 90% decrement to be a more definite end point; furthermore, this degree of pupil area decrease has operational significance. However, because Callaway and Dirnhuber (1971) do not document incidence data, neither an EC_{50} nor an EC_{90} for a given percentage miosis, as defined by current experimental protocols, can be reliably determined for their exposed rabbit population.

Primary experimental data for GB:VX comparisons for the same end point are available for five mammalian species (human, rat, mouse, guinea pig, rabbit; see Table 1-23). In all cases, agent VX is more potent than agent GB (range of 2.7 to 33).

Human Estimates of GB and VX Toxicity

Estimates of lethality and severe effect levels in humans are based on extrapolations from animal data and on modeling studies. Several of these estimates are presented in Table 1-24, together with the limited human experimental data (vapor inhalation, oral, intra-arterial, and intravenous exposures) for ChE_{50} levels. The GB:VX ratios for these experimentally derived end points fall in the range of 2.7 to 6.5. For the end point of miosis, ECt_{50} estimates range from 0.06 mg·min/m^3 to 0.09 mg·min/m^3 for VX and 0.5 mg·min/m^3 to 1.5 mg·min/m^3 for GB, resulting in overall GB:VX ratios of 5.6 to 25 (secondary sources and nonverifiable data).

Human inhalation exposures (Oberst et al. 1968; Bramwell et al. 1963), human oral exposures (Grob and Harvey 1958; Sidell and Groff 1974), and human intra-arterial and intravenous exposures (Grob and Harvey 1958;

TABLE 1-24 Human Toxicity Estimates for Agents GB and VX

Toxicity End Point (Exposure Time)	GB (mg·min/m^3)	VX (mg·min/m^3)	GB:VX Ratio
Inhalation ChE_{50}[a]	42[b]	~6.5[c]	6.5
Oral RBC-ChE_{50}[a]	10 µg/kg[d]	2.3 µg/kg[e]	4.3
Intra-arterial/intravenous RBC-ChE_{50}[a]	3 µg/kg[d]	1.1 µg/kg[e]	2.7
LCt_{50} (2-10 min)	35[f]	15[f]	2.3
ECt_{50} (2-10 min)	25[f]	10[f]	2.5
LCt_{05}	20[g]	6[g]	3.3
ECt_{05} (severe)	1[g]	5[g]	3
LCt_{01}	10[d]	–	–
ECt_{05} (mild)	8[g]	3[g]	2.7
No deaths	6[h]	–	–
ECt_{05} (ocular, miosis)	1.5[g]	0.06[g]	25
Ocular threshold	1.0[i]	0.04[i]	25
ECt_{50} (ocular, miosis; 2-10 min)	0.5[f]	0.09[f]	5.6
ECt_{50} (ocular, miosis)	>0.5[k]	0.09[k]	>5.6

[a]Experimental data with human subjects; all other estimates are extrapolations based on animal data.
[b]Oberst et al. (1968); resting men, breathing 7 L/min.
[c]Bramwell et al. (1963); estimated from tabulated data—not verifiable.
[d]Grob and Harvey (1958).
[e]Sidell and Groff (1974).

[f]Reutter and Wade (1994).
[g]Wells et al. (1993).
[h]DA (1987).
[i]DA (1990b).
[k]NRC (1997).

Sidell and Groff 1974) are included in the experimental database summarized in Tables 1-23 and 1-24; reported end points for each human study were ChE_{50}. The Bramwell et al. (1963) study of VX inhalation toxicity is considered a flawed and nonverifiable source because the human subjects were not exposed to a rigorously controlled atmosphere (breathing zone concentrations could not be determined and potential effects of the carrier solvent [benzene] on agent absorption by subject was not evaluated, etc.). In consequence, the GB:VX ratio for inhalation ChE_{50} (which includes the VX Ct from Bramwell et al. [1963]) is not as credible as the comparable ratio derived from the well-conducted human oral exposure studies of Grob and Harvey (1958) and Sidell and Groff (1974).

4.3.3. Comparison of Exposure Standards

G-Series Agents

The current occupational exposure limits for the G-series nerve agents, as published by the CDC (DHHS 1988) are 0.0001 mg/m^3 for GB and GA to be applied as a no-adverse-health-effect level for 8-h continuous workplace exposure. The resulting GB:GA ratio is 1.0. The current general population exposure limits for the G-series nerve agents, as published by the CDC (DHHS 1988) are 0.000003 mg/m^3 for GB and GA, to be applied as a no-adverse-health-effect level for 24-h continuous exposure (provides a GB:GA ratio of 1.0). Agents GD and GF are not part of the unitary stockpile and were not evaluated by the CDC in 1988.

The U.S. Department of the Army has prepared a health criteria document for the G-series agents (Mioduszewski et al. 1998) in which exposure limits for the G agents were derived using the relative potency approach and the currently accepted exposure limits for agent GB. As part of a regularly scheduled review process, the 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 currently (September 2002) in progress, and the CDC has not yet released a final position.

Agent VX

The current occupational exposure limit for VX, as published by the

CDC (DHHS 1988), is 0.00001 mg/m^3. Compared with the CDC recommended value of 0.0001 mg/m^3 for agent GB, the resulting GB:VX ratio is 10. The current general population exposure limits for VX and GB, as published by the CDC (DHHS 1988), are 0.000003 mg/m^3 for GB and 0.000003 mg/m^3 for VX, resulting in a GB:VX ratio of 1.

The U.S. Department of the Army has prepared a health criteria document for VX (Reutter et al. 2000) in which exposure limits for VX are derived using the relative potency approach and the currently accepted exposure limits for agent GB (see Mioduszewski et al. [1998] and DHHS [1988], as detailed above). The exposure limits developed by Reutter et al. (2000) were based on minimal effect levels, and miosis was considered to be the most appropriate end point to use for comparison. Reutter et al. (2000) consider a ratio of 10 to be a protective estimate of the relative potency of miosis for agents GB and VX.

Embedded within the Army's logic (USACHPPM 1998; Reutter et al. 2000) regarding the choice of 10 for the relative potency of VX:GB are two elements: downward adjustment to allow for the greater effect of VX on the eye, and upward adjustment to allow for the more rapid recovery (reversibility) of eye effects from VX exposure compared with recovery following GB exposure. These adjustments are based on the human intravenous studies of Kimura et al. (1960) and a calculational model based on ChE activity recovery (McNamara et al. 1973). Ocular exposure to VX vapor is estimated to cause eye effects at approximately one-twenty-fifth of the GB Ct required to attain the same effect. VX "ages" (irreversibly binds to cholinesterase) very slowly ($t_{½}$ of 48 h) when compared with agent GB ($t_{½}$ of 5 h) (Sidell and Groff 1974), and some spontaneous enzyme recovery occurs even in the absence of antidote. In general, recovery from the effects of VX vapor exposure is 4 times greater than that for agent GB (McNamara et al. 1973). Thus, an effective concentration of VX relative to GB is four-twenty-fifths, or 0.16, or approximately one-sixth. The ratio of 1:10 used by the Army in deriving exposure criteria for VX (Reutter et al. 2000) was to allow for a greater margin of safety.

4.3.4. Selection of Nerve Agent Potency Values for Use in Deriving AEGLs

Recent publication of science policy by the EPA Office of Pesticides to guide the use and application of data on cholinesterase inhibition (EPA 2000) recommends a weight-of-evidence approach for evaluating toxicity

end points for anticholinesterase compounds. This approach is consistent with that of Storm et al. (2000), who consider that the most defensible means of deriving (occupational) inhalation exposure limits for organophosphates should be based on weight-of-evidence. In the weight-of-evidence approach, first priority is given to clinical signs and physiological or behavioral effects in humans and animals followed by

- Symptoms in humans.
- Cental nervous system acetylcholinesterase inhibition.
- Peripheral nervous system acetylcholinesterase inhibition.
- Red blood cell acetylcholinesterase inhibition.
- Plasma cholinesterase inhibition in humans and animals.

In general, the guidelines consider blood ChE inhibition to be an imperfect measure because of the need for individual baseline measurements for comparison and the fact that there is no fixed percentage of blood ChE activity change that can distinguish adverse from nonadverse effects (EPA 2000; Storm et al. 2000). A number of nerve agent exposure investigations have noted the poor association between blood (RBC and plasma) cholinesterase activity and anticholinesterase intoxication (Koelle 1994; Sidell 1992; Rubin and Goldberg 1957; Mioduszewski et al. 2002b). Circulating ChE activity does not parallel tissue ChE activity, and minimal blood ChE activity has been observed in association with normal tissue function (Sidell 1992). In the recent GB vapor exposure study of Mioduszewski et al. (2002b), "miosis was not correlated with, or even accompanied by, significant reduction of circulating AChE, BuChE, or CaE" as a consequence of GB vapor whole-body exposure to SD rats. These results further document the fact that miosis alone, and in the absence of signs such as ChE or CaE activity inhibition, is a local effect and reflects an exposure much less than that required to produce a systemic clinical effect. Thus, selection of the local effect of miosis as a critical AEGL end point allows a greater margin of protection against the potential for exposures that would generate systemic effects.

The findings of Mioduszewski et al. (2002b) are consistent with those for human volunteers exposed to GB vapor in the study of Rubin and Goldberg (1957).

Although RBC-ChE inhibition in the blood is an acceptable surrogate for central nervous system inhibition, plasma ChE is more labile and is considered a less reliable reflection of enzyme activity change at neuro-

effector sites (EPA 2000; Young et al. 1999; California Environmental Protection Agency 1998). In consequence, plasma-ChE activity inhibition is considered a biomarker of effect and is here rejected as a critical end point from which to develop a reliable estimate of relative potency. Relative RBC-AChE inhibition or an observable sign (i.e., miosis) in a test species are considered more appropriate end points for deriving relative potency estimates.

G Agents

Experimental determination of miosis (90% decrease in pupil area) in the eyes of albino rabbits directly exposed to a range of GB, GD, and GF vapor concentrations for periods of time ranging from 2-10 min to 5-6 hours (Callaway and Dirnhuber 1971) is a suitable study for estimating relative potency between the G-series nerve agents.

Although there are acknowledged analytical weaknesses in the protocol and data of Callaway and Dirnhuber (1971), this experiment is the only study found in the literature for which the same end point is measured in the same species following exposure to each of several G-series agents. There are no comparable human experimental data. The resulting potency ratios, estimated from cumulative exposure (Ct) values in the literature, are presented in Table 1-21.

For AEGL-1 and AEGL-2 effects, GB and GA are considered equipotent, and GD and GF are each considered equipotent to each other, and more potent than GB by a factor of 2.0 for miosis (see Table 1-22 and the review by Mioduszewski et al. [1998]). Thus, for an equivalent effective concentration (EC) for miosis

EC of GA (mg/m^3) = EC of GB (mg/m^3);
EC of GD (mg/m^3) = EC of GB (mg/m^3) ÷ 2; and
EC of GF (mg/m^3) = EC of GB (mg/m^3) ÷ 2.

For AEGL-3 effects, GB, GD, and GF are considered equipotent, while GA is considered less potent than agent GB by a factor of 2 (see Table 1-22 and the review by Mioduszewski et al. [1998]). As previously discussed in Section 3.1.3, a secondary and short-term GD vapor inhalation study of rat lethality was performed for GD dynamic chamber exposure times of ≤30 min (Aas et al. 1985). In addition, a recent study of GF vapor inhalation

lethality in male and female SD rats reported 24-h LC_{50} and LCt_{50} values for 3 durations of exposure (10, 60, and 240 min) (Anthony et al. 2002). The assumptions for agent GD and GF lethal potency relative to agent GB is generally supported by analysis of the Aas et al. (1985) and Anthony et al. (2002) rat lethality data. Thus, for lethal concentrations (LC) of the G agents

LC of GB (mg/m^3) = LC of GD (mg/m^3) = LC of GF (mg/m^3); and
LC of GA (mg/m^3) = LC of GB (mg/m^3) × 2.

Agent VX

The AEGL standing operating procedures (NRC 2001) state the following: "It is important to emphasize that only toxicity data obtained directly from a primary reference source is used as the basis for 'key' toxicity studies from which the AEGL values are derived. Additionally, all supporting data and information important to the derivations of an AEGL value is obtained solely from the primary references." In the studies listed in Tables 1-23 and 1-24, the verifiable experimental data for humans, rats, and rabbits provide a range of VX:GB relative potencies (RPs) of 2.7 to 33.

Of the various animal data available for developing a GB:VX relative potency factor, the rabbit miosis study of Callaway and Dirnhuber (1971) offers advantages in that VX and GB vapor were tested by the same investigators using the same protocols and test species (see Table 1-23). Nevertheless, it is understood that agent measurements collected during the study were hampered by the limited capabilities and techniques for determining agent vapor concentrations in the early 1970s. Furthermore, when compared with current low-light digital methods, the protocols employed to measure rabbit miosis in Callaway and Dirnhuber (1971) are today considered semisubjective. In addition, the study documentation does not fully report miosis incidence in the agent-exposed rabbit population.

When making cross-compound comparisons for use in developing human exposure guidelines, there is a preference for human data sets. Three exposure routes have been examined in the analysis presented in Tables 1-23 and 1-24. Remarkable concordance (RP range of 2.7 to 6.5) is noted.

The flawed and nonverifiable study of Bramwell et al. (1963) was described in previous sections. The GB:VX ratio for inhalation ChE_{50}

(which includes the VX Ct from Bramwell et al. [1963]) is not as credible as the comparable ratio derived from the well-conducted human oral and intra-arterial and intravenous exposure studies of Grob and Harvey (1958) and Sidell and Groff (1974). In addition, the oral exposure studies evaluate the effects of known agent doses (μg/kg). The GB:VX ratio resulting from the oral exposure studies is considered more protective (RP = 4.3) than that derived from the direct systemic intra-arterial and intravenous studies (RP = 2.7). Of the values derived from available human data, the GB:VX ratio calculated from oral dose exposures needed to achieve RBC-ChE_{50} is the most appropriate for the present application.

With no adjustments for differences in recovery or reversibility (aging), direct application of experimental data from human subjects for the ChE_{50} end point supports a GB:VX RP estimate approximating 4.3. With rounding, the GB:VX RP equals 4.0. Because the ChE_{50} end point is part of the continuum of response for these anticholinesterase compounds, it is consistent to apply the same RP for estimating AEGL-1, AEGL-2, and AEGL-3 values for agent VX.

Until additional data from well-conducted experimental studies are available, the current relative potency approach (RP = 4) is reasonable, is supported by existing human experimental data, and meets the requirements of the standing operating procedures for developing AEGLs (NRC 2001).

4.4. Structure-Activity Relationships

Mager (1981) conducted a quantitative structure-activity analysis of organophosphorus compounds having anticholinesterase properties. The toxicity end point used in the analysis was the intraperitoneal LD_{50} value for the mouse. The calculated values were similar to the observed values. The observed values (-log LD_{50}) were 0.70, 0.25, 0.22, -0.01, and 2.30 for GB, GD, GA, GF, and VX respectively; the calculated values (-log LD_{50}) were 0.43, 0.19, 0.11, 0.01, and 2.41 for GB, GD, GA, GF, and VX, respectively. In this analysis, agent GB was determined to be 3-4 times more toxic than GD and GA; however, it was noted by Mager (1981) that only the L-enantiomorph of GB was tested and that that isomer is 10-20 times more toxic than the D-isomer. The relative potency of the L-isomer is not necessarily reflective of the relative potency for different mixtures of GB isomers. The optical stability of the isomers can be maintained in the laboratory only under special storage conditions involving solvent solutions and

temperature control (Boter et al. 1966). Those same conditions are not maintained in munition storage or the field.

The toxicokinetics of VX stereoisomers [(±)-] have been examined and preliminary results documented in recent reports from the TNO Prins Maurits Laboratory (Benschop 1999; Benschop et al. 2000). Benschop and his colleagues studied the toxicokinetics of several VX stereoisomers [(±)-] in hairless guinea pigs (intravenous and percutaneous exposures) and marmosets (intravenous exposures only). Following an intravenous dose of 28 µg/kg (marmosets) or 56 µg/kg (guinea pigs), VX was found in the blood at toxicologically relevant levels after 6 h. Detoxification proceeded at a slower rate in marmosets than in guinea pigs. Desethyl-VX was found after incubation of VX in plasma of all species tested; however, because of its slow rate of formation, Benschop et al. (2000) concluded that it would be unlikely that this compound would be present at toxicologically relevant levels after administration of VX in vivo.

4.5. Other Relevant Information

4.5.1. Breathing Rates and Toxicity

For chemicals that are as acutely toxic as the nerve agents, and for which the concentration-response curves are expected to be very steep, a critical factor associated with the estimation of the potential inhalation toxicity is the breathing rate of individuals who might be exposed. In the case of the nerve agents, the vapor concentration producing a similar level of effect can be considerably different depending on the inhalation rate. In studies conducted on 125 human volunteers exposed to nerve agent GB, Oberst et al. (1968) demonstrated that the same end point (50% of RBC-ChE depression) could be attained with 2-min exposures to GB concentrations as high as 16.2-22.7 mg/m^3 (average 20.7 mg/m^3) in men breathing 5.6-8.4 L of air per minute; however, concentrations of only 3.9-4.53 mg GB/m^3 (average 4.19 mg/m^3) were needed to produce the same effect in exercising men breathing 41.5-64.9 L of air per minute. Oberst et al. (1968) reported that the retained dose (mg/kg) in both test groups was very similar.

Minute volumes up to about 25 L/min should cover most situations involving civilian populations; however, breathing rates may be higher under stressful evacuation conditions. Dosimetric adjustments based on

breathing rate are not normally considered by the AEGL protocol (NRC 2001, 57-62). In the case of the nerve agents, such a dosimetric adjustment would not be necessary for the AEGL-1 (and, to some extent, the AEGL-2) values, which are based on a local effects to the eye (miosis) as the most sensitive indicator of direct vapor exposure toxicity (see also Section 2.7 of this document). Changes in breathing rate would not affect this end point.

As is true for AEGL-3 determinations for agent GB, the composite UF applied in the determination of an AEGL-3 for agent VX does not include any adjustment for interspecies differences in dosimetry due to species differences in breathing rates, minute volumes, and body weight. For systemic poisons that are 100% absorbed, the minute volume-body weight normalization method results in a human equivalent concentration approximately 3.5 times greater than that for rats for the same end point (NRC 2001). However, for high exposure levels, such as those at the AEGL-3 level, absorption may be less than 100% and the estimated human equivalent exposure may be excessively high, resulting in an underestimation of the toxicity of the compound (NRC 2001). Another possible dosimetric adjustment is one using the inhaled dose against the body weight raised to the three-fourth power (EPA 1992). This approach is supported by the results of chronic toxicity studies but may not be relevant for acute lethality end points (NRC 2001). When applied to breathing rates, the adjustment predicts that rats would receive a dose about 4 times greater than humans. When this adjustment for breathing rate is combined with the adjustment for toxicity (EPA 1992), the two cancel each other out, and the conclusion is reached that equivalent exposures result in equivalent results in both rats and humans (NRC 2001). Use of the EPA RfC dosimetric method for systemically acting Category 2 gases (gases that are moderately water soluble and intermediate in reactivity and would therefore be distributed throughout the respiratory tract and readily absorbed into the blood stream) results in the prediction that humans would receive a dose ranging from 6,000 to 50,000 times greater than a rodent (depending on the species) for an equivalent exposure (NRC 2001). These numbers are not considered biologically reasonable or scientifically credible by the NRC (2001).

Given the uncertainties surrounding the issue of dosimetric adjustment across species, and the fact that no dosimetric correction would be the most conservative public-health approach, the NAC/AEGL committee decided that it would not use dosimetry corrections across species unless there were sufficient data on a specific chemical to support their use. Dosimetric

adjustments for nerve agents are complicated by the fact that species response to cholinesterase inhibitors are affected to an extent by levels of endogenous enzymes that bind with the inhibitors. Some of these detoxification pathways are present in rodents but not in humans (see Section 4.5.3). Therefore, a dosimetric adjustment alone may be insufficient to account for interspecies differences in response to nerve agents. In consequence, no dosimetric adjustment is required for these compounds, including nerve agents.

4.5.2. Delayed Neuropathy

Exposure to some organophosphate ChE inhibitors results in delayed neurotoxic effects (distal neuropathy, ataxia, and paralysis, which has been referred to as organophosphate-induced delayed neuropathy [OPIDN]) several days to several weeks after exposure. These effects, characterized by axon and myelin degeneration, are not associated with the inhibition of AChE and had been thought to be a consequence of the inhibition (and subsequent aging) of an enzyme known as neuropathy target esterase (NTE) (Abou-Donia 1993; Ehrich and Jortner 2002). As pentavalent phosphorous-containing compounds, the G agents and agent VX may also indirectly generate neurotoxic effects through a noncholinergic mechanism involving the kinase-mediated protein Ca^{2+}/calmodulin kinase II (Ca^{2+}/CaM kinase II) (de Wolff et al. 2002; Abou-Donia and Lapadula 1990). The Ca^{2+}/CaM kinase II protein becomes activated by OP-induced phosphorylation, and reacts (proteoloysis) with the cytoskeletal proteins found in neurofilaments to produce axonal swelling and degeneration in the large-diameter tracts of the spinal cord. The proteolysis and axonal degeneration are accompanied by accumulation of myelin debris, perturbed ionic gradients, and cellular edema (de Wolff et al. 2002).

For some OP compounds, delayed neuropathy can be induced in experimental animals at relatively low exposure levels, whereas for others the effect only is seen following exposure to supralethal doses, when the animal is protected by antidotes from acute cholinergic effects caused by ChE inhibition. In either case, there is evidence that a threshold exists below which delayed neuropathy does not occur. Studies reviewed by Somani et al. (1992) indicate that, in chickens (a species particularly susceptible to delayed neuropathic effects), a 70% decrease in brain NTE activity 24-48 h after exposure is related empirically to the subsequent development of

delayed neuropathy. According to Husain et al. (1995), a minimum of 45% NTE inhibition is associated with delayed neuropathy after multiple exposures.

G Agents

It has been shown that agents GB, GA, and GD inhibit NTE in vitro (Vranken et al. 1982). Supralethal doses of all three G agents produced delayed neuropathy in antidote-protected chickens in vivo (Gordon et al. 1983; Willems et al. 1984). Doses of 120 × LD_{50} for agent GA resulted in mild neuropathic signs, and delayed neuropathy was observed at 120-150 × LD_{50} for GD in a single surviving hen, but not at GD doses of 38 × LD_{50}. Delayed neuropathy was also observed in chickens administered agent GB at 30-60 × LD_{50}. In all of these challenge tests, nerve agents were administered to adult chickens previously protected from lethality by large antidote doses (Gordon et al. 1983; Willems et al. 1984). Because chickens are considered a sensitive species for this effect, it would appear that the potential for delayed neuropathy would be a concern only for those human individuals surviving a single exposure to concentrations greater than 30 × LD_{50} for the G agents. There are also some delayed neuropathy data for animals receiving serial exposures.

Although not comparable to the single, one-time exposure assumption basic to AEGL determinations, the serial exposure data are useful to illustrate the high-concentrations of G agents necessary to induce delayed neuropathy. Signs indicative of delayed neuropathy have been observed in chickens receiving serial subcutaneous injections of one-tenth LD_{50} of agent GB on each of 10 successive days (a total of 1 × LD_{50}) (Husain et al. 1995) and in mice exposed to GB vapors at 5 mg/m^3 (one-sixth LD_{50}) for 20 min/d on each of 10 successive days (a total of 1.66 × LD_{50}; Husain et al. 1993). Rats receiving daily gavage doses of GB for 90 d at the maximum tolerated (nonlethal) dose (MTD) did not exhibit neuropathy (Bucci et al. 1991; Bucci and Parker 1992). Of the four G agents evaluated in this report, agent GB has the greatest potential for inducing delayed neuropathy after single, large exposures in excess of those necessary to cause death.

Another type of delayed neuropathy that has been associated with exposures to some organophosphate anticholinesterase agents is referred to as an "intermediate syndrome" (Senanayake and Karalliedde 1987; Brown and Brix 1998). Recovery of muscle function after a well-defined cholin-

ergic phase has been followed by reappearance of paralysis between 24 and 96 h postexposure (Baker and Sedgwick 1996; Senanayake and Karalliedde 1987). This delayed response has involved respiratory and proximal limb muscles, neck flexors, and motor cranial nerves (Senanayake and Karalliedde 1987). Paralytic symptoms have been documented to persist as long as 18 d, and some cases require ventilatory support (Senanayake and Karalliedde 1987). Intermediate syndrome is considered to be a reversible neuromuscular effect resulting from a nondepolarizing neuromuscular block. For the purposes of AEGL estimation, single fibre electromyographic changes observed in humans following agent GB vapor exposures are considered a subclinical and protective indication of syndrome onset (Baker and Sedgwick 1996).

Agent VX

No clinical or experimental evidence is available to indicate that VX causes delayed neuropathy in humans (see Munro et al. [1994] for review). Delayed neuropathy was not observed in three strains of antidote-protected chickens given a single subcutaneous dose of VX as large as 0.15 mg/kg (estimated to be 5-10 times the lethal level). Repeated intramuscular injections of VX (0.04 mg/kg/d and equivalent to 1.3 × LD_{50} for this species per day, 3 d/wk for 30 d or 5 d/wk for 90 d) also did not produce any signs of OPIDN (Goldman et al. 1988; Wilson et al. 1988). For comparison, the LD_{50} value for an intramuscular injection of VX in chickens is about 0.03 mg/kg (Goldman et al. 1988).

In 90-d subchronic studies conducted on Sprague-Dawley rats, Goldman et al. (1988) found no incidence of tissue degeneration in brain, spinal cord, or peripheral nerves that could be associated with daily subcutaneous injections of VX at up to 4 µg/kg for 5 d/wk. However, in tests conducted on rats, Lenz et al. (1996) found that continuous subcutaneous exposure (via an osmotic pump) to 57 µg/kg/d (1.3 times the subcutaneous LD_{50} of 45 µg/kg) for 14 d resulted in 75-90% reduction in NTE in the brainstem, midbrain, and soleus muscle. Myopathy was seen in the soleus muscle of the test animals.

There is no clinical or experimental evidence that agent VX induces a delayed neuropathy of the "intermediate syndrome" type.

In summary, delayed neuropathy was not observed in three strains of antidote-protected chickens given a single subcutaneous dose of VX equiv-

alent to 5-10 times the lethal dose. Further, repeated supralethal intramuscular injections of VX (each injection being equivalent to 1.3 times the LD_{50}) for either 3 d/wk over 30 d or 5 d/wk over 90 d produced no signs of organophosphate-induced delayed neuropathy (Goldman et al. 1988; Wilson et al. 1988). It is true that, in rats, continuous subcutaneous exposure via osmotic pump to a *daily* supralethal dose equivalent to 1.3 times the subcutaneous LD_{50} for 14 d is reported to generate myopathy in the soleus muscle (Lenz et al. 1996). Nevertheless, application of the Lenz et al. (1996) results seems appropriate only for individuals who survive exposures to lethal concentrations (which are well above final AEGL-3 values).

4.5.3. Intra- and Interspecies Variability in Esterase Activity and Response to Nerve Agents

Intraspecies Variability

Differences between individuals in blood cholinesterase activity may affect their susceptibility to the toxic effects of nerve agents. It has been shown that a small subpopulation of men and women possess genetically determined variants in their plasma ChE resulting in very low activity levels (Harris and Whittaker 1962; Lehmann and Liddell 1969) (see also Jokanović and Maksimović [1997] for review). Studies reviewed by Bonderman and Bonderman (1971) indicate that homozygous individuals have plasma-ChE activity reduced to less than 25% of the normal value. For heterozygous individuals, mean plasma-ChE activity is 64 % of normal (range 28-114%) (Lehmann and Liddell 1969). Morgan (1989) reported that about 3% of individuals may have genetically determined low levels of plasma cholinesterase and may therefore be unusually sensitive to some anticholinesterase compounds. The frequency of the atypical homozygous phenotype is estimated at 0.025% (Hayes 1982).

Several studies indicate that plasma- and RBC-ChE activity is significantly lower in women than in men (Rider et al. 1957; Reinhold et al. 1953; Augustinsson 1955; Kaufman 1954; all as cited in Hayes 1982 and Wills 1972). Gender differences of 10% in plasma- or RBC-ChE activity have been reported (Wills 1972). Plasma-ChE activity may also be depressed in pregnant women and in individuals with liver disease, heart disease, allergic conditions, and neoplasms (Wills 1972). Such individuals may also be

at a greater risk from exposure to OP compounds. Although some investigators consider gender differences in plasma ChE activity to be confined to young persons (Shanor et al. 1961), data are available suggesting that adult females may be more susceptible to nerve agents than males. Yokoyama et al. (1998c) reported vestibulocerebellar effects (increased postural sway) in a small population of patients tested 6-8 mo after being exposed to agent GB (sarin) during the Tokyo subway terrorist attack. Both female and male patients (nine of each gender) had similar levels of plasma cholinesterase inhibition following the attack, and in both genders, postural sway was correlated with plasma-ChE activity; however, only in females was the increase in sway significantly greater than controls.

Females are here considered to be part of the susceptible subpopulation. In the Mioduszewski et al. (2000, 2001, 2002a,b) studies on rats, females were statistically more sensitive than males for the lethality end point. For agent GF, LCt_{50} values were generally lower in adult female rats than in adult male rats (Anthony et al. 2002). The observed increased susceptibility of females is taken into account by the intraspecies uncertainty factor (UF) for susceptible subpopulations in AEGL estimation. Additional gender comparisons found in the literature are included in Table 1-25.

While the biological role of plasma cholinesterase is at present unknown, it is acknowledged that plasma cholinesterase may likely serve as a buffer to offset the binding of nerve agents (and preferential binding of agent VX) to RBC-AChE. For example, pretreatment with human plasma cholinesterase has protected laboratory rats (Ashani et al. 1993) and monkeys (Raveh et al. 1997) from lethal and other acute toxic effects of VX exposure. Thus, variability in plasma cholinesterase activity is a parameter of concern for characterization of population susceptibility to nerve agent exposure.

As discussed in Section 4.1, A-esterases (paraoxonase/arylesterase) present in the blood and liver are also capable of hydrolyzing phosphate esters (Cashman et al. 1996; Davies et al. 1996; Wang et al. 1998; and Pond et al. 1995). Further, paraoxonase is known to be polymorphic in human populations, and individuals express widely different enzyme levels (see Section 4.1) (LaDu et al. 1986, as cited in Davies et al. 1996; Furlong et al. 1988, 1989; Kujiraoka et al. 2000).

Individuals expressing certain isomeric forms of the enzyme with low hydrolyzing activity are considered to be more susceptible to organophosphate anticholinesterase poisoning (Yamasaki et al. 1997). The polymor-

TABLE 1-25 Comparison of Acute (1-10 min) Lethal Inhalation Toxicity Values for G Agents for Male and Female Rats

	Toxicity Value LCt_{50} (mg·min/m^3)								
	Agent GB			Agent GD			Agent GF		
Species	Females	Males	Ratio F:M	Females	Males	Ratio F:M	Females	Males	Ratio F:M
Rat (5-min)[a]	164	230	0.71						
Rat (10-min)[a]	181	226	0.80						
Rat (1-min)[b]	118	220	0.54	135	196	0.69	110	181	0.61
Rat (10-min)[c]	184	231	0.80				253	368	0.69
Geometric Mean			0.70			0.69			0.64

Note: Entries from primary sources and known experimental data.

[a]Mioduszewski et al. (2000, 2001, 2002a).

[b]Callaway and Blackburn (1954).

[c]Mioduszewski et al. (2001) for agent GB; Anthony et al. (2002) for agent GF; 24-h lethality.

phic paraoxonase gene (PON1) has an important role in the detoxifying metabolism of nerve agents and OP insecticides. The $PON1_{R192}$ paraoxonase isoform hydrolyzes agents sarin (GB) and soman (GD) slowly compared to the $PON1_{Q192}$ isoform (Furlong et al. 2002; Davies et al. 1996). The human population can be organized into three PON1*192 genotypes: $PON1_{Q192}$ homozygotes; heterozygotes; and $PON1_{R192}$ homozygotes (Furlong et al. 2002; Allebrandt et al. 2002). Frequency distributions of the PON1*192 variants have been examined in ethnically diverse populations (Allebrandt et al. 2002). The allele expressing low activity for agent GB and agent GD hydrolysis ($PON1_{R192}$) is significantly more frequent in African-Americans (sampled in Brazil and North America) and Asians (sampled in China, Japan, and Canada) than in individuals of Indo-European origin (sampled in East India, Turkey, Canada, Russia, Germany, North America, England, France, the Netherlands, Brazil). Nevertheless, Furlong et al. (2002) point out that "genotyping alone provides no information about PON1 levels, which can vary up to 13-fold between individuals" (homozygous for the low-activity allele) (see also Furlong et al. [1989] and Davies et al. [1996]).

Some investigators have previously considered that low levels of paraoxonase in newborns may contribute to the observed sensitivity of newborn rats to organophosphate insecticides (Benke and Murphy 1975; Burnett and Chambers 1994, as cited in Davies et al. 1996). A recent investigation (Chanda et al. 2002) presents in vitro and in vivo evidence that carboxylesterases "are critical for explaining age-related sensitivity" of rat pups to the OP insecticide chlorpyrifos. Further, the presence of low carboxylesterase activity, although important, does not sufficiently characterize the greater susceptibility of rat pups to neurotoxic effects of certain OP insecticides (Chanda et al. 2002).

Distribution of the low sarin-hydrolysis allele ($PON1_{R192}$) appears to be somewhat ethnically related. The Japanese population has a higher frequency of the low sarin-hydrolysis isoform (allele frequency of 0.66) (Yamasaki et al. 1997) than Caucasian groups documented in the literature (0.24 to 0.31) (Serrato and Marian 1995; Ruiz et al. 1995; Antikainen et al. 1996).

Carboxylesterases are another enzyme group capable of binding with certain OP compounds and are present in human erythrocytes and monocytes as well as in human liver, kidney, lung, skin, and nasal tissue (Cashman et al. 1996; Chanda et al. 2002; Kaliste-Korhonen et al. 1996; Munger et al. 1991). As detailed in Section 4.1, the detoxification potential

of carboxylesterases is multifaceted and is an area requiring further experimental characterization.

Interspecies Variability

Differences exist among animal species in the types of esterases found in the blood as well as in their relative activity, and those differences may affect a species' susceptibility to specific OP compounds. Baseline RBC-AChE activity in humans is slightly higher than that in monkeys but much higher than levels measured in sheep, rats, and other species (see Table 1-26) (Ellin 1981). Species differences also exist in plasma cholinesterase levels. In humans, about 50% of the total blood ChE consists of plasma ChE (Osweiler et al. 1985). Plasma-ChE activity constitutes about 40% of the total blood ChE in dogs, about 30% in rats, and 20% in monkeys, but only 10% in sheep, horses, and cows (Wills 1972). Cohen et al. (1971) reported that plasma ChE activity in humans was 2 times greater than that in mice and 4 times greater than that in rats. Because of its more rapid turnover time when compared with RBC-AChE, plasma ChE may function as a repository and primary detoxification pathway for many OP compounds. This logic also applies to the carboxylesterases, discussed more fully in the earlier section on intraspecies variability.

It is acknowledged that the CaE profile in humans is not well known and that there are few data from which to characterize the contributions that CaE may make to human protection from anticholinesterase poisoning. Chanda et al. (2002) consider that full characterization of CaE amount, affinity, and IRE in human tissues will be necessary before accurate predictions can be made regarding CaE detoxification potential following anticholinesterase exposures to humans. Interspecies variation in response to some nerve agents may be accounted for largely by carboxylesterase binding (Somani et al. 1992). The G agents readily bind with carboxylesterases (Fonnum and Sterri 1981; Jokanović 1989; Clement 1994; Maxwell et al. 1987; Jokanović et al. 1996), and Maxwell (1992) demonstrated that endogeneous carboxylesterase activity provided rats with protection against the lethal effects of agents GA, GB, and GD, but not VX. In rodents, detoxification of G agents might be accounted for largely by carboxylesterases binding, and in the case of GD, binding appears to occur specifically with the most toxic stereoisomer of the agent (Cashman et al. 1996). Inhibition of carboxylesterase activity significantly increased the

TABLE 1-26 Baseline RBC-ChE Activity in Different Species[a]

Species	RBC-ChE Activity (μmol/mL/min)	Optimum Substrate Concentration (M)
Human	12.6	2 × 10-3
Monkey	7.1	2 × 10-3
Pig	4.7	1 × 10-3
Goat	4.0	2 × 10-3
Sheep	2.9	2 × 10-3
Mouse	2.4	2 × 10-3
Dog	2.0	2 × 10-2
Guinea pig	2.7	2 × 10-3
Rabbit	1.7	5 × 10-3
Rat	1.7	5 × 10-3
Cat	1.5	5 × 10-3

[a]Ellin (1981); Acetylthiocholine iodide concentration for maximum RBC-ChE activity.

acute toxicity of GD, GB, and GA to laboratory animals (Clement 1984; Jokanović 1989; Maxwell et al. 1987), and induction of carboxylesterase activity by pretreatment with phenobarbital substantially reduced the acute toxicity of GD and GA, but not GB (Clement 1983, 1984; Jokanović 1989). In contrast, selective inhibition of acetylcholinesterase or butyrylcholinesterase had no effect on the acute toxicity of GD to mice (Clement 1984). Because rodents have high levels of plasma carboxylesterases, they may be less susceptible to the G agents than humans.

Interspecies variability in response to nerve agents can be evaluated in terms of lethal and nonlethal end points.

G-series Agents

Available experimental agent GB LCt_{50} data for the monkey, dog, and rat are presented in Table 1-13. Data for rats (Table 1-25) show that females of these species are more susceptible than males. Comparisons of female rat LCt_{50} values with those of dogs and monkeys indicate that, in

terms of lethality, adult female SD rats are less susceptible to agent GB than adult dogs or monkeys by approximate factors of 2.0 to 4.0. Because rats are a CaE-rich species and dogs and monkeys were once thought to possess no plasma carboxylesterase (Augustinsson 1959), these differences in susceptibility may be due, in part, to species differences in CaE.

In the case of human lethality estimates, Bide et al. (1999) estimated GB inhalation toxicity values for humans by application of allometric model extrapolation from extensive experimental animal data. The calculated 2-min adult human LCt_{50} was approximately 31 mg·min/m^3, equivalent to a 2-min LC_{50} of 15.5 mg/m^3. In contrast, the 2-min LC_{50} for female SD rats is 52 mg/m^3 (derived from a 2-min LCt_{50} of 104 mg/m^3 as reported by Mioduszewski et al. [2000, 2001, 2002a]). Therefore, the ratio of the 2-min LC_{50} values for female rats and humans is approximately 3.4 (52/15.5). This comparison indicates that, when challenged with a lethal concentration of GB vapor, adult female SD rats are likely to be more resistant than adult humans by a factor between 3.0 and 3.5.

Few comparative studies have been conducted for nonlethal end points. However, some information is available on the miotogenic potency of agent GB in several species, including humans. In a study conducted by Johns (1952), 128 adult male volunteers were exposed to agent GB concentrations ranging from 0.05-3.0 mg/m^3 for 2-20 min in an exposure chamber. 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. At the lowest test exposure level (0.05 mg/m^3 for 20 min, equal to a Ct of 1 mg·min/m^3) there was a mean maximum decrease in pupil diameter of 0.82 mm in the right eye and 1.00 mm in the left eye (total of eight observations) compared with 0.36 mm in the right eye and 0.33 mm in the left eye in controls (55 observations). Although mild miosis (defined by the author as a decrease of 1 to 2 mm in pupil diameter) was observed in some subjects at a Ct of 1.0 mg·min/m^3, other subjects exposed to the same Ct exhibited mean maximal pupil decreases of <1 mm.

Callaway and Dirnhuber (1971) evaluated the "miotogenic potency" of GB vapor in humans and rabbits exposed to GB "under goggles" (62 miosis responses in 26 human volunteers and 43 miosis responses in 10 albino rabbits). Nevertheless, it is understood that agent measurements collected during this study were hampered by the limited capabilities and techniques for determining agent vapor concentrations in the early 1970s. Further, when compared with current low-light digital methods, the protocols em-

ployed to measure rabbit miosis in Callaway and Dirnhuber (1971) are considered semisubjective. In addition, the study documentation does not fully report miosis incidence in the agent-exposed rabbit population. 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% decrement in pupil area in humans at a Ct of 3.13 mg·min/m^3 (with 95% confidence limits of 2.15-4.57 mg·min/m^3) and in rabbits at a Ct of 2.33 mg·min/m^3 (with 95% confidence limits of 1.65-3.31 mg·min/m^3). A 90% decrement in pupil area occurred in humans at a Ct of 13.85 mg·min/m^3 (with 95% confidence limits of 6.00-32.02 mg·min/m^3) and in rabbits at a Ct of 7.68 mg·min/m^3 (with 95% confidence limits of 4.90-19.50 mg-min/m^3). Callaway and Dirnhuber (1971) reported that comparison of the values for 90% area decrement suggests that the human eye "may be somewhat less sensitive to GB than the rabbit eye in that it appears to be more difficult to produce a maximal miosis with low concentrations of GB vapor in humans than in rabbits, but this has not been validated statistically."

Van Helden et al. (2001, 2002) exposed marmosets and guinea pigs (whole-body) to GB vapor concentrations at 0.05 to 150 µg/m^3 for 5 h. In guinea pigs, the LOAEL for miosis (5% decrement in pupil size compared with controls; estimated to be equivalent to approximately 10% decrement in pupil area; $p < 0.05$) was reported to be 1.8 ± 0.3 mg·min/m^3. In marmosets, the LOAEL for miosis (10% decrease in pupil size compared with controls; estimated at approximately 20% decrement in pupil area; $p < 0.05$) was reported to be 2.5 ± 0.8 mg·min/m^3. Van Helden et al. (2001, 2002) reported that the guinea pig and marmoset LOAEL values were not significantly different.

Mioduszewski et al. (2002b) exposed young adult male and female SD rats (whole-body) to a range of GB vapor concentrations (0.01-0.48 mg/m^3) for three time durations (10, 60, and 240 min). The results (ECt_{50} for miosis) are summarized in Table 1-15.

The results of the Callaway and Dirnhuber (1971), van Helden et al. (2001, 2002) and Mioduszewski et al. (2002b) studies suggest that, in terms of miosis, the response of mammalian eyes appears to be quantitatively very similar across species (including humans).

Agent VX

Interspecies differences in susceptibility to VX have also been reported. In subacute inhalation studies conducted on rats, mice, guinea pigs, and rabbits (exposures were 6 h/d, 5 d/wk, for 2 wk), Crook et al. (1983) determined from calculated LCt_{50} values that mice (LCt_{50} = 0.9 mg·min/m^3) were more sensitive to VX than rats (LCt_{50} = 24.9 mg·min/m^3), and rats were more sensitive than guinea pigs (LCt_{50} = 238.6 mg·min/m^3). Rabbits were more resistant than guinea pigs.

The detoxification potential of endogenous carboxylesterase to protect against the lethal effects of nerve agent exposure was tested by Maxwell (1992) in (male) SD rats. Nerve agents GA, GB, GD, or VX in isotonic saline were administered by subcutaneous injection. The degree of in vivo CaE inhibition was measured in the plasma, lung, and liver of exposed rats. In vivo protection provided by endogenous CaE was estimated by comparing differences in LD_{50} following nerve agent exposures to rats with inhibited CaE activity (following administration of the probe, 2-(O-cresyl)-4H-1,3,2-benzodioxaphosphorin-2-oxide) versus nerve agent exposures to rats without inhibited CaE activity. Maxwell determined that endogenous CaE in the rat provided no significant protection against in vivo lethal exposures to nerve agent VX under the experimental protocol employed; furthermore, Maxwell concluded that "CaE detoxification does not appear to be important" against exposures to lethal concentrations of agent VX.

In conclusion, the SD rat in vivo experimental results of Maxwell (1992) indicate that endogenous CaE in this species confers no protection against lethal exposures of nerve agent VX. Thus, rats exposed to VX should not be considered more robust than other species possessing a different CaE profile (e.g., humans).

4.5.4. Unique Physicochemical Properties

As discussed by Somani et al. (1992), organophosphate nerve agents consist of stereoisomers resulting from the presence of a chiral phosphorus atom in the molecule. Limited data (mainly from studies with agents GD and GB) indicate that the stereoisomers may differ considerably in their toxic potency. In general, most toxicity studies have utilized racemic mixtures of these agents.

The volatility of agent VX is 10.5 mg/m^3 at 25 °C (DA 1990). The Department of the Army considers agent VX to be "about 2,000 times less volatile than [nerve agent] GB" (DA 1990). A volatility of 3.0 ± 0.5 mg/m^3 was reported for a temperature of 25 °C in tests in which the vapor phase was in equilibrium with the aerosol phase (Frostling 1974).

4.5.5. Concurrent Exposure Issues

Two issues might be of concern: (1) simultaneous exposure to multiple nerve agents or related organophosphate compounds, and (2) simultaneous exposure through multiple exposure pathways.

Multiple Exposures to Similar Chemicals

Because of their similarity in mechanism of action, it can be expected that the toxic effects of the nerve agents would be additive. Clement (1994) and Luo and Liang (1997) reported that the toxicity of agents GB and GF were basically additive when administered together by subcutaneous injection to mice. Nevertheless, the various nerve agents are deliberately stored in separate locations and will undergo demilitarization and destruction at separate times. Furthermore, the agents are deliberately stored and secured separately prior to destruction. Thus, the chance for the release of more than one agent while under storage or during the disposal process is minimal.

The acute toxicity for numerous organophosphate insecticides in current use is identical to that of the nerve agents (i.e., initiated by cholinesterase inhibition). The vapor concentrations of insecticides causing acute toxic effects are considerably higher than nerve agent vapor concentrations producing the same end points. Information on lethality levels for some organophosphate insecticides, listed on the Registry of Toxic Effects of Chemical Substances (RTECS) (NIOSH 1999), are shown in Table 1-27. The most acutely toxic of the insecticides listed in Table 1-27 is methyl parathion, for which a 4-h LC_{50} value of 34 mg/m^3 has been reported for rats. In comparison, the 10-min LC_{50} values for rats for agents GA, GB, and GD are 45 mg/m^3, 22 mg/m^3, and 23 mg/m^3, respectively. Using a direct linear extrapolation, the corresponding 4-h LC_{50} values can be estimated to be 1.9 mg/m^3 for GA, 0.92 mg/m^3 for GB, and 0.95 mg/m^3 for GD,

TABLE 1-27 Inhalation Lethality Values for Organophosphate Pesticides

Chemical	Species	Exposure Time (h)	LC_{50} (mg/m^3)	Reference
Tetraethyl dithiopyrophosphate	Rat (f)	4	38	Kimmerle and Klimmer 1974
	Rat (m)	4	59	
Methyl parathion	Rat	4	34	EGESAQ 1980
Parathion	Rat	4	84	AMRL 1977
Phosmet	Rat	4	54	Izmerov et al. 1982
Pirimiphos-methyl	Rat	4	>150	Kagan et al. 1983
Methamidophos	Rat	4	162	Hartley and Kidd 1983-1986
Disulfoton	Rat	NA	200	Klimmer 1971
Ethion	Rat	NA	864	FCH 1991
Naled	Mouse	6	>1,500	Hartley and Kidd 1983-1986
Fonophos	Rat	1	1,900	Hartley and Kidd 1983-1986
Acephate	Mouse	5	>2,200	Berteau and Chiles 1978

Abbreviation: NA, not available.

or approximately 15- to 30-fold more toxic than methyl parathion. The toxicity of VX is considerably greater; the 10-min LCt_{50} value in mice is only 4 mg·min/m^3. Thus, the organophosphate insecticides are considerably less potent than nerve agents.

Comparison of the large differences in LC_{50} values between the G agents, agent VX, and commercial insecticides illustrates that the effects of concurrent exposure would be dominated by the more potent nerve agents. In consequence, concurrent exposure is of far less significance than exposure to each nerve agent alone.

Multiple Exposure Through Different Exposure Pathways

Nerve agents can be absorbed through the skin as well as through the respiratory tract. The extent of skin absorption of a vapor depends on the physicochemical characteristics of the agent and the presence of moisture on the skin. A comparison of the relative toxicity of the nerve agent vapors through inhalation and skin absorption can be made by evaluating the reported LCt values for each pathway.

In studies on human subjects, Freeman et al. (1954) reported that doses up to 400 mg of liquid agent GA applied to the skin of the forearm (5 mg/kg) and allowed to evaporate to dryness caused no clinical signs but resulted in a 30% decrease in RBC-ChE activity. The degree of liquid versus vapor absorption through the skin was not measured in the Freeman et al. (1954) study.

Although the human exposure study of Bramwell et al. (1963) might have provided potential percutaneous ECt_{50} values for severe or threshold effects in humans, the study is flawed by a defective protocol (no reliable estimate of agent exposure to the subjects; see discussion in Section 2.2.2).

Information on the percutaneous toxicity of the G series nerve agents and agent VX was reviewed by a subcommittee of the National Research Council Committee on Toxicology in *Review of Acute Human-Toxicity Estimates for Selected Chemical-Warfare Agents* (NRC 1997). Following evaluation of relevant human and animal studies, the NRC summarized human toxicity estimates. Differences between ECt_{50} values for mild effects resulting from vapor inhalation exposures (GA and GB, 0.5 mg·min/m^3; GD and GF, 0.2 mg·min/m^3) and the ECt_{50} values for threshold effects resulting from percutaneous vapor exposures (GA, 2,000 mg·min/m^3 ; GB, 1,200 mg·min/m^3; GD and GF, 300 mg·min/m^3) are all in excess of 10^2. The NRC

(1997) considered the GD and GF percutaneous vapor values to be in need of further research and the inhalation vapor estimates to be "low." Nevertheless, the NRC recommendations suggest that, for mild effects, the vapor inhalation pathway is several orders of magnitude (approximately 10^3) more effective than the percutaneous vapor pathway. There are similar order-of-magnitude differences for severe effects (NRC 1997).

In Chapter 6 of NRC (1997), "Review of Acute Human-Toxicity Estimates for VX," relevant human and animal studies are summarized. The NRC reported percutaneous vapor VX LCt_{50}s of 11.5 mg·min/m^3 for mice and 100-150 mg·min/m^3 for clipped goats (body-only) (Koon et al. 1960). In comparison, a whole-body (inhalation and percutaneous) vapor LCt_{50} of 9.2 mg·min/m^3 was reported for goats; the comparable value for mice was 4.0 mg·min/m^3 (Koon et al. 1960). It appears that VX vapor exposures involving inhalation are more effective in causing lethality than percutaneous vapor exposures alone; the difference in effectiveness for the lethality end point is approximately 3 for mice and between 11 and approximately 16 for goats.

Human toxicity estimates listed by NRC (1997) include a VX ECt_{50} value of 0.09 mg·min/m^3 for mild effects resulting from vapor inhalation exposures and an ECt_{50} value of 10 mg·min/m^3 for threshold effects resulting from percutaneous vapor exposures. The latter value was considered by NRC to have an associated low degree of confidence, and further research was recommended. However, these recommendations suggest that for mild effects, the vapor inhalation pathway is several orders of magnitude more effective than the percutaneous vapor pathway. For severe effects, the NRC (1997) presented an ECt_{50} value of VX at 10 mg·min/m^3 for vapor inhalation exposures and an ECt_{50} value of 25 mg·min/m^3 for percutaneous vapor exposures as interim values (low confidence, further research recommended), indicating that for this end point, the inhalation pathway is 2.5 times as effective as the percutaneous pathway for the severe effects ECt_{50}.

The issue of differential toxicity associated with physical states of the same compound has been illustrated in the case of certain industrial compounds; an example is *n*-butyl acetate (OXO Process Panel 1995). *n*-butyl acetate has commercial use in fine furniture manufacture as a vehicle in spray finish application. In studies of rats exposed to *n*-butyl acetate atmospheres generated by either evaporation (vapor exposure) or "atomization" (submicron aerosol exposure), lethality was profoundly different and almost entirely dependent on the physical state of *n*-butyl acetate to which

rats were exposed. Irritation and hypoactivity were noted in animals exposed to *n*-butyl acetate as the vapor (6,800 ppm for 4 ho), but the animals recovered within 1 d and went on to gain weight during the 14-d recovery period. Exposure to comparatively low concentrations (approximately 150 ppm v/v) of *n*-butyl acetate as the aerosol resulted in severe lung damage and mortality. During industrial spray application of finishes in wood furniture manufacture, aerosol particles of *n*-butyl acetate are extremely short-lived, and measurement of worker breathing zone exposure found only the vapor (OXO Process Panel 1995). In consequence, it was determined that toxicity information on the vapor form of *n*-butyl acetate was more appropriate than information on the aerosol form in establishing *n*-butyl acetate occupational exposure limits (ACGIH 1996).

The above examples support the need for research characterizing the emissions profile expected during VX release. Parameters essential to accurate quantification by modern methods and protocols include the following: generation and yield of vapors versus aerosols; rate of aerosol conversion to the vapor; atmospheric degradation half-times; deposition rates; and rates of degradation as influenced by humidity, temperature, and ultraviolet light. Until these parameters are more fully characterized in determinations of differential toxicity of VX vapor and aerosols, AEGL determinations will necessarily be based on the assumption of exposure to VX as the vapor.

It is acknowledged that droplets and/or aerosols may be present during certain release events. Nevertheless, the community emergency preparedness need for guidelines is presently focused on vapor exposure. There is interest and potential for developing a comparable guideline for exposures to nerve agent aerosols at some future time.

4.5.6. Critical Effect End Point

Blood cholinesterase levels are too variable to use as critical effect end points in deriving AEGLs for the nerve agents. Although there are some estimates of enzyme inhibition levels that are associated with acute effects, individual response will vary not only with baseline ChE levels but also with certain characteristics of physiological status (e.g., anemia, liver dysfunction or infection, pregnancy, etc.) which are transient and thus result in dynamic individual susceptibility through time (Lessenger and Reese 1999; Bakerman 1984; Rider et al. 1957; Ciliberto and Marx 1998; Haboubi and Thurnham 1986; Phillips 1995). Local effects on the eyes

(miosis) and upper respiratory tract (rhinorrhea) are more sensitive and consistent indicators of exposure. A number of investigators consider both miosis and rhinorrhea to be early signs of exposure to cholinesterase inhibitors. The presence of rhinorrhea can be indicative of inhalation exposure and/or development of systemic effects, although miosis alone 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 added advantage of being readily recognized and quantifiable.

The logic of not using ChE depression as a critical effect is consistent with the science policy of EPA's Office of Pesticide Programs (EPA 2000). According to EPA, there is no predetermined percentage of enzyme activity inhibition that separates adverse from nonadverse effects. The weight-of-evidence analysis advocated by this science policy document for selection of critical effects considers first the "clinical signs and other physiological and behavioral effects in humans and animals," after which "symptoms in humans" are considered, and then changes in blood cholinesterase. The recommended sequence is as follows:

1. Clinical signs and other physiological and behavioral effects in humans and animals.
2. Symptoms in humans.
3. Cental nervous system acetylcholinesterase inhibition.
4. Peripheral nervous system acetylcholinesterase inhibition.
5. Red blood cell acetylcholinesterase inhibition.
6. Plasma cholinesterase inhibition in humans and animals.

Miosis can be observed before significant ChE depression can be measured; setting AEGL values on the basis of miosis (a local effect) will protect against significant ChE activity depression (a systemic effect).

5. DATA ANALYSIS FOR AEGL-1

5.1. Summary of Human Data Relevant to AEGL-1

G-Series Agents

Candidate human data from which to develop AEGL-1 values for the G agents are available in the studies of Harvey (1952) and Johns (1952),

the study of McKee and Woolcott (1949), and the study of Baker and Sedgwick (1996). In the study described by Harvey (1952) and Johns (1952), several male volunteers who were exposed to GB at 0.05 mg/m^3 for 20 min experienced mild effects including miosis, rhinorrhea, and tightness in the chest (see Tables 1-7 and 1-8). Miosis and rhinorrhea were clinically observed. Harvey (1952) and Johns (1952) quantified miosis as the maximal decrease in pupil diameter measured with a modified fixed focus prism telescope in a clinical setting. In the study of McKee and Woolcott (1949), five male subjects were exposed to GB at 0.062 mg/m^3 for 20 min/d without any signs of clinical effects until day 4, when miosis was observed. A single exposure to GB at 0.6 mg/m^3 for 1 min or 0.06 mg/m^3 for 40 min resulted in miosis and slight tightness of the chest. In the Baker and Sedgwick (1996) study, eight healthy male servicemen who were exposed to GB at 0.5 mg/m^3 for 30 min developed miosis and several also exhibited photophobia and dyspnea. In addition, RBC-ChE activity was inhibited to approximately 60% of individual baseline at 3 h and 3 d postexposure, and small but measurable changes occurred in single fibre electromyography (SFEMG) of the forearm. The latter, which were detectable in the lab between 4 and 15 mo postexposure, were not considered clinically significant by Baker and Sedgwick. The SFEMG changes were not detectable after 15-30 mo.

In tests on 125 volunteers, Oberst et al. (1968) observed no signs or symptoms of toxicity in resting men (breathing rate about 7 L/min) following 2-min exposures to an average GB concentration of 20.7 mg/m^3 or in exercising men (breathing rate 50 L/min) following 2-min exposures to an average GB concentration of 4.19 mg/m^3. Linear extrapolation of the lower concentration results in a 30-min exposure to GB at 0.27 mg/m^3, less than the exposure used in the Baker and Sedgwick (1996) study.

The above studies do not agree in identifying the concentration of GB at which effects first appear, and there are even inconsistencies within some of the studies. One possibility for the conflicting results is human variability, since few subjects were used in each study. Another possibility is that the analytical measurements were not accurate. There are no details on analytical procedures in the study of Harvey (1952) or Johns (1952), where it is stated that "known concentrations" were used. The Baker and Sedgwick (1996) study provides a description of the analytical method and indicates that exposure concentrations were verified before and after exposure.

Agent VX

Clearly defined human concentration-response data for low-level inhalation exposures to agent VX are not available. The human toxicity studies that have been conducted with VX are not considered adequate for deriving exposure limits. The study conducted by Bramwell et al. (1963) suggests that an inhalation dose of about 8 μg/kg causes a 50% ChE depression as well as signs of toxicity including miosis, rhinorrhea, and nausea; however, this suspect study involved multiple exposures to the same individuals, short exposure durations (maximum of 7 min), and an experimental protocol (open tunnel rather than exposure chamber) in which the individual exposures to VX may have varied. The Bramwell et al. (1963) study is therefore not considered credible because of its seriously flawed exposure protocol.

Other experimental data indicate that inhalation exposures equivalent to internal doses in the range of 0.01-0.13 μg/kg result in mild signs of toxicity and no change in ChE (Koon et al. 1959). As extrapolated from historical animal data, the human ECt_{50} for miosis has been estimated at 0.09 mg·min/m^3 (Reutter and Wade 1994).

5.2. Summary of Animal Data Relevant to AEGL-1

G-Series Agents

Acute inhalation toxicity data are available for agents GA and GB for several animal species. In most cases, the studies were designed to estimate LCt_{50} values, and they are not directly suitable for application to an AEGL-1 estimation. Several studies, however, have identified minimal effect levels. Van Helden et al. (2001, 2002) reported LOAELs for miosis of 2.5 ± 0.8 mg·min/m^3 for marmosets and 1.8 ± 0.3 mg·min/m^3 for guinea pigs exposed to agent GB for 5 h. The LOAEL values for miosis in the two species were not statistically different (van Helden et al. 2001, 2002). Mioduszewski et al. (2002b) reported ECt_{50} values for miosis in male and female SD rats exposed (whole-body) to GB vapor for time durations of 10, 60, and 240 min. Miosis was defined by the authors as "post-exposure pupil diameter 50% or less of the pre-exposure pupil diameter." The ECt_{50} determinations for both genders are summarized in Table 1-21.

In studies conducted by Harris et al. (1953), dogs were able to tolerate daily exposures to GB at an average Ct of 10.5 mg·min/m^3 (equivalent to an average concentration of 0.53 mg/m^3 for 20 min), 5 d/wk, for 2 mo. The only reported clinical sign was miosis, which appeared with each exposure but disappeared before the next exposure. However, when each daily exposure was increased to 15 mg·min/m^3, toxic signs (body tremors, dyspnea, loss of muscle control, convulsions) occurred within 7-10 d and several dogs died. Henderson et al. (2000, 2001, 2002), Conn et al. (2002), and Kalra et al. (2002) exposed male F344 rats to GB at 0.2 mg/m^3 or 0.4 mg/m^3 (nose-only) for 1 h/d for 1 d, 5 d, or 10 d, with sacrifices at 1 d after exposure and at 1 mo after exposure. Henderson et al., Conn et al., and Kalra et al. reported that there were no overt signs or symptoms of neurotoxicity (tremors) under non-heat stress conditions at either GB exposure concentration and that single GB exposures "did not alter body weight, breathing patterns, routine brain histopathology, or apoptosis in brain cells."

Agent VX

There are no single exposure studies available for deriving AEGL-1 values for VX. In a nonverifiable study, Crook et al. (1983) reported no signs of toxicity except miosis in rats, mice, guinea pigs, or rabbits exposed to VX vapor concentrations up to 0.0002 mg/m^3 for 6 h/d, 5 d/wk, for 2 wk. A test concentration of 0.004 mg/m^3 resulted in rat and mice mortality. The available animal data indicate that VX does not cause reproductive or developmental toxicity, and there is no evidence suggesting that VX is genotoxic or carcinogenic.

In an examination of miotogenic potency, Callaway and Dirnhuber (1971) consider that agent VX is an order of magnitude more effective than agents GB or GD at producing miosis in the eyes of male and female albino rabbits.

5.3. Derivation of AEGL-1 for Agent GB

The estimation of interim AEGL-1 values relied on the Harvey (1952) study (66 Fed. Reg. 21940 [2001]). Of 14 individuals exposed to the lowest concentration for the longest exposure time (0.05 mg/m^3 for 20 min), the

following signs and symptoms were reported: two headaches, two eye pain, three rhinorrhea, one tightness in the chest, one cramps, one nausea, and two malaise. Of human studies available, this analysis gave the lowest LOAEL of 0.05 mg/m^3 for a 20-min exposure and was chosen as the basis for deriving the interim AEGL-1 values. The miosis effects data of Johns (1952) were considered as supportive. The subjects were male "normal human volunteer" service personnel between the ages of 22 and 59 and under clinical supervision during the periods of exposure as well as for postexposure periods of several months. Derivation of the interim values is detailed in Appendix A.

The final analysis relies on the Mioduszewski et al. (2002b) study of miosis induction to young adult SD rats as the basis for AEGL-1 estimation, with retention of van Helden et al. (2001, 2002; marmosets), Harvey (1952) (humans) and Johns (1952) (humans) as secondary and supportive studies.

The selection of miosis induction as the basis for deriving final AEGL-1 values is supported by the evaluation of a U.S. Surgeon General's review panel on agent exposure limits convened by the Chemical Demilitarization Branch of the National Center for Environmental Health of the Centers for Disease Control and Prevention (CDC) (67 Fed. Reg. 894 [2002]; DHHS 2002). Although the CDC has not yet finalized its position, the review panel generally concluded that cholinesterase activity depression is too variable for application as a critical effect in the estimation of nerve agent exposure limits and that miosis is an appropriate and readily quantified critical effect.

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 (0.01-0.48 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 employed in this well-conducted study documenting highly credible protocols for GB vapor generation and measurement. A sufficient number of individual animals were exposed at each interval (10 min, 52 female SD rats; 60 min, 35 female SD rats; 240 min, 55 female SD rats). Analysis of rat pupil diameters assessed pre- and postexposure allowed generation of EC_{50} determinations 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 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). As the female rat appears to be more susceptible than the male for at least two of the AEGL exposure durations of interest, the AEGL-1 estimations are calculated from the female data set. This data set selection for the most susceptible gender will provide a more protective estimation of AEGL-1. This is a well-defined animal end point in a susceptible gender and is transient, reversible, and nondisabling. (Further details of this study are provided in Section 3.2.3.)

Data from the GB vapor study of nonhuman primates (marmosets; 5 h exposures to GB vapor concentrations of 0.05 to 150 µg/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 of 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]). The studies of Harvey (1952) and Johns (1952) also show that miosis is transient and reversible, with reversibility occurring within hours to days (depending on degree of miosis). This is consistent with other human data documenting miosis after nerve agent vapor exposures. In consequence, with the knowledge that the EC_{50} exhibited by rats in the study of Mioduszewski et al. (2002b) is also transient and reversible, the determination is made that EC_{50} for miosis in female SD rats is an appropriate end point for estimating AEGL-1 values (Mioduszewski et al. 2002b).

Because exposure-response data were unavailable for all of the AEGL-specific exposure durations, temporal extrapolation was used in the development of AEGL values for 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 to 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 time durations of 10 min to 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, while 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 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, due to uncertainties associated with some of the exposure measurements in these earlier studies, the Mioduszewki et al. rat data were determined to be the best source of an estimate for n.

Derivation of AEGL-1 Values Using Animal Data

AEGL-1 values can be derived from the data set presented by van Helden et al. (2001, 2002) for GB-induced miosis in marmosets exposed to agent GB vapor for 5 h, as well as the data set presented by Mioduszewski et al. (2002b) for rats exposed to GB vapor for 10, 60, and 240 min.

Van Helden et al. (2001, 2002) reported a LOAEL for threshold miosis of 2.5 ± 0.8 mg·min/m^3, and considered miosis to be significantly different ($p < 0.05$) from controls when a 10% decrease in marmoset pupil size was observed (estimated to be equivalent to an approximate 20% decrement in pupil area). Van Helden et al. (2001, 2002) also reported that there was no significant difference between the LOAEL for miosis in marmosets and in guinea pigs. The EPA IRIS database and the NLM Hazardous Substances Databank were searched for additional information on the miotogenic response of marmosets to cholinesterase inhibitors, but no relevant data were found.

The recent miosis and lethality data of Mioduszewski et al. (2000, 2001, 2002a,b) in rats have been subjected to regression analysis (see Appendix B). In consequence, the nonlethality (miosis) and lethality data of Mioduszewski and his colleagues are determined to be the best source of

an estimate for the *n* value for GB response. The Mioduszewki et al. (2000, 2001, 2002a,b) data sets are robust and compound-specific for the most completely characterized G-series nerve agent, agent GB. As outlined earlier, the mechanism of mammalian toxicity for nerve agents is known, and all end points observed in human and animal studies represent a response continuum to anticholinesterase exposure. Accordingly, it is valid to apply an *n* value derived from compound-specific miosis and lethality data to time scaling for nonlethal as well as lethal effects. This position is consistent with that of the recently published science policy of the EPA Office of Pesticide Programs (EPA 2000). Furthermore, this approach is preferable to the use of default values.

For AEGL-1 derivation, an interspecies uncertainty factor (UF) of 1 and an intraspecies UF of 10 were used, resulting in a composite UF of 10. To estimate an interspecies UF, miosis data for a number of species were compared. Van Helden et al. (2001, 2002) exposed marmosets and guinea pigs (whole-body) to GB vapor to estimate a LOAEL for miosis in both species. They determined that there was no significant difference between guinea pigs and marmosets at the 5% level. Contact with leading investigators in the field (H. van Helden, Pulmonary and CNS Pharmacology Lab, TNO, the Netherlands, personal communication; S. Tattersall, Biomedical Sciences Division at Porton Down, United Kingdom, personal communication) was performed to determine availability of experimental data characterizing miosis following nerve agent vapor exposure to mammals. Dr. Tattersall pointed out that Porton Down has not performed systematic measurements of miosis in recent years, and that the only other extant report of relevant data was Callaway and Dirnhuber (1971), cited in this document. These investigators have independently concluded that the miotogenic response of mammalian eyes to agent GB vapor exposure is quantitatively similar across species, including standard laboratory animals (rabbits and guinea pigs), nonhuman primates (marmosets), and humans (please see Table 1-21). In consequence, the interspecies UF for the AEGL-1 end point of miosis in young adult female SD rats is set equal to 1.

The intraspecies UF of 10 used in the derivation of the AEGL-1 is based on the known polymorphic 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 protection of susceptible populations.

The database for agent GB is reasonably complete. Strong arguments for not incorporating an additional modifying factor include the following:

- Data are available for multiple species.
- Data characterizing both lethal and nonlethal end points have been used in the analysis; the end points possess exposure-response data.
- The mechanism of toxicity is known.
- The *n* value is derived from experimental data and is not the default.
- There are no uncertainties regarding reproductive and developmental effects or issues of carcinogenicity.

In consequence, no modifying factor was used in the estimation of AEGL-1 values.

For comparison, from the marmoset data of van Helden et al. (2001, 2002), *k* was derived using a composite UF of 10.

$([0.0083\ mg/m^3]/10)^2 \times (5\ h) = k;$
$k = 3.4 \times 10^{-6}\ mg/m^3 \times h.$

From the experimental data of Mioduszewski et al. (2002b), *k* was derived as follows for the 10-min to 30-min extrapolation:

$([0.068\ mg/m^3]/10)^2 \times (10/60)\ h = k;$
$k = 7.7 \times 10^{-6}\ mg/m^3 \times h.$

For the 4-h to 8-h extrapolation, *k* was derived as

$([0.012\ mg/m^3]/10)^2 \times 4\ h = k;$
$k = 5.8 \times 10^{-6}\ mg/m^3 \times h.$

The Interim AEGL-1 estimates and the estimates from the van Helden et al. (2001, 2002) (marmoset; 5-h exposure) and Mioduszewski et al. (2002b) (female SD rat; 10-min, 60-min, and 240-min exposures) data sets are summarized for comparison in Table 1-28 below. The interim values (66 Fed. Reg. 21940 [2001]) are bolded.

Comparison of AEGL estimates from this rich database for GB vapor-induced miosis in the eyes of mammals exhibits remarkable concordance and corroboration across species. There is little to no change between the interim estimates derived from historical human data (Harvey 1952; Johns 1952; 66 Fed. Reg. 21940 [2001]) and that derived from the female rat miosis data published in 2002 (Mioduszewski et al. 2002b). Any differences are usually a single digit in the fourth decimal place. Estimates based

TABLE 1-28 Alternate AEGL-1 Estimates for Nerve Agent GB

Time Period	Interim Value (66 Fed. Reg. 21940 [2001])[a] (mg/m^3)	Alternate 1[b] (mg/m^3)	Alternate 2[c] (mg/m^3)
10 min	0.0069	0.0045	0.0068
30 min	0.0040	0.0026	0.0039
1 h	0.0028	0.0019	0.0020
4 h	0.0014	0.00092	0.0012
8 h	0.0010	0.00065	0.0010

Note: $n = 2$; interspecies UF = 1 (research staff of TNO and Porton Down consider miosis response in all mammal eyes exposed to nerve agent vapors to be similar across species); intraspecies UF = 10 (adjustment for possible susceptible individuals); total UF = 10.

[a]Determined using human data from Harvey (1952) and Johns (1952) (20-min exposure). See Appendix A for details of derivation.

[b]Determined using marmoset miosis data from van Helden et al. (2002) (5-h exposures).

[c]Determined using female SD rat miosis data from Mioduszewski et al. (2002b) (10-min, 60-min, and 240-min exposures).

on marmoset data (a single exposure period of 5 h) differ from the interim values by an approximate factor of 1.5. Given that variation of this magnitude in the AEGL estimates does not reflect response differentiation with any precision, the GB interim values for AEGL-1 are considered adequately representative and protective against miosis resulting from GB vapor exposure to the public. The female rat miosis experiment of Mioduszewski et al. (2002b) is the critical study for final AEGL-1 determination.

The recommended AEGL-1 values are summarized in Table 1-29. The calculations of exposure concentrations for female SD rats and humans scaled to AEGL-1 time points are shown in Appendix A.

5.4. Derivation of AEGL-1 Values for Agents GA, GD, and GF

The relative potency approach was used to estimate AEGL-1 values for agents GA, GD, and GF. A discussion of the relative toxic potencies for these agents is given in Section 4.3. It was determined that for the end point of miosis, the effect usually observed at the lowest exposure concen-

TABLE 1-29 AEGL-1 Values for Agent GB (mg/m^3 [ppm])

10 min	30 min	1 h	4 h	8 h
0.0069 mg/m^3 (0.0012 ppm)	0.0040 mg/m^3 (0.00068 ppm)	0.0028 mg/m^3 (0.00048 ppm)	0.0014 mg/m^3 (0.00024 ppm)	0.0010 mg/m^3 (0.00017 ppm)

trations, the potency of GA is identical to that of GB. Agents GD and GF are each considered approximately twice as potent as agents GB or GA for miosis, and equipotent to each other for AEGL-1 effects. Thus, the AEGL-1 concentration values for agents GD and GF are equal to 0.5 times the values derived for agents GA and GB (Table 1-30).

5.5. Derivation of AEGL-1 for Agent VX

Because of inadequacies in the human and animal toxicologic database for agent VX, the present analysis recommends that the AEGL-1 for agent VX be derived from the critical study (Mioduszewski et al. 2002b) for the agent-GB AEGL-1 using a relative potency approach. The experimental protocol for the Mioduszewski et al. (2002b) study is described fully in Section 3.2.1.

A relative potency (RP) of 4 is used to derive the AEGLs for VX. The well-conducted (and clinically supervised) human exposure studies of Grob and Harvey (1958) and Sidell and Groff (1974) report RBC-ChE_{50} values following single oral or intra-arterial/intravenous exposures to GB and VX (see analysis presented in Tables 1-23 and 1-24). Of the values derived from available human data, the GB:VX ratio (RP = 4.3, rounded to 4.0) calculated from oral dose exposures needed to achieve RBC-ChE_{50} is the most appropriate for the present application. Details of this logic are provided in Section 4.3. The comparative miosis study of Callaway and Dirnhuber (1971) is considered secondary and supportive of the concept that agent VX is more potent than GB for the miosis end point.

By applying an RP factor of 4 to the miosis data set of Mioduszewski et al. (2002b), the comparative concentrations for VX were estimated to be one-fourth that of GB, or 0.017 mg/m^3, for a 10-min exposure, 0.005 mg/m^3 for a 60-min exposure, and 0.003 mg/m^3 for a 240-min exposure. The VX concentrations were further adjusted by a composite UF of 30; 1 for

TABLE 1-30 AEGL-1 Values for Agents GA, GD, and GF (mg/m^3 [ppm])

Agent	10 min	30 min	1 h	4 h	8 h
GA	0.0069 mg/m^3 (0.0010 ppm)	0.0040 mg/m^3 (0.00060 ppm)	0.0028 mg/m^3 (0.00042 ppm)	0.0014 mg/m^3 (0.00021 ppm)	0.0010 mg/m^3 (0.00015 ppm)
GD	0.0035 mg/m^3 (0.00046 ppm)	0.0020 mg/m^3 (0.00026 ppm)	0.0014 mg/m^3 (0.00018 ppm)	0.00070 mg/m^3 (0.000091 ppm)	0.00050 mg/m^3 (0.000065 ppm)
GF	0.0035 mg/m^3 (0.00049 ppm)	0.0020 mg/m^3 (0.00028 ppm)	0.0014 mg/m^3 (0.00020 ppm)	0.00070 mg/m^3 (0.00010 ppm)	0.00050 mg/m^3 (0.000070 ppm)

interspecies uncertainty (miosis response is similar across species), 10 for intraspecies variability to accommodate known human variation in ChE and carboxylesterase activity (protection of susceptible populations), and a modifying factor of 3 for the sparse VX data set. To derive AEGL-1 values for different time periods (10min to 30 min and 4 h to 8 h), the data were scaled using the relationship $C^n \times t = k$ (ten Berge et al. 1986). An n value has not been determined experimentally for VX; however, because the primary mechanism of action (cholinesterase inhibition) is the same as that for agent GB, the n value of 2 used in the derivation of the AEGL values for GB is also appropriate for deriving all AEGL values for VX. 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 is here used as the scaling function for the agent-VX AEGL-1 values, rather than a default value. Until additional data from well-conducted experimental studies are available, the current value of n is reasonable, is supported by existing data, and meets requirements of the standing operating procedures for estimating AEGL values (NRC 2001).

The10-min to 30-min extrapolation was

$C^2 \times t = k$;
([0.017 mg/m^3]/30])2 × (10/60) h = k;
$k = 5.0 \times 10^{-8}$ $mg/m^3 \times h$.

The 4-h to 8-h extrapolation was

$$C^2 \times t = k;$$
$$([0.003 \text{ mg/m}^3]/30)^2 \times 4 \text{ h} = k;$$
$$k = 4.0 \times 10^{-8} \text{ mg/m}^3 \times \text{h}.$$

The resulting AEGL-1 values for VX are summarized in Table 1-31. The calculations of exposure concentrations for humans scaled for all AEGL-1 time points are shown in Appendix A.

6. DATA ANALYSIS FOR AEGL-2

6.1. Summary of Human Data Relevant to AEGL-2

Human data to derive an AEGL-2 for the G agents are provided in the studies of Harvey (1952), Johns (1952), and Baker and Sedgwick (1996). In the Harvey (1952) study an array of signs and symptoms, including headache, eye pain, dimness of vision, twitching of eyelids, rhinorrhea, salivation, throat irritation, tightness in the chest, cramps, nausea, vomiting, giddiness, difficulty in concentrating, and malaise were reported in individuals exposed to GB at 0.3 mg/m^3 for 20 min. Twelve subjects were exposed at this GB concentration—all experienced rhinorrhea, eight suffered from headaches, and seven reported dimness of vision. In the Baker and Sedgwick (1996) study, eight healthy male servicemen who were exposed to GB at 0.5 mg/m^3 for 30 min developed miosis, and several also exhibited photophobia and dyspnea. In addition, RBC-ChE activity was inhibited to approximately 60% of individual baseline at 3 h and 3 d postexposure, and small but measurable changes occurred in single fibre electromyography (SFEMG) of the forearm. The latter effects, which were detectable in the lab between 4 and 15 mo postexposure, were "not significantly different from the control value," with "control" defined as preexposure baseline readings for each individual subject (Baker and Sedgwick 1996). The SFEMG changes were not detectable after 15-30 mo.

6.2. Summary of Animal Data Relevant to AEGL-2

Animal inhalation data are insufficient to derive AEGL-2 values.

TABLE 1-31 AEGL-1 Values[a] for Agent VX (mg/m^3 [ppm])

10 min	30 min	1 h	4 h	8 h
0.00057 mg/m^3 (0.000052 ppm)	0.00033 mg/m^3 (0.000030 ppm)	0.00017 mg/m^3 (0.000016 ppm)	0.00010 mg/m^3 (0.0000091 ppm)	0.000071 mg/m^3 (0.0000065 ppm)

[a]The AEGL values are for vapor exposures only.

6.3. Derivation of AEGL-2 for Agent GB

The present analysis applies the Baker and Sedgwick (1996) study as the basis of the AEGL-2 values. Of the human studies conducted on GB that were available for evaluation, the Baker and Sedgwick study is recent, was conducted following a rigorous experimental protocol, and used modern analytical methods for determining the exposure concentrations (GB at 0.5 mg/m^3 for 30 min). Furthermore, this study was performed under Helsinki accords and clinical supervision and was conducted with the cooperation of fully informed human subjects ($N = 8$, "fit male servicemen"). The observed effects included miosis in eight of eight subjects, dyspnea and photophobia in some individuals (number not given), inhibition of RBC-ChE to approximately 60% of individual baseline at 3 h and 3 d postexposure in (eight of eight subjects), and small but measurable changes in single fibre electromyography (SFEMG) of the forearm (in five of eight subjects). Nevertheless, the fact that the SFEMG abnormalities were detectable in the lab between 4 and 15 mo postexposure makes these effects long-lasting, and they are therefore included under the definition of AEGL-2.

Respiratory effects resolved within minutes, and visual effects resolved within 48 h. 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 to be a possible early indicator or precursor of the nondepolarising neuromuscular block found associated with intermediate syndrome paralysis in severe organophosphorous insecticide poisoning cases (Senanayake and Karalliedde 1987). The study concluded that these electromyographic changes were persistent (>15 mo), but that they were reversible and subclinical. Subclinical and reversible effects are not normally included within the definition of AEGL-2 effects. However, because SFEMG changes may be a precursor of intermediate syndrome (see Section

4.5.2), and because of the steepness of the dose-response curve for nerve agents, the use of this end point for establishing AEGL-2 values is considered a protective approach. This concept of added precaution for steep dose-response is consistent with emergency planning guidance for nerve agents previously developed by the National Center for Environmental Health of the Centers for Disease Control and Prevention (Thacker 1994).

As previously described in the development of AEGL-1 values for the G agents (Sections 5.3 and 5.4), an *n* value of 2 derived from a linear regression of both miosis and lethality data for GB vapor exposure to female SD rats (Mioduszewski et al. 2000, 2001, 2002a,b) is appropriate for use as a scaling function for all nerve agents. AEGL-2 values for exposure times different from the experimental time of 30 min were thus scaled using an *n* of 2.

A composite UF of 10 was used in the calculation. 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). Because human data were used, an interspecies UF was not required. The database for agent GB is reasonably complete. As was true for the AEGL-1 estimations, there are strong arguments for not incorporating an additional modifying factor. In consequence, no modifying factor was used in the estimation of AEGL-2 values.

From the experimental data, *k* was derived as

$([0.5\ mg/m^3]/10)^2 \times (0.5\ h) = k;$
$k = 0.0013\ mg/m^3 \times h.$

The resulting estimates of AEGL-2 are summarized in Table 1-32.

6.4. Derivation of AEGL-2 Values for Agents GA, GD, and GF

The relative potency approach is used to estimate AEGL-2 values for agents GA, GD, and GF. A discussion of the relative toxic potencies for these agents is given in Section 4.3. It was determined that for the end point of miosis, the effect usually observed at the lowest exposure concentrations, the potency of GA is identical to that of GB. Agents GD and GF are each considered approximately twice as potent as agents GB or GA for

TABLE 1-32 AEGL-2 Values for Agent GB (mg/m^3 [ppm])

10 min	30 min	1 h	4 h	8 h
0.087 mg/m^3 (0.015 ppm)	0.050 mg/m^3 (0.0085 ppm)	0.035 mg/m^3 (0.0060 ppm)	0.017 mg/m^3 (0.0029 ppm)	0.013 mg/m^3 (0.0022 ppm)

miosis, and equipotent to each other for AEGL-2 effects. Thus, the AEGL-2 concentration values for agents GD and GF are equal to 0.5 times those values derived for agents GA and GB (Table 1-33).

6.5. Derivation of AEGL-2 Values for Agent VX

Acute inhalation toxicity studies on animals have identified median lethal concentrations; however, these studies are inadequate for deriving AEGL-2 values because of the lack of dose-response data for the appropriate time periods. Some information for agent VX is available from a repeat exposure study in which a VX concentration of 0.004 mg/m^3 for 6 h/d, 5 d/wk, for 2 wk resulted in severe signs of toxicity (tremors, convulsions, salivation, and bloody tears) and 100% mortality of mice, 35% mortality in rats, and 3% mortality in guinea pigs (Crook et al. 1983). Exposure to 0.0002 mg/m^3 under the same experimental protocol resulted in no toxic signs but miosis and ChE depression. The Crook data set is considered nonverifiable. An AEGL-2 effect for a single 6-h exposure would most likely fall within the range of 0.0002 and 0.004 mg/m^3.

There are no definitive data identifying the minimal exposure level at which severe, irreversible, or escape-impairing effects of acute exposure to agent VX would occur. Because of the inadequacy of the human and animal toxicologic database for agent VX, the AEGL-2 for agent VX is derived from the AEGL-2 for agent GB using a relative potency approach.

The Baker and Sedgwick (1996) study of GB vapor exposure in human volunteers is used as the basis of the AEGL-2 values for agent VX, as described in Section 6.3.

By applying a relative potency of 4, the comparable VX exposure is one-fourth that of GB, or 0.125 mg/m^3, for a 30-min exposure. The VX concentration was adjusted by a composite UF of 30; 1 for interspecies uncertainty (human data), 10 for intraspecies variability to accommodate known human variation in ChE activity (protection of susceptible populations), and a modifying factor of 3 for the sparse VX data set. To derive AEGL-2 values for different time periods, the data were scaled using the

TABLE 1-33 AEGL-2 Values for Agents GA, GD, and GF (mg/m^3 [ppm])

Agent	10-min	30-min	1-h	4-h	8-h
GA	0.087 mg/m^3 (0.013 ppm)	0.050 mg/m^3 (0.0075 ppm)	0.035 mg/m^3 (0.0053 ppm)	0.017 mg/m^3 (0.0026 ppm)	0.013 mg/m^3 (0.0020 ppm)
GD	0.044 mg/m^3 (0.0057 ppm)	0.025 mg/m^3 (0.0033 ppm)	0.018 mg/m^3 (0.0022 ppm)	0.0085 mg/m^3 (0.0012 ppm)	0.0065 mg/m^3 (0.00085 ppm)
GF	0.044 mg/m^3 (0.0062 ppm)	0.025 mg/m^3 (0.0035 ppm)	0.018 mg/m^3 (0.0024 ppm)	0.0085 mg/m^3 (0.0013 ppm)	0.0065 mg/m^3 (0.00091 ppm)

relationship $C^n \times t = k$ (ten Berge et al. 1986). An n value has not been determined experimentally for VX. However, because the mechanism of action (cholinesterase inhibition) is the same as that for agent GB, the n value of 2, as used in the derivation of the AEGL values for GB, is also appropriate for deriving AEGL values for VX. In consequence, the experimentally derived $n = 2$ from the Mioduszewski et al. (2000, 2001, 2002a,b) rat lethality data set for agent GB is here used as the scaling function for the agent VX AEGL-2 values, rather than a default value; therefore

$C^2 \times t = k$;
$([0.125 \text{ mg/m}^3]/30)^2 \times 0.5 \text{ h} = k$;
$k = 8.7 \times 10^{-6} \text{ mg/m}^3 \times \text{h}$.

The resulting AEGL-2 values are summarized in Table 1-34. The calculations of exposure concentrations for humans scaled for all AEGL-2 time points are shown in Appendix A.

7. DATA ANALYSIS FOR AEGL-3

7.1. Summary of Human Data Relevant to AEGL-3

Human lethality data resulting from exposure to any of the G agents were not available for deriving an AEGL-3.

TABLE 1-34 AEGL-2 Values[a] for Agent VX (mg/m^3 [ppm])

10 min	30 min	1 h	4 h	8 h
0.0072 mg/m^3 (0.00065 ppm)	0.0042 mg/m^3 (0.00038 ppm)	0.0029 mg/m^3 (0.00027 ppm)	0.0015 mg/m^3 (0.00014 ppm)	0.0010 mg/m^3 (0.000095 ppm)

[a]The AEGL values are for vapor exposures only.

7.2. Summary of Animal Data Relevant to AEGL-3

Agent GB

Data on the lethality of GB are available for several laboratory species (see Table 1-9). Mioduszewski et al. (2000, 2001, 2002a) reported LCt_{50} and LC_{50} values for rats for exposure time periods of 10, 30, 60, 240, and 360 min. Bide et al. (1999) (see also Yee et al. [1999]), determined LC_{50} values for mice for time periods of 1 s to 30 min and estimated LC_{50} values for five other laboratory species and humans using a three-dimensional probit model.

Agent GD

In an experimental exposure 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 $mg \cdot min/m^3$. Aas et al. (1985) graphically present their data as an LCt-versus-mortality curve. As estimated from this curve, the lethality threshold for rats exposed to GD is about 335 $mg \cdot min/m^3$. Because the reported GD air concentration was fixed at 21 mg/m^3, the exposure time corresponding to the threshold was back-calculated to equal 16 min.

Note that 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. 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).

Agent GF

A recent study of GF vapor inhalation toxicity in male and female SD rats reported 24-h postexposure LC_{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 accommodations for the lesser volatility of agent GF. For female rats, Anthony et al. (2002) report 24-h postexposure 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 postexposure 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. The preliminary data of Anthony et al. (2002) document 24-h lethality and LC_{50} only (Table 1-16). In consequence, these data are not comparable to the 14-d postexposure rat LC_{01} information available from the Mioduszewski et al. studies for GB vapor inhalation lethality. Furthermore, the preliminary nature of the Anthony et al. (2002) documentation precludes LC_{01} determination by benchmark dose analysis at this time.

7.3. Derivation of AEGL-3 for Agents GB and GD

Agent GB

The most complete lethality data set for the relevant time periods is that presented by Mioduszewski et al. (2000, 2001, 2002a). The final report of this study (Mioduszewski et al. 2001, 2002a) is further documentation of the findings presented below. 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. 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 54 mg/m^3. Agent concentrations were confirmed in the exposure chamber by three procedures to allow point and continuous determinations (Mioduszewski et al. 2000, 2001, 2002a). Lethality was assessed at 24 h and at 14 d postexposure. Female rats were reported to be more sensitive to GB vapor toxicity than males over the range of exposure concentrations and durations studied. Please note that

comparison of LCt_{50} values for male and female rats exposed to vapor concentrations of GB from Mioduszewski et al. (2000, 2001, 2002a) and Callaway and Blackburn (1954) reports indicates that the range of ratios (F:M) is 0.54 to 0.80, with a geometric mean of 0.67 (see Table 1-25). Gender differences for lethality are reported by Mioduszewski et al. (2000, 2001, 2002a) to be statistically 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 agent 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. Based on a probit analysis of the data (Mioduszewski et al. 2000), the estimated LC_{01} values for the females are as follows: 11.537 mg/m^3 for 10 min, 5.836 mg/m^3 for 30 min, 4.006 mg/m^3 for 60 min, 2.087 mg/m^3 for 4 h, and 1.761 mg/m^3 for 6 h. Mioduszewski et al. (2000) note that these estimates of LC_{01} are associated with large error bars.

The AEGL-3 for agent GB was derived from the lethality data for female Sprague-Dawley rats Mioduszewski et al. (2000, 2001, 2002a).

Regarding selection of the species to be used in neurotoxicity tests, the EPA Health Effects Test Guidelines (OPPTS 870.6200, *Neurotoxicity Screening Battery*) published by the Office of Prevention, Pesticides and Toxic Substances (EPA 1998) state that "in general, the laboratory rat should be used." The experimental protocol of Mioduszewski et al. (2000, 2001, 2002a) followed the OPPTS guidelines concerning the species, age, gender, and number of animals per dose and control group. Furthermore, in their recent review of organophosphates insecticide toxicity data, Storm et al. (2000) consider rat organophosphate inhalation data to be a defensible basis for developing (occupational) exposure limits, especially in the absence of human exposure data.

As previously discussed in the text on AEGL-1 and AEGL-2 values, the recent miosis and lethality data of Mioduszewski et al. (2000, 2001, 2002a,b) are determined to be the best source of an estimate for the n value for GB response (see Appendix B). Therefore, $n = 2$ is used as the scaling function for AEGL-3 derivations in the equation ($C^n \times t = k$) according to the methods of ten Berge et al. (1986) to derive an 8-h AEGL-3 from the 6-h LC_{01}. All the other time-specific AEGL-3 values were derived directly from the LC_{01} values for female SD rats in the Mioduszewski et al. (2000) study.

Given that the AEGL-3 estimation for the G-series nerve agents is derived from a lethal inhalation toxicity study of adult female SD rats (Mioduszewski et al. 2000, 2001 2002a), it is reasonable to consider the

whole-organism response of lethality as an appropriate end point by which to compare data for rats (a CaE-rich species) with data for monkeys and dogs, two experimental species considered in earlier studies to possess no plasma carboxylesterase (Augustinsson 1959). Available experimental LCt_{50} data for the monkey, dog, and rat are presented in Table 1-13. As shown in Table 1-13, LCt_{50} values for 10-min exposures to GB are 310 mg·min/m^3 in mice, 181-226 mg·min/m^3 in rats, 60 mg·min/m^3 in dogs, and 74 mg·min/m^3 in monkeys. There is a 2- to 3-fold difference between rats and monkeys, and a 3- to 4-fold difference between rats and dogs. These comparisons indicate that, when challenged with a lethal concentration of GB vapor, adult female SD rats are more resistant than adult dogs or monkeys by approximate factors of 2 to 4. Species differences in carboxylesterase concentrations may account, in part, for these observed differences. Please see Section 4.5.2 for a more detailed discussion of carboxylesterases as detoxification enzymes for nerve agent exposures.

In the case of human lethality estimates, Bide et al. (1999) estimate GB inhalation toxicity values for humans by application of allometric model extrapolation from extensive experimental animal data. Their study estimates that a 2-min adult human LCt_{50} approximates 31 mg·min/m^3 (a 2-min LC_{50} of 15.5 mg/m^3). The resulting 2-min LC_{50} ratio with the female SD rat from Mioduszewski et al. (2000, 2001, 2002a) (2-min LCt_{50} of 104 mg/m^3 or 2-min LC_{50} at 52 mg/m^3) is

female SD rat:human (estimated) = 52/15.5 = 3.4.

This comparison indicates that, when challenged with a lethal concentration of GB vapor, adult female SD rats (Mioduszewski et al. 2000, 2001) are likely to be more resistant than adult humans by a factor between 3.0 and 3.5.

The following summarizes the above analysis of interspecies UF for AEGL-3 estimates:

- The literature regarding carboxylesterase (CaE) in lab animals and humans indicates that CaE is present in human plasma as well as numerous other human tissues and organs (including those where exposure and distribution leading to death by G agent vapor toxicity would likely occur).
- Interspecies data for comparison of the whole-organism response of lethality indicates that, when challenged with a lethal concentration of GB vapor, adult female SD rats are more resistant than adult dogs or monkeys by approximate factors of 2 to 4. Species differences in carboxyl-

esterase concentrations may account for these differences. Model predictions of human LCt_{50} indicate a rat:human ratio of between 3.0 and 3.5.

- The known detoxification potential of carboxylesterases is multifaceted and encompasses consideration of CaE amount, affinity, and inhibitor resistant esterase activity. The present state of incomplete characterization for human CaE precludes accurate prediction regarding CaE detoxification potential in a population of humans exposed to anticholinesterase compounds.

In conclusion, recent literature indicates that CaE detoxification potential exists in numerous human organs and tissue, including blood plasma. It is acknowledged that further experimental characterization of CaE detoxification potential in humans will be necessary before accurate prediction of the contributions CaE may make to human protection from anticholinesterase poisoning. Interspecies comparisons of lethality data for rats and monkeys (as well as estimated human LCt_{50} values) has been performed. The results indicate that an interspecies UF (rat-to-human) of approximately 3 for AEGL-3 determination is a reasonable characterization of the present state of knowledge for this parameter.

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). Because a modifying factor is not applicable for reasons previously outlined for AEGL-1 and AEGL-2, the composite UF for AEGL-3 determination for agent GB is equal to 30. From the experimental data, k was derived from the 6-h LC_{01} as

$$([1.761 \text{ mg/m}^3]/30)^2 \times 6.0 \text{ h} = k;$$
$$k = 0.021 \text{ mg/m}^3 \times \text{h}.$$

The resulting AEGL-3 estimates for agent GB are summarized in Table 1-35.

Benchmark Exposure Analyses

A benchmark exposure calculation has been performed on the female rat 14-d vapor lethality data presented in the Mioduszewski et al. (2001)

TABLE 1-35 AEGL-3 Values for Agent GB (mg/m^3 [ppm])

10 min	30 min	1 h	4 h	8 h
0.38 mg/m^3 (0.064 ppm)	0.19 mg/m^3 (0.032 ppm)	0.13 mg/m^3 (0.022 ppm)	0.070 mg/m^3 (0.012 ppm)	0.051 mg/m^3 (0.0087 ppm)

report in accordance with guidance provided in the NRC standing operating procedures (NRC 2001, 45). For comparison, a NumberCruncher Statistical System analysis has also been completed.

There appears to be some degree of controversy around using the BMD approach for acute lethality data. The SOP workgroup will address this issue in the future.

There are eight models that accept dichotomous data in the Benchmark Dose software package available on the EPA Web site (http://cfpub.epa.gov/ncea/cfm/bmds.cfm): gamma, logistic, log-log multistage, probit, log-probit, quantal-linear, quantal-quadratic, and Weibull. Evaluations were performed with all eight (multiplied by five time points, times the 5% response for the 95% Lower Confidence Limit (LCL) and the 1% Maximum Likelihood Estimate (MLE), as per the SOP). The Weibull and gamma programs would not run with the input data; contact with the EPA Webmaster eventually revealed, through systems testing, that these two models require entry of a zero-concentration effect value in order to converge. The Mioduszewski et al. (2001) data set does not contain any zero-concentration effects data, and its addition would be an artificial alteration of the data set. It was concluded that the content of the data set is not compatible with requirements of the Weibull and gamma models; thus no analyses of the vapor lethality data were performed with these two models.

Tables summarizing the statistical results of the Benchmark Exposure Concentration analysis are included in Appendix C. Table C-1 is a summary of LC_{01} values obtained from all the Benchmark Dose software routines; the ones on the lower tier of the table (logistic, multistage, quantal-linear, quantal-quadratic) are poor fits and are rejected from any further consideration. The first column following the exposure times is the set of MLE LC_{01} values used to develop the AEGL-3 estimates published in the *Federal Register* notice of May 2, 2001 (66 Fed. Reg. 21940 [2001]). The LC_{01} values in the second column following the exposure times are those published by Mioduszewski et al. (2001). All remaining values presented in Table C-1 were based on the raw experimental data presented in Mioduszewski et al. (2001).

The MINITAB log probit seems to be a reasonable fit with the lethality data, and the experimental results on which this analysis is based are published in Mioduszewski et al. (2001, 2002a).

Because the statistical routines used to evaluate the data in Mioduszewski et al. (2000) differ slightly from those used in Mioduszewski et al. (2001), the LC_{01} values employed in developing interim AEGL-3 determinations also differ slightly. The resulting LC_{01} and AEGL-3 estimates developed with the same calculational approach—with the UF and *n* values applied in the AEGL-3 determinations presented earlier (see Appendix A)—are summarized in Tables C-1 and C-2 of Appendix C. The *Federal Register* interim values for AEGL-3 (see Table C-2) are consistently lower or equal to the Mioduszewski et al. (2001) log probit derived estimates, with the single exception of the 4-h value. In the case of the 4-h value, the NAC interim AEGL-3 (0.070 mg/m^3) is somewhat greater than the 4-h AEGL-3 estimate derived from the Mioduszewski et al. (2001) log probit derivation (0.059 mg/m^3; see Table C-2), by 0.011 mg/m^3. The variation is slight.

The LC_{01} values presented in Mioduszewski et al. (2001), although slightly different from the preliminary results considered (Mioduszewski et al. 2000), represent a better documented and more widely accessible data set. These differences are acknowledged.

Agent GD

A relative potency approach is used to estimate AEGL-3 values for agent GD, and a discussion of the relative potency of agent GD and GB is provided in Section 4.3. The lethal potency of agent GD is considered equivalent to that of agent GB (see Table 1-22).

A secondary and short-term GD inhalation study of rat lethality for exposure times ≤30 min (Aas et al. 1985) lends support to the assumption of lethal equipotency for agents GB and GD when used as a secondary study for derivation of 10-min and 30-min AEGL-3 values and as a comparison with the values derived by the relative potency method. Aas et al. (1985) calculated an LCt_{50} of 400 $mg \cdot min/m^3$ for GB and graphically presented their data as an LCt-versus-mortality curve. As estimated from this curve, the lethality threshold for rats exposed to agent GD (six animals tested at each of three exposure levels for periods of time <30 min) is about 335 $mg \cdot min/m^3$. Because the GD air concentration was fixed at 21 mg/m^3,

the exposure time corresponding to the threshold could be back-calculated, and was found to be16 min. This lethality threshold was used to derive a comparative estimate of the AEGL-3.

Regression analysis of the data of Aas et al. (1985) was not possible from the information provided. Because the principal mode of action (cholinesterase inhibition) for the G agents is identical, $n = 2$ was used for deriving comparative AEGL-3 values from the GD data of Aas and his colleagues. Because of the sparse data set for GD, the full default values for interspecies (10) and intraspecies (10) uncertainty were applied. Because a modifying factor is not applicable, a composite UF of 100 was used in deriving comparative 10-min AEGL-3 and 30-min AEGL-3 estimates for agent GD from the data provided by Aas et al. (1985).

AEGL-3 values for exposure times different from the experimental times were scaled using an n of 2. From the experimental data, k was derived as

$$([21\ \text{mg/m}^3]/100)^2 \times (16/60)\ \text{h} = k;$$
$$k = 0.012\ \text{mg/m}^3 \times \text{h}.$$

The resulting comparative AEGL-3 estimates include a 10-min AEGL-3 estimate of 0.27 mg/m^3 and a 30-min AEGL-3 estimate of 0.15 mg/m^3. Details of the comparative derivation are provided in Appendix A.

The values derived from the Aas et. al (1985) study are in good agreement with those derived by means of relative potency comparison with agent GB for the same time periods.

The recommended AEGL-3 value estimates are those derived by relative potency comparison with agent GB (with agent GD being considered equipotent to agent GB for lethal effects), and are summarized in Table 1-36 below.

7.4. Derivation of AEGL-3 Values for Agents GA and GF

A relative potency approach is used to estimate AEGL-3 values for agents GA and GF. A discussion of the relative potency of these agents to cause lethality is given in Section 4.3 and summarized in Table 1-22. The lethal potency of GA is considered to be one-half that of GB, and agent GF is considered to be equipotent to GB for lethality (Table 1-37). The preliminary GF lethality report of Anthony et al. (2002) is not sufficiently com-

TABLE 1-36 AEGL-3 Values for Agent GD (mg/m^3 [ppm])

10-min	30-min	1-h	4-h	8-h
0.38 mg/m^3 (0.049 ppm)	0.19 mg/m^3 (0.025 ppm)	0.13 mg/m^3 (0.017 ppm)	0.070 mg/m^3 (0.0091 ppm)	0.051 mg/m^3 (0.0066 ppm)

plete (no documentation of threshold lethality) to support an independent AEGL-3 estimation for agent GF (see Section 7.2).

7.5. Derivation of AEGL-3 Values for Agent VX

Credible data on the acute vapor exposure lethality of agent VX are available for only two laboratory species, mice and goats. The LCt_{50} values are 4.0 $mg \cdot min/m^3$ for mice and 9.2 $mg \cdot min/m^3$ for goats for 10-min exposures (Koon et al. 1960). However, LC_{01} estimates cannot be derived from the Koon et al. study, and the analytical methods employed in measurement of experimental VX concentrations are not considered acceptable by modern standards.

Because of inadequacies in the human and animal toxicological database for agent VX, the AEGL-3 for agent VX is derived from the AEGL-3 for agent GB using a relative potency approach. The AEGL-3 for agent GB was derived from the lethality data for female Sprague-Dawley rats (Mioduszewski et al. 2000, 2001, 2002a), as discussed more fully in Section 7.3.

Probit analysis (MINITAB, version 13) presented in Mioduszewski et al. (2000) gave the following 14-day LC_{50} values for female rats exposed to agent 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.

Based on a probit analysis of the data (Mioduszewski et al. 2000), the estimated 14-day 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, and 1.76 mg/m^3 for 6 h.

By applying the relative potency of 4 as described earlier, the estimated 14-day LC_{01} for female SD rats exposed to VX vapor are as follows: 2.89 mg/m^3 for 10 min, 1.46 mg/m^3 for 30 min, 1.00 mg/m^3 for 60 min, 0.52 mg/m^3 for 4 h, 0.44 mg/m^3 for 6 h.

TABLE 1-37 AEGL-3 Values for Agents GA and GF (mg/m^3 [ppm])

Agent	10 min	30 min	1 h	4 h	8 h
GA	0.76 mg/m^3 (0.11 ppm)	0.38 mg/m^3 (0.057 ppm)	0.26 mg/m^3 (0.039 ppm)	0.14 mg/m^3 (0.021 ppm)	0.10 mg/m^3 (0.015 ppm)
GF	0.38 mg/m^3 (0.053 ppm)	0.19 mg/m^3 (0.027 ppm)	0.13 mg/m^3 (0.018 ppm)	0.070 mg/m^3 (0.0098 ppm)	0.051 mg/m^3 (0.0071 ppm)

The derived LC_{01} values above were adjusted by a total UF of 100. 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 for the interspecies UF because the mechanism of toxicity in both laboratory rodents and humans is cholinesterase inhibition, and the experimental results of Maxwell (1992) indicate that endogenous carboxylesterases in rats confer no protection against lethal exposures of nerve agent VX. To accommodate known variation in human cholinesterase activity, the full default value of 10 for intraspecies uncertainty was considered necessary to protect susceptible populations. 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.

To derive an AEGL-3 for a time periods not included with the experimental protocol of Mioduszewski et al. (2000, 2001, 2002a) (i.e., the 8-h AEGL), the 6-h data were scaled using the relationship $C^n \times t = k$ (ten Berge et al. 1986). An n value has not been determined experimentally for VX. However, because the mechanism of action (cholinesterase inhibition) is the same as that for agent GB, the n value of 2 used in the derivation of the AEGL values for GB is appropriate for deriving AEGL values for VX. 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 here as the scaling function for the agent-VX 8-h AEGL-3 values, rather than a default value. Therefore, using the 6-h estimated LC_{01},

$C^2 \times 6\ h = k$;
$([0.44\ mg/m^3]/100)^2 \times 6\ h = k$;
$k = 1.16 \times 10^{-4}\ mg/m^3 \times h$.

The resulting AEGL-3 values are summarized in Table 1-38. The calculations of exposure concentrations for humans scaled for all AEGL-3 time points are shown in Appendix A.

8. SUMMARY OF AEGLS

8.1. AEGL Values and Toxicity End Points

A summary of the AEGLs for agents GA, GB, GD, GF and VX is shown in Table 1-39.

G-Series Agents

In consultation with experimental investigators at Porton Down (United Kingdom) and the TNO Prins Maurits Laboratory (Netherlands), the current 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. In consequence, the interspecies 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.

For the development of AEGL-2 values, the database for toxicological effects in humans is more complete for agent GB than for any of the other G agents. Sufficient human data are available to directly derive AEGL-2 values for agent GB. The toxicity end points used to derive the value were considered to be appropriate, and the lowest of the available exposure concentrations was used. The data were limited, however, by the maximum time of exposure of 30 min (Baker and Sedgwick 1996). 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 the relative potency approach based on the potency of the agents to induce miosis. Agents GA and GB were considered to have an equivalent potency for causing miosis. Agents GD and