Chapter Five

NERVE AGENTS

The military nerve agents are a family of highly toxic phosphoric acid esters, structurally related to the larger family of organophosphate compounds. In fact, development of nerve agents was a by-product of insecticide research and development (OSRD, 1946; Hayes, 1982). Germany developed nerve agents just before and during World War II; subsequently, several countries, including the United States and the Soviet Union, made them the subject of intense research and development and stockpiled them as weapons (SIPRI, 1971; SIPRI, 1973).

Nerve agents have been used in some wars since that period (SIPRI, 1971; UN, 1984; Cordesman and Wagner, 1990, 1991); to suppress internal uprisings in Iraq (Macilwain, 1993); and more recently, in large-scale terrorist attacks (Ohtomi et al., 1996; Morita et al., 1995, Okumura et al., 1996). Nerve agents have also been the subject of much concern during and since the Gulf War. They occasioned considerable defensive efforts and, later, concerns that coalition forces might have been exposed to them during the war (Riegle and D'Amato, 1994; House, 1997; Senate, 1994). The concern was increased by the discovery that U.S. forces had unknowingly destroyed a substantial amount of nerve agents in demolitions at the Iraqi depot at Khamisiyah shortly after the end of the Gulf War, resulting in possible exposure to low concentrations of nerve agents over a large area (OSAGWI, 1997a; CIA, 1997).

There is a great deal of literature on nerve agents and organophosphate pesticides, including several recent books on chemical agents (Somani, 1992; Marrs et al., 1996; Sidell, Takafuji, and Franz, 1997), with one giving a detailed summary of human studies in the UK and United States (Marrs et al., 1996; Smart, 1997; Sidell, 1997; Dunn et al., 1997; Sidell and Hurst, 1997).

This chapter provides an overview of nerve agent effects but looks especially at information about the effects of low, or inapparent, exposures on mood, memory, thinking, strength, and behavior. It also pays particular attention to information that may provide insight on mechanisms for long-term neuropathy.

HISTORY

The first nerve agent of military significance was discovered by Dr. Gerhard Schrader, a chemist conducting insecticide research with organophosphates in 1937. He synthesized ethyl-N-dimethyl-phosphoroamidocyanate, which has had a number of names since then but is most commonly called tabun. The toxicity was personally experienced by the investigators, who found that a small drop of tabun, spilled on a laboratory bench, resulted in pinpoint pupils, dim vision, headache, and difficulty breathing (OSRD, 1946; Harris and Paxman, 1982). Later animal tests showed rapid lethality. Under German law, these findings were reported to the War Ministry, which subsequently developed tabun (in 1939) and a related nerve agent, sarin, later. A third agent, soman, was discovered in 1944 (SIPRI, 1971; SIPRI, 1973). The designation "G" arose from the markings on German chemical weapons found after the war: GA for tabun, GB for sarin, and GD for soman (SIPRI, 1971).

A pilot plant at Munster Lager provided enough tabun for field trials in 1939 (OSRD, 1946). Later, larger production plants were built but encountered considerable delays, with full production of tabun beginning in 1942. Sarin proved more difficult to produce, and only in 1945 were the Germans able to produce several hundred tons of it. Soman was not produced in quantity (SIPRI, 1971; SIPRI, 1973).

Several hundred accidents occurred during the production of nerve agents, and ten workers were killed. Exposure to low levels of tabun was so common that workers were given extra milk and fat rations because it was observed that larger fat consumption had a protective effect (Harris and Paxman, 1982).

The United States and the UK conducted extensive research during World War II on some related compounds, diisopropyl flurorophosphate (DFP) (also designated as agent P-3) being the best known (OSRD, 1946), but these less-toxic variants appeared most suitable as incapacitating agents because of their ocular effects. Achieving lethal concentrations was difficult.

After the war, the United States, the UK, and the former Soviet Union conducted extensive classified research and development. The German plants and technical information were in the part of Germany the Soviets occupied. That appears to have contributed to a very large postwar Soviet chemical effort (Seagrave, 1981; Harris and Paxman 1982; SIPRI, 1971).

The United States began producing sarin on a large scale in the early 1950s; occupational exposures from that period also provided useful data. No worker died, but nearly 1,000 sustained some exposure. Illnesses were generally brief, usually only a few days, sometimes a few weeks (Craig and Freeman, 1953; Gaon and Werne, 1955; Craig et al., 1959; Holmes, 1959; Marrs et al., 1996,

Sidell, 1997). These workers have been subject to only limited follow-up, using small groups and controls (Metcalf and Holmes, 1969).

Defensive research into detection, decontamination, and treatment continues. The perception that soman was a key element in the Soviet arsenal, coupled with recognition of its high toxicity and resistance to therapy, resulted in research emphasis on this agent. The rapidity of action and resistance to oxime therapy lead to the development of pretreatment drugs (carbamate reversible inhibitors), as well as deployment of diazepam drugs with some NATO forces (NATO, 1973; Gall, 1981; Marrs et al., 1996; Sidell, 1997; Dunn et al., 1997).

Problems related to aging chemical munitions in stockpiles and decisions in many countries to eliminate chemical weapons have resulted in research into lower-dose exposures and the longer-term implications of exposure of nonmilitary populations (SIPRI, 1980; Watson et al., 1989; Dacre, 1989).

Meanwhile, development and use of organophosphate-based insecticides has proliferated, and they continue to be widely used in agriculture (Hayes, 1982). Although these insecticides are less toxic than the nerve agents, the illnesses they produce clinically resemble those nerve agents produce (Grob and Harvey, 1953; Hayes, 1982). The toxicity of these insecticides to humans is thus relevant (Haley and Kurt, 1997; Haley, Kurt, and Horn, 1997), and this chapter includes information from pesticide studies where it seems helpful. However, Sidell stresses the clinical differences between the organophosphate insecticides and nerve agents, noting that cholinergic crises from pesticides last much longer than those from military nerve agents (Sidell, 1997; Sidell and Hurst, 1997). On the other hand, reviews of possible long-term effects of nerve agents have regarded organophosphate pesticide experience as being informative (NAS, 1982; Karczmar, 1984; Boskovic and Kusic, 1980; Jamal, 1995b).

It was recognized early that the clinical-pharmacological effects of nerve agents and related organophosphate pesticides resembled the strong actions of the neurotransmitter acetylcholine (ACh). This chemical activates specialized receptors at the nerve synaptic junction, promoting discharge of the nerve on the other side of the synapse and stimulating the action of the nerve. ACh is rapidly destroyed by the enzyme acetylcholinesterase (AChE) (one of a family of serine esterase enzymes), which plays a regulatory role to limit the effects of ACh.

A key mechanism of action of nerve agents is their inhibition of AChE, which results in physiological-pathological overstimulation by excessive ACh (OSRD, 1946; Somani, 1992, Ch. 4). This common mechanism explains the similar effects of many nerve agents and their response to therapy with atropine and oximes.

These agents also inhibit a variety of other enzyme systems (e.g., serine esterases), and their effects impinge on other biological systems via mecha-

nisms that the inhibition of AChE does not fully explain. Increased understanding of neurobiology and neurotransmitters has aided the understanding of these agents (O'Neill, 1981; Prioux-Guyonneau et al., 1982).

WEAPONIZATION

The earliest nerve agents, tabun and sarin, were considerably more toxic than the existing chemical gas weapons, such as phosgene, by a factor of 7 to 40 (Franke, 1967; OSRD, 1946). These agents were hard to detect; even when exposures were insufficient for rapid fatality, they injured and incapacitated soldiers. Liquid contamination of soils, clothing, and material could provide a secondary vapor hazard for variable periods. Artillery shells that detonated the same as ordinary shells could deliver these agents effectively (OSRD, 1946). During World War II, the Germans used aerial bombs and spray tanks for delivery. The vapor density allowed the agent to flow into lower terrain, trenches, bunkers etc., extending the hazard after the attack, which the Germans regarded as desirable.

Subsequently, many agents and potential agents were synthesized and tested. Toxicities turned out to be rather similar (Callaway and Blackburn, 1954). The several G agents varied in the threat they posed via the skin (sarin was not very effective), and efforts were made to mix them with other agents that might enhance skin penetration, such as mustards or lewisite (SIPRI, 1973; Krustanov, 1962). However, a variety of other factors, such as stability, ease of production, and physical properties, may have been more important than toxicity in weaponization decisions (SIPRI, 1971, 1973). Efforts were made to thicken the nerve agents with additives to increase their persistence and penetration (SIPRI, 1973). In the end, several countries adopted sarin, while the former Soviet Union produced soman and thickened soman (SIPRI, 1971, 1973).

The later development of the V agents, such as VX, provided a number of very toxic compounds. Although not very volatile, these could be disseminated in aerosols and provided a very high percutaneous hazard with an environmental persistence far greater than the G agents. Both Western and Soviet forces adopted these agents.

Nerve agents can be delivered by free rockets, guided missiles, and mines, as well as mortar and artillery shells, aerial bombs and submunitions, and spray tanks. Weaponized nerve agents are suitable for a large variety of military operations and for both tactical and strategic use.

Defensively, nerve agents can be used to disorganize forces in assembly areas and reserve formations. The more persistent agents can impede advancing forces, especially by reinforcing other obstacles. During the Gulf War, commanders were reasonably concerned that operations to breach Iraqi defenses might be subject to chemical attack (Clancy and Franks, 1997).

Because of the hazards and difficulties of deploying chemical weapons, the United States (and perhaps other countries) developed so-called binary weapons during the 1970s and 1980s. The ingredients to produce a nerve agent were stored separately in the munitions and then were combined to produce the agent shortly before impact (Rutman, 1976; Eyring, 1976). There are reports that Saddam Hussein claimed Iraq had such weapons,[1] but UNSCOM found none (UNSCOM, 1991, 1992, 1995). The United States found the development of such weapons to be challenging. A variety of ingredients were potentially involved, and some of the reaction by-products were also toxic (Rutman, 1976; McNamara et al., 1979). Sarin and VX are the most commonly discussed binary agents in the U.S. stockpile, but other theoretically highly toxic, although less stable, agents might be produced (Lohs, 1975).

There have been reports of a highly toxic Soviet binary nerve agent, called Novichok, designed to be undetectable by U.S. detectors (Smart, 1997). The information came from a émigré who indicated that Iraq might have acquired agents of this family. Information about these newer Soviet agents (33 and 232) is only to be found in press reports interviews and Internet postings (Englund, 1992a, 1992b; Adams, 1996; Tucker, 1996). No detailed or peer-reviewed scientific data are available.

RELEVANCE TO THE GULF WAR

Nerve agents are relevant to illnesses in Gulf War veterans for two reasons: Iraq had developed a chemical capability and had used nerve agents prior to the Gulf War, and there was some potential for exposure of U.S. troops during the conflict.

Iraq's Capability

Iraq's acts against the Kurds were an early indication of its chemical capability,[2] while the Iran-Iraq War showed considerable and improving Iraqi use of a variety of agents, not all of which were identified (Cordesman and Wagner, 1990). Tabun was definitely used against Iranian forces (UN, 1984). Typical nerve agent casualties were independently confirmed, and tabun was identified in a bomb, mixed with chlorobenzene (a stabilizer) in a percentage quite similar to what the Germans used in World War II (OSRD, 1946). In later fighting, Iraq appears to have used nerve agents with some success in attacks in the southern

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[1] "Iraqi Threat of Chemical Warfare with Israel" (1990).

[2] "Iraq: How Iraq is Defying the World Concerning the Alleged Use of Chemical Weapons by its Armed Forces Against the Kurdish Population" (1988); Macilwain (1993).

sector (Cordesman and Wagner, 1990). Although sarin and cyclosarin might have been used in these attacks, there was reason to be concerned that Iraq's large chemical program might also have produced soman. More recently, UNSCOM suspected and later documented that Iraq had produced VX. Iraq initially admitted to some research on VX, but admitted to UNSCOM late in 1996 that it had produced 3.9 tons of VX as well as 58.5 tons of precursor chemicals (Miller, 1998).

After the Gulf War, the UN became aware that Iraq had substantial stocks of tabun, sarin, and cyclosarin—with sarin and cyclosarin being present in the 122-mm rockets destroyed at Khamisiyah. A barrage of such rockets can rapidly establish a lethal concentration over a large area, representing great danger to personnel not wearing respirators. Cyclosarin can also be a more persistent threat than sarin and is a greater percutaneous hazard (U.S. Army, 1990).

It seems unlikely that potential use of such agents against coalition forces had been the reason Iraq chose them. Development of weapons takes considerable time, and the coalition formed rapidly. Tabun has some persistence and is the easiest agent to produce. It is also capable of producing incapacity for many military functions at levels well below lethal concentrations (OSRD, 1946). As with other Iraqi nerve agents, tabun is suitable for both offensive and defensive use.

The discovery that Iraq had substantial stocks of cyclosarin was interesting because, although this agent was fairly well known, no major power had adopted it. Iraq may have selected it to provide a more persistent and percutaneously effective agent than sarin, one that also has formidable inhalation toxicity. With a sarin production capability, Iraq may have found it easier to produce cyclosarin than to develop VX. However, Sidell, Takafuji, and Franz (1997) indicates that Iraq may have produced cyclosarin because precursor chemicals for sarin—but not those for cyclosarin (e.g., cyclohexyl alcohol)—had been embargoed.

Coalition forces offered many potential targets to Scud missiles: airfields, ports, assembly areas, and logistic facilities, some proximate to urban areas. The Iraqi Scuds had payloads sufficient to place considerable agent on target, although not with great accuracy. Before the air war began, Iraq had a substantial air force, which had demonstrated some ability to deliver chemical agents (Cordesman, 1990; UN, 1984; Zilinskas, 1997), which threatened both the same targets as missiles did and other tactical targets.

Writings after the war indicated that U.S. commanders were concerned about the threat of chemical agents to their forces, especially during initial efforts to breach Iraqi defenses, when friendly forces would be concentrated in identifiable locations and not be moving rapidly (Clancy and Franks, 1997). Training

emphasized protective equipment and, as the attack risk increased, the use of pretreatment medications.

Potential for Exposure

Both before and after the start of the air war, there were many alarms from chemical-agent detection systems. The significance of these alarms remains controversial. They apparently resulted from other environmental contaminants, and confirmatory tests generally did not find proof of an agent, although some allied force reports continue to raise doubts (Riegle and D'Amato, 1994). Some have alleged that a nerve agent was present, perhaps from attacks on Iraqi chemical storage facilities, while the general position of DoD and other analysts has been that sarin would be unlikely to present a hazard after being dispersed over hundreds of kilometers and thus having the opportunity to disperse and to hydrolyze (OSAGWI, 1998b; PAC, 1996b).

In two separate accidental exposures during the 1950s at Dugway Proving Ground, workers developed signs, symptoms, and laboratory evidence of mild nerve agent exposure in a test area three days after a sarin test, when it was thought safe to work without protection. In both incidents, it was noted there was a lot of dust blowing at the time of exposure, but the exact locations with respect to test area were not indicated. At the time, it was suspected that sarin had survived longer than expected because it was trapped on dust particles. No environmental samples were taken. The severity resembled that seen with a vapor exposure CT of 15 mg-min/m^3. This suggests that, in some circumstances, sarin trapped on dust particles may persist for a long time and represent a hazard when stirred into the air (Brody and Gammill, 1954; Craig and Freeman, 1953). Craig and Freeman (1953) described an exposure that took place 24 hours after a test: The safety officer had thought it safe to be in the immediate test area without protection because the weather was warm. Again, exposure seemed to be from dust.

Earlier military research had shown that sarin and the organophosphate pesticide paraoxon trapped on small inert particles were highly toxic to experimental animals (Asset and Finklestein, 1951). Particle delivery is a key means of distributing pesticides. No information was available about the details of the models used to estimate agent dispersion from Iraq to Saudi Arabia, or for the Khamisiyah event, or whether particle trapping is even considered relevant to such models. There are indications that sarin trapped on dust may persist and be dangerous longer than is commonly thought. Declassified reports (Defense Intelligence Agency, 1997) indicate an awareness of Iraqi "dusty mustard," but also noted that other agents might be used in dusty form.

The DoD position has been that Iraq did not use chemical weapons, and there do not appear to have been any readily recognizable casualties from nerve

agent attacks. However, as discussed in detail below, there is precedent for misinterpretation of low-level exposures (Gaon and Werne, 1955), and there is some reason to think that the pyridostigmine bromide (PB) pretreatments U.S. troops received could have reduced the intensity of response to low-level challenges (Gall, 1981; Husain, Kumar, et al., 1993; Vijayaraghavan, Husain, et al., 1992).

Controversy thus remains about Iraqi use of chemical weapons,[3] with allegations that there might have been some. Even if the Iraqi higher command had explicitly instructed troops not to use chemicals, they appear to have been present in the operational area.[4] At least some of these lacked distinctive markings, making accidental release feasible. There is no proof that this occurred, however. UNSCOM, as cited in PAC (1997), indicates there were no chemical agents in Kuwait or in Iraq south of Khamisiyah. OSAGWI has also extensively investigated all suspected cases and to date has not been able to confirm chemical weapon exposure except one case for a single individual and in the case of Khamisiyah (OSAGWI, 1997d).

Khamisiyah

It is clear that U.S. forces unknowingly destroyed Iraqi chemical weapons in March 1991 at the Khamisiyah depot, thinking that these were conventional munitions (OSAGWI, 1997a). Rockets containing sarin and cyclosarin were destroyed by explosive charges, releasing some agent into the atmosphere. Several studies have attempted to model exposures from this incident (Babarsky, 1998; CIA, 1997). One study under way (Gray et al., 1998) used the plume analysis to identify troops who had been more and less exposed to the sarin. The case narrative (OSAGWI, 1997a) had indicated that there were no reports of immediate clinical effects on the nearest troops. In an effort to rule out longer-term effects from low-level exposure, the researchers are now comparing the hospitalization experiences of the 61,000 personnel potentially exposed to the plume with a group of 250,000 who had been in the region but not in the plum pattern. Low-level exposure effects are discussed later in this review.

Hypotheses

Unexplained illnesses in personnel returning from the Gulf War generally do not "fit" the pattern of readily recognized disorders associated with nerve agents. Many hypotheses are being tested. Congressional testimony (Riegle

[3] "Researcher Claims Iraq Fired Chemical Weapons During Gulf War" (1997).

[4] "Iraq May Have Moved Chemical Weapons into Southern Kuwait" (1990).

and D'Amato, 1994; House, 1997; Senate, 1994) questioned whether a combination of exposures to chemicals might have produced a new delayed-onset disease. The chemicals may have included pesticides, such as the personal repellent diethyl-*m*-toluamide (DEET), the anti–nerve agent prophylactic PB, and perhaps chemical warfare agents.

Veteran Reports

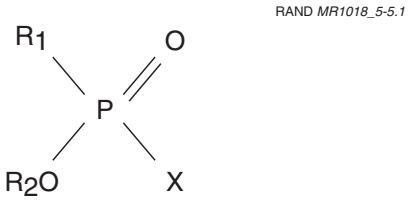
Studies of selected, defined small groups of ill Gulf veterans and controls in the United States (Haley, Kurt, and Horn, 1997; Haley, Horn, et al., 1997; Haley and Kurt, 1997; Hom, Haley, and Kurt, 1997) both found epidemiological indications of unusual exposures (e.g., flea collars, being outside during attacks) and identified three to six clinical syndromes. They found subtle indications of diffuse neurological injury in a smaller group of 23 veterans and suggested a variant of delayed organophosphate neuropathy, with the suggestion, based on animal research, that nerve agents could not be ruled out as being involved (Abou-Donia et al., 1996; Husain, Kumar, et al., 1993; Husain, Vijayaraghavan, et al., 1993). Jamal et al. (1996), studying ill UK Gulf veterans, found indications of subtle neurological injury, with sensory peripheral neuropathy being the most striking, although exposure studies were not reported. Where the Jamal studies correspond to those of Haley and Hom, they do not always agree; for example, Haley and Hom did not detect sensory neuropathy, while Jamal did not find the abnormal evoked responses that Haley and Hom did. There was, however, evidence of some organic neurological disorder in both groups of ill veterans. The significance of these findings is controversial; while the authors considered them statistically significant, others question the statistical techniques.

The RAND report on pesticides (Cecchine et al., 2000) will document the use of anticholinesterase pesticides in the Gulf theater, while self-reported exposure interviews (Haley and Kurt, 1997) document some unauthorized use of commercial "flea-collar" devices that contained chlorpyrifos. Pesticides are discussed here only with respect to possible interactions with nerve agents.

This review cannot determine the causes of illnesses in Gulf War veterans, but it provides a background of information about nerve agent effects to help analyze hypotheses and plan further studies. PB is discussed only in the context of interactions with nerve agents; a separate report has been issued on PB (Golomb, 1999).

CHEMICAL CHARACTERISTICS

The common structural framework for the agents under consideration is shown in Figure 5.1 (key in Table 5.1), with a table showing the particular groups attached in the various agents. The thio-analogs (e.g., thiosarin) substitute



SOURCE: AD Little (1986, Ch. 5).
NOTE: See Table 5.1

Figure 5.1—Chemical Structures of Nerve Agents

Table 5.1

Nerve Agent Chemical Structure

Agent	X	R_1	R_2
Tabun (GA)	CN	$N(CH_3)_2$	C_2H_5
Sarin (GB)	F	CH_3	$CH(CH_3)_2$
Soman (GD)	F	CH_3	$CH(CH_3)C(CH)_3$
Cyclosarin (GF)	F	CH_3	Cyclohexyl
VX	$SCH_2CH_2N[CH(CH_3)_2]_2$	CH_3	C_2H_5

SOURCE: SIPRI (1973).
NOTE: Keyed to Figure 5.1.

double-bonded S for double-bonded O. The chemical and physical properties are given in Appendix B.

A carbon-phosphorous bond is common to the nerve agents but is rare in the less-toxic organophosphate pesticides (SIPRI, 1973). Thousands of organophosphate compounds have been synthesized. Because of their chemical characteristics, nerve agents slowly degrade in water, with half-lives from 5 to 40 hours, depending on pH.

The military agents are racemic mixtures of stereoisomers. There are (+) and (–) forms of tabun and sarin, while soman has four chiral forms (Benschop, Berends, and de Long, 1981). The different isomers and mixtures thereof have important toxicological and kinetic differences; for example, the (–) isomer of sarin is more toxic than the racemic mixture (SIPRI, 1973; Boter and Dijk, 1969).

RELATED CHEMICALS

Many chemicals are related to the nerve agents. Some are even more toxic (e.g., Tammelin esters, fluorophosphocholines, phosphothiocholates) (SIPRI, 1973; Binenfeld, 1967). Others include such agents as GE, VM, VS, Gd42, Gd83, and

DFP and such pesticides as amidon, parathion, malathion, paraoxon, chlorpyrifos, systox, tetraethyl pyrophosphate. Of note is TOCP, an organophosphate chemical that is a very weak inhibitor of AChE compared to most of the other organophosphates. TOCP, however, produces delayed neuropathy (Hayes, 1982).

DETECTION

Sight and smell are unreliable means of detecting nerve agents (although some have characteristic odors). It has been difficult to develop detectors that are more sensitive than the miotic response of the human eye although that has been the goal.

MILITARY SYSTEMS

A number of measurement systems are not used operationally (Department of the Army, 1996). Field systems include ion mobility spectrometers (the M8A1 alarm and the CAM device), chemical reaction kits (M256A1), enzyme-based detection (the M256A1 and some foreign systems, such as the Czech GSP11), and mass spectrometers (the MM-1 system in Fox vehicles) (DSB, 1994; OSAGWI, 1997f; OSAGWI, 1998b; OSAGWI, 1997c).

The M8A1 alarm system, which U.S. forces use widely, is designed to detect nerve agents as vapors or aerosols. It responds within less than 2 minutes to G agents in the range of 0.1 to 0.2 mg/m^3 and to VX at 0.4 mg/m^3. The bias is toward sensitivity, not specificity, and a large number of interfering chemicals can produce false alarms (smokes, fuels, insecticides, paint fumes, cologne) (OSAGWI, 1997f).

The M256A1 system is used not as an alarm but for confirmatory testing. This is a slower response system than the M8A1 alarm, taking 15 minutes for nerve agent analyses, but it is able to detect nerve agent vapors at 0.005 mg/m^3 of G agents and 0.02 mg/m^3 of V agents. This system is less influenced by the interferants that affect the M8A1 (DSB, 1994).

The UK's CAM also uses ion mobility spectrometry but responds to nerve agents at or below 0.1 mg/m^3 within less than a minute. This device also can detect mustard agent vapors (DSB, 1994).

The Fox nuclear, biological, and chemical reconnaissance vehicle is optimized to detect and mark surface contamination by chemical weapons. The system uses a mass spectrometer that is configured to determine suspected threat chemicals promptly, then determine the spectra of specific agents more definitively. This system is less sensitive than the alarms—requiring levels of about 62 mg/m^3 and 45 seconds to respond to nerve agents. Events during the Gulf War

showed that oil fires and oil vapors could interfere with the system and cause some false alarms. The vehicle was also equipped with the M8A1 alarm (OSAGWI, 1997c).

There are also detector papers used to detect droplets, and according to DSB (1994), M8 paper responds to G or V droplets of 0.02 ml with a color change within 20 seconds or less. M9 paper responds with a color change but to smaller 100 μm drops.

Regarding the sensitivity of detectors in comparison with human thresholds for eye effects, the Subcommittee on Toxicity Values for Selected Nerve and Vesicant Agents (NAS, 1997) estimates miosis levels as follows:

- VX—0.09 mg-min/m^3
- cyclosarin—0.2 mg-min/m^3
- soman—0.2 mg-min/m^3
- sarin—0.5 mg-min/m^3 (or somewhat higher)
- tabun—0.5 mg-min/m^3 (or somewhat higher).

This suggests that, for the M8A1 at least, it is quite possible for miosis to occur from VX before detector alarming, with miosis from cyclosarin and soman occurring sooner or at the same time as detection. In the cases of tabun and sarin, the detection is likely to precede miosis.

TISSUES

There has recently been progress in documenting the presence of sarin in body fluids from patients exposed in the Japanese attacks. Frozen serum samples taken from hospitalized cases were found to contain sarin in the range of 0.2 to 4.1 ng/ml. The methodology, too complex to detail here, may be useful for biological monitoring of exposed workers, for confirming exposures, and in patient care (Polhuijs, Langenberg, and Benschop, 1997).

Urinary metabolites of sarin were followed in a patient from the Matsumoto, Japan, incident. Two metabolites—methylphosphonic acid (MPA) and isopropylmethylphosphonic acid (IMPA)—were identified on the first day after the attack. By day 3, MPA was barely detectable, but IMPA was measured for one week. Total excretion was 2.1 mg for IMPA and 0.45 mg for MPA. Estimated total sarin exposure was 0.05 mg/kg (Nakajima, Sasaki, et al., 1998).

Analysis of urine samples from four Tokyo victims not only documented sarin and sarin metabolites, such as IMPA, but also detected chemicals associated with sarin products, such as ethyl-sarin and its metabolite, ethyl methylphos-

phonic acid. Other contaminants associated with sarin production were found in substantial amounts: ethyl alcohol, isopropyl alcohol, isopropyl methyl phosphonate, and diethylphosphonate (Minami et al., 1998).

Although congressional testimony (Riegle and D'Amato, 1994) emphasized agent alarms after the start of the air war, there were alarms before then, probably false positives. To conserve battery power, fewer detectors were turned on before the air war and therefore one would expect fewer alarms before the air war (OSAGWI, 1999).

ENVIRONMENTAL EFFECTS AND PERSISTENCE

Compared to other organophosphate compounds, nerve agents are highly to extremely toxic. Nerve agents are highly toxic to vertebrates and invertebrates, and their persistence in soil and water can be quite harmful. Demilitarization research has considered these factors. Table 5.2 describes the persistence of the various agents. The threat that persistent agents pose can affect military operations, e.g., by restricting the use of contaminated facilities.

Table 5.2

Persistence of Nerve Agents

Agent	Persistence
Tabun	Heavily splashed liquid lasts one to two days, depending on weather. Takes 20 times as long as water to evaporate. Persists in water one day at 20°C and six days at 5°C.
Sarin	Little persistence. Evaporates as fast as water or kerosene.
Soman	Heavily splashed liquid lasts one to two days (depending on weather). Takes four times as long as water to evaporate. Thickeners can extend the duration of persistence.
Cyclosarin	Heavily splashed liquid lasts one to two days (depending on weather). Takes 20 times as long as water to evaporate.
Thiosarin	Unknown
VX[a]	Splashed liquid can persist for weeks to months. Calculated to evaporate 1,500 times slower than sarin.

SOURCE: U.S. Army (1990).

[a]Other V agents are similar.

TOXICOLOGY AND TOXICOKINETICS

Nerve agent effects were satisfactorily modeled in the 1940s and 1950s, providing an understanding of the mechanisms of action and the clinical pathological findings and guiding therapy. But this knowledge, based on weapon development and efforts to improve casualty care, focuses on higher-level exposures, rather than the mild to subclinical exposure levels that might be relevant for Gulf War studies. Also, interactions with common pharmaceuticals and other environmental toxins have not been studied, although interactions with heat, cold, and exercise have been.

There are differences in opinion as to how pertinent studies of organophosphate pesticides are to understanding nerve agents. Clinical differences between organophosphate pesticides and nerve agents should be kept in mind, as will be discussed later. Sidell (1997) emphasized the rapid onset of nerve agent effects compared with those of organophosphate pesticides and noted the longer and more-difficult-to treat course of serious organophosphate pesticide poisoning. Likewise, no seriously poisoned nerve agent casualty has been reported to have the intermediate syndrome that Senanayake and Karalliedde (1987) described as arising from pesticide exposure.

Cholinesterase inhibitors have been used therapeutically in the past for glaucoma and are used currently to treat myasthenia gravis (Harrison, 1997). Cholinesterase inhibitors are currently the most established treatment strategy in Alzheimer's disease—several drugs, including tacrine, donazepil, and rivastigmine are in use, and many others are under study (Nordberg and Svensson, 1998).

REASONS FOR CONSIDERING ORGANOPHOSPHATE PESTICIDE EFFECTS

Although organophosphate pesticides are less-potent inhibitors of AChE than nerve agents and have less-steep dose-response curves, the two groups share many features in common: inhibiting AChE and other serine esterases and interacting with receptors similarly. Ignoring the human experience with organophosphate chemicals in looking at lower-dose effects and the possibility of longer-term effects from such exposures and assessing effects from unrecognized exposures would neglect much of the relevant evidence, in the opinion of this author.

At the clinical level, the early signs and symptoms of nerve agents and those of organophosphate pesticides are identical, so there is no ready distinction between them. Early investigators such as Grob and Harvey (1953, 1958) showed equal interest in other organophosphate pesticides and at times

included them in studies using nerve agents. Organophosphate pesticides are sometimes used in laboratory models to understand nerve agent effects. Since follow-up information on nerve agents is limited, the somewhat greater human experience with organophosphate pesticides may be instructive in looking for possible effects. Those interested in organophosphate pesticides have also noted common features of longer-term effects (Korsak and Sato, 1977). It is of course impossible to discuss delayed neuropathy without considerable reference to non–nerve agent organophosphates; likewise, experience with organophosphate pesticides illuminates understanding of the common mechanisms of tolerance both classes share. Interactions between environmental exposures to organophosphate pesticides and the effects of nerve agents are also possible.

The model is that these agents inhibit AChE, resulting in excessive ACh effects within the nervous system. The peripheral cholinergic systems are best understood, while the central nervous system picture continues to evolve. Central cholinergic systems are important in protective systems, locomotion, alertness, and memory and in the regulation of a number of cyclic and periodic behaviors (Petras, 1984).

NON-AChE EFFECTS

It became evident over time that inhibition of AChE did not completely explain the effects of nerve agents. Van Meter, Karczamar, and Fiscus (1978) showed that administering sarin or DFP to rabbits, even when the brain AChE was profoundly inhibited by a first dose of these agents with atropine treatment to prevent seizures, that a second dose of agent produced seizures although there was no AChE to inhibit. In their related studies, even when the enzyme was fully protected by physostigmine pretreatment, large doses of the agents were lethal. Therefore, lethality was not closely related to AChE inhibition. They concluded that the agents could act directly on central nervous system sites (see later discussions).

It has also been recognized that nerve agents can also inhibit enzymes outside of the cholinergic system, chiefly serine esterases. O'Neill (1981) reviewed data that indicated a role for anticholinesterase compounds, such as DFP or nerve agents, in altering the metabolism and persistence of important neuropeptides, such as endorphins, enkephalins, and substance P, which are degraded by serine esterases—producing some symptoms of agent exposure that do not respond to atropine. Experimental support of this concept of non-ChE effects on the brain is provided by Clement and Copeman (1984), who found a long-lasting analgesia in mice following exposure to soman and sarin, which was reversed by the opiate antagonist nalaxone. They also infer that inhibition of

proteases may increase the effects of endogenous opioids. No information was available on changes in opioid receptors following exposure to anti-cholinesterase drugs.

Before the discovery of nerve agents, it was known that some organophosphorus compounds (e.g., TOCP) could cause a delayed neuropathy occurring weeks after exposure, in people and animals, the effect being quite separate from any AChE effects. This disorder, organophosphate-induced delayed neuropathy (OPIDN), has been the subject of much study (Abou-Donia, 1981; Johnson, 1975; Johnson, 1992). The disorder was complex, with sensitivities varying according to age, species, and chemical. The hen came to be the standard screening model (Johnson, 1975).

An enzyme, neuropathy target esterase (NTE), was recognized as playing a role in the disorder. Naturally, the nerve agents came under scrutiny for their ability to induce neuropathy (see later discussions). Different agents have been found to vary considerably in their ability to inhibit NTE and to produce neuropathy. It was shown (in hens) that repeated subneurotoxic doses of DFP or sarin, in animals protected by atropine and the oxime P2S, could develop delayed neuropathy even at dosing intervals of 16 days (Davies and Holland, 1972).

Prior to the Gulf War, the prevailing view was that some military agents could acutely produce delayed neuropathy only at very high levels, many times the lethal doses of the agents (and requiring "heroic" treatment efforts for the cholinergic problems) (Marrs, Maynard, and Sidell, 1996; Sidell, Takafuji, and Franz, 1997, Ch. 8; Bucci, Parker, and Gosnell, 1992b, 1992c; Gordon et al., 1983). The reviewer did not find discussions of hazards from repeated low-dose exposures, although neuropathy from repeated low doses of other neurotoxic chemicals was known.

But the concern with illnesses after the Gulf War has turned attention toward that possibility. Haley et al. have suggested that an atypical delayed neuropathy might be involved in some Gulf War illnesses (Haley, Horn, et al., 1997; Haley and Kurt, 1997; Haley, Kurt, and Hom, 1997; Hom, Haley, and Kurt, 1997; and Somani, 1997). There is a report that inhaled sarin in mice at daily doses that do not produce cholinergic signs of illness can produce typical delayed neuropathy after 10 days of exposure (the does would be very symptomatic in humans) (Husain, Vijayaraghavan, et al., 1993); see later discussions.

Regardless of the proximate mechanism of action, a complex cascade of effects can follow once the toxic effect of the nerve agent is initiated: seizures and hypoxia, with the excitotoxins the seizures release producing neuronal injury (Lipton and Rosenberg, 1994).

MECHANISM OF ACTION—ACUTE EFFECTS

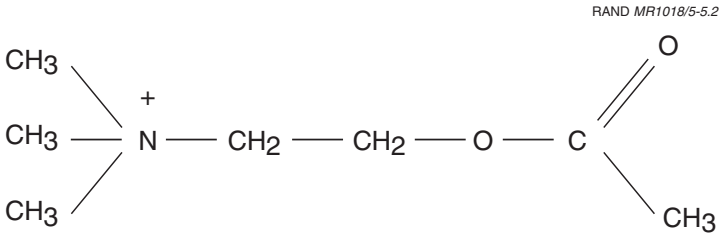
ACh (Figure 5.2) serves as the neurohumeral transmitter at the endings of postganglionic parasympathetic nerve fibers, between somatic motor nerves and skeletal muscle, at preganglionic fibers of both sympathetic and parasympathetic nerves, and at synapses within the central nervous system (Goodman and Gilman, 1990). Normally, as a nerve impulse arrives at a cholinergic synapse, or neuroeffector junction, ACh is liberated in packets from storage vesicles, crosses the synaptic cleft, and stimulates specialized choline receptors of the adjacent neuron, depolarizing the postsynaptic membrane (Figures 5.3 and 5.4). ACh is almost immediately inactivated by the enzyme AChE, producing choline and acetic acid. Transmission of the impulse ceases, and the membrane repolarizes and is ready to respond again.

Nerve agents and organophosphate pesticides bind to the enzyme, first in a reversible way. Many agents then "age" the enzyme, producing a very difficult-to-reverse bond (Figure 5.5). The aging rate varies with the agent and is an important therapeutic consideration. VX ages slowly (many hours), while soman ages rapidly (6 minutes) (SIPRI, 1976; De Jong, 1987). The carbamate inhibitors, such as eserine and PB, also inhibit the enzyme, but reversibly.

As a consequence of nerve agent inhibition of AChE, ACh accumulates at synapses, giving rise to uncoordinated bursts of signals, initially stimulating function and then paralyzing it. This brings about the characteristic signs and symptoms, which are usually grouped as follows (Klaassen, 1996; Goodman and Gilman, 1990; Marrs et al., 1996; Koelle, 1994):

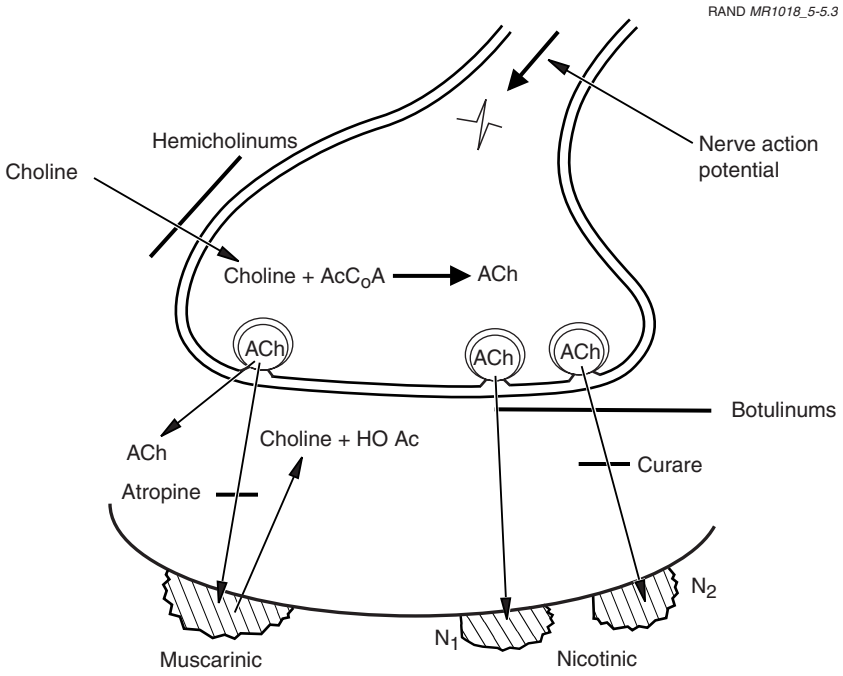
1. **muscarinic**—miosis, increased lachrymation, increased nasal secretions, tightness in chest, wheezing, increased bronchial mucus, increased gastrointestinal tone, cramps, peristalsis, nausea, vomiting, diarrhea, increased sweating, frequent urination, bradycardia, and heart block
2. **nicotinic**—muscle weakness, fasciculations, fibrillation, cramps, difficulty breathing, pallor, elevated blood pressure, and tachycardia
3. **central**—headache, dizziness, impaired memory and alertness, anxiety, tension, irritability, emotional instability, lethargy, ataxia, seizures, insomnia, excessive dreaming, coma, respiratory depression, and paralysis.

The predominant findings in acute intoxication are explained by AChE inhibition as manifested by the above responses and are similar with both nerve agents and organophosphate pesticides. However, the situation in the central nervous system is complex. Receptors activated by ACh can also modulate the release of other neurotransmitters within the brain (Goyal, 1989). Nerve agents may directly affect the release of other transmitters by mechanisms unrelated to cholinergic receptors (Prioux-Guyonneau et al., 1982).



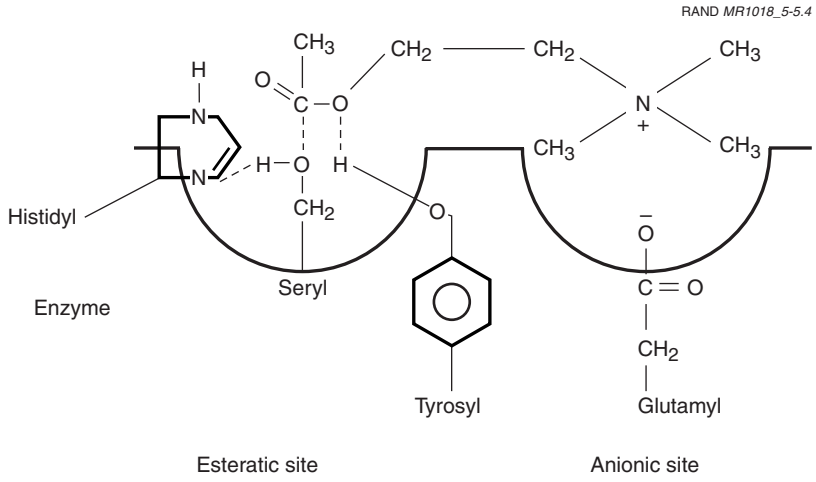
SOURCE: AD Little (1986, Ch. 5).

Figure 5.2—Chemical Structure of ACh



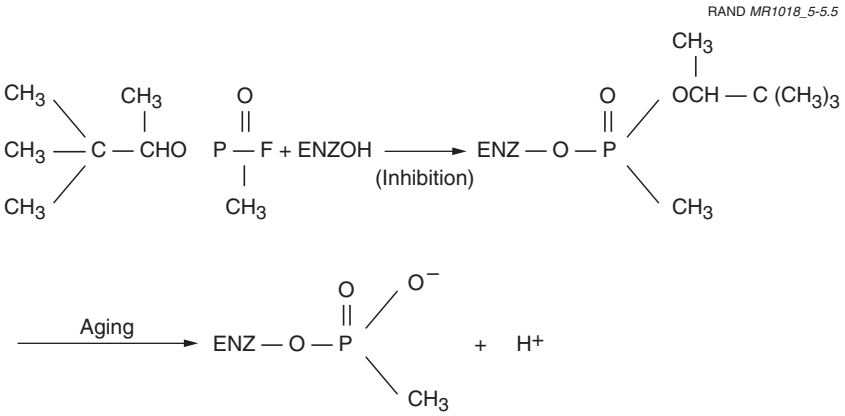
SOURCE: Adapted from AD Little (1986, Ch. 5).

Figure 5.3—Cholinergic Action and Effects of Drugs and Toxins



SOURCE: Adapted from AD Little (1986, Ch. 5).

Figure 5.4—AChE Active Site, with ACh



SOURCE: Adapted from AD Little (1986, Ch. 5)

Figure 5.5—Reaction of Nerve Agents with AChE

AChE INHIBITORS

A common index of the relative potency of various agents is the negative log of the molar concentration required to inhibit 50 percent of the AChE *in vitro* (the pI_{50}). The higher the pI_{50}, the more potent the ability to inhibit cholinesterase and, in general, the greater the toxicity. Because this is a logarithmic measure, a

difference of 1 in pI_{50} is a difference of a factor of 10 in actuality. Table 5.3 is more precise in predicting the inhibition of the enzyme than in predicting toxic effects.[5] Animal studies show a correlation between potency and toxicity. Many drugs and chemicals are weak cholinesterase inhibitors, with pI_{50}s of about 2 (e.g., cysteine, glutathione, streptomycin, chloramphenicol, penicillin, quinine, quinidine, antimalarials, antihistamines, and ranitidine) (Root and Hofmann, 1967).

Alkylating agents used in chemotherapy and for immune suppression, such as cyclophosphamide, inhibit AChE and serum cholinesterase, requiring modifications of anesthesia given proximate to their use (PDR, 1998). Sulfur mustard also inhibits cholinesterase (Krustanov, 1962; Dacre and Goldman, 1996).

Toluene, an organic solvent, at levels of 2,000 parts per million, has been shown *in vivo* and *in vitro* to inhibit AChE bound to red blood cell and synaptic membranes (Korpela and Tahti, 1988). Other aromatic and chlorinated hydrocarbons inhibited red cell AChE *in vitro* (Korpela and Tahti, 1986a, 1986b). Another study showed *in vitro* red cell inhibition from a variety of hydrocarbons (including benzene, xylene, and trichloroethylene); ethanol also slightly inhibited the enzyme (Korpela and Tahti, 1986a, 1986b). Lower levels of toluene (300 parts per million) *in vitro* also inhibited red cell AChE.

A comparison of rates of inhibition of eel AChE by oxono (P = O) inhibitors and thiono analogs (P = S) of several agents (paraoxon, fonofos, sarin, and soman) showed that the oxono compounds had substantially higher phosphorylation rate constants than their thiono analogs. This was thought to be due to differ-

Table 5.3

Anticholinesterase Potency of Organophosphates

Agent	pI_{50}
Tabun	8.6
Sarin	8.9
Soman	9.2
Thiosoman	8.9
Cyclosarin	10.1
VX	8.8
DFP	6.5
Parathion	4.9

NOTE: See text for definition of pI_{50}.
SOURCES: (Dacre, 1984; SIPRI, 1973).

[5]Other measures of relative potency, such as LD_{50} ratios, have also been explored.

ences in hydrophobicity of the analogs (Maxwell and Brecht, 1992). Aging of the enzyme was not reported.[6] Note that AChE is found in a number of tissues that lack neural connections, such as erythrocytes, lymphocytes, basophils, spermatozoa, and placenta (Sastry and Sadavongvivad, 1979). The functional role of the enzyme in such tissues is not known, and although it is inhibited there by nerve agents, the biological consequences are little understood (Sastry and Sadavongvivad, 1979; Meier et al., 1985).

PRETREATMENTS AND TREATMENTS FOR NERVE AGENT POISONING

This review is not concerned with treatment of nerve agent injury, but the basic pretreatment approach is described below.[7] The term *pretreatment* is used to describe an intervention made before exposure to assist with treatment required after exposure. This is distinct from prophylactic intervention, which may protect to the point that therapy is not required. At one time, there was serious consideration of using atropine (or atropinelike drugs) or oximes (reactivators) as pretreatments (Karakchiev, 1973, SIPRI, 1976).

The main current approach is to use a drug like PB that binds to cholinesterase in a reversible manner, in an amount that leaves many functional sites untouched, avoiding toxicity. When a soldier encounters a nerve agent, such as soman, that binds irreversibly to the enzyme, the sites occupied by the drug are protected from attack. After the dose of nerve agent has reacted elsewhere, the reversible drug PB leaves the enzyme site, restoring function. This approach is needed for agents, such as soman, that "age" and bind rapidly to the enzyme in a way that oxime drug treatment cannot reverse.

Koster (1946) used the carbamate physostigmine to protect cats from DFP. The very rapid aging of the enzyme bound by soman (about 6 minutes) caused great interest in NATO countries in using carbamates to pretreat for this Soviet threat agent. Several countries, including the United States, chose PB (used to treat myasthenia gravis) (Sidell, Takafuji, and Franz, 1997). This drug, the subject of a separate RAND report (Golomb, 1999), ordinarily does not cross the blood-brain barrier because of its polar nature (Goodman and Gilman, 1990).

No discussion was found on the situation of continued PB use after unrecognized exposure to nerve agent. One concludes that an unstated assumption within the research and medical community about PB use was that attacks would be obvious and that PB would not be used after intoxications.

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[6]This is the only information that emerged from this review on properties of thiosarin. It suggests that thiosarin and thiosoman may be somewhat less toxic than their better-known analogs.

[7]For more information, see the accompanying report on PB (Golomb, 1999).

Adverse Health Effects

Considerable effort went into developing administration regimens for PB that would be safe and effective and that would not impair military performance (Gall, 1981). But the actual use of PB during the war has produced unexpected responses and concern. These issues have been exhaustively investigated in a separate volume of this series (Golomb, 1999).

Treatments

Atropine, the mainstay of treatment agents (Marrs, Maynard, and Sidell, 1998; Grob and Harvey, 1953), antagonizes the effects of ACh on muscarinic receptors. It does not act on nicotinic receptors. Oximes, a second class of treatment drugs, are used to reactivate AChE by displacing the agent from the enzyme, but only before aging has occurred. As noted, the treatment of soman poisoning is difficult, and it was for this agent primarily that pyridostigmine was used as a pretreatment. U.S. forces use injectable forms of atropine, pralidoxime, and diazepam. Diazepam is used for its anticonvulsant effect (U.S. Army Medical Research Institute of Chemical Defense, 1995).

It is known that cyclosarin is somewhat resistant to treatment with some common oximes, including pralidoxime, based on animal and *in vitro* studies (Coleman et al., 1966; Clement, 1992; Worek et al., 1998; Kassa and Bajgar, 1995). However, rhesus monkeys pretreated with pyridostigmine and challenged with intramuscular doses of 5 LD_{50} of cyclosarin, followed by treatment with atropine and 2-PAM (pralidoxime) had 100 percent survival (Koplovitz et al., 1992). This suggests that the treatment means available to U.S. forces during the Gulf War would have been effective against cyclosarin. Oximes have a number of other effects on allosteric sites and receptors and block overstimulated ganglia.

As mentioned above, however, AChE inhibition does not explain all aspects of nerve agent toxicity (Van Meter, Karczmar, and Fiscus, 1978; Kaufer et al., 1998; O'Neil, 1981). Several nerve agents appear to be weak direct agonists of receptors, with VX acting strongly on nicotinic receptor ion channel sites (Albuquerque et al., 1983, 1985; Eldefrawdi et al., 1985). There are also indications of direct agent binding to synaptic membranes (Anderson and Chamberlain, 1988), and soman has also been shown to act directly on the receptor (Hoskins, 1982). Cholinergic stimulation by inhibition of cholinesterase in effect stimulates expression of the proto-oncogene c-fos in the brain. The long-term significance of this is uncertain, as will be discussed later (Kaufer et al., 1998; Friedman et al., 1996).

Nerve agents can also inhibit other serine esterases (e.g., trypsin, chymotrypsin, and thrombin) (Meier et al., 1985; O'Neill, 1981; Walday, Aas, and Fonnum,

1991; Pasternack and Eisen, 1985) and serine proteases involved in regulating neuropeptides (e.g., substance P and met-enkephalin (O'Neill, 1981; Clement and Copeman, 1984). The functional significance of these findings is unclear, although O'Neil (1981) suggests that some signs and symptoms of nerve agent poisoning might be mediated by enkephalins, whose persistence in the brain is prolonged by exposure to DFP, for example.

Delayed Neuropathy and Neuropathy Target Esterase (NTE)

Of particular interest is NTE, which has been implicated in a form of delayed neuropathy known as OPIDN (Abou-Donia, 1981; Johnson, 1975; Johnson, 1992). It has been hypothesized that some of the neurological findings in Gulf War illness patients arise because of combined chemicals including nerve agents that may have produced this type of neuropathy (discussed further under "Clinical Findings," below).

In general it has been very difficult to produce delayed neuropathy in animals using nerve agents. Doses vary in excess of lethal levels, requiring pretreatment and treatment (Gordon et al., 1983). The natural substrate of NTE and the detailed mechanism of toxicity are not known. Toxicity only occurs when a sufficiently large amount of the enzyme is inhibited. There is no known treatment. The enzyme is widely distributed in the nervous system and has also been demonstrated in lymphocytes and platelets, which have been used in screening and toxicity studies (Bertoncin et al., 1985; Lotti, 1991). The hen has become the standard research animal for NTE studies (e.g., Olajos, DeCaprio, and Rosenblum, 1978).

Johnson (1972) noted that only a tiny amount of toxin was required to produce an effect, the rest being dissipated via nonspecific reactions and degradation mechanisms. He raised concern that if another compound overloaded or blocked such pathways, the threshold dose of the neurotoxic compound would decrease.

Phenylmethylsulfonyl fluoride has been used as a pretreatment protective of NTE but increases toxicity if given after a NTE inhibitory agent, such as TOCP or mipafox (Pope and Padilla, 1990). There has been speculation that administration of PB after a toxic exposure might have the same effect (Haley, Kurt, and Horn, 1997; Halley, Horn, et al., 1997; Halley and Kurt, 1997).

The temporal properties of delayed neuropathy are complex. Classically, after an acute exposure to TOCP or DFP at sufficient doses, there is a 10- to 14-day delay before onset of signs and symptoms. In human TOCP cases, there is weakness and then paralysis, chiefly involving the lower extremities. There is degeneration of axons both peripherally and in the spinal cord. Recovery is rare but does occur (Hayes, 1982). Animal studies with organophosphate chemicals

have shown that cumulative effects can produce such lesions; in some studies, doses six weeks apart were cumulatively able to produce the neuropathy (Lotti, 1991). Although no reports of the effects of combinations of different chemicals that inhibit NTE emerged, there seems to be some potential for complex interactions.

The classical findings of delayed neurotoxicity have generally been found from the medulla to the periphery (Abou Donia, 1981). Some possibility remains that higher brain centers might be affected. Prendergast, Terry, and Buccafusco (1997, p. 116), point out in the review section of their paper that impairment of AChE does not predict cognitive impairment well in animals and suggest that NTE, which has also been associated with cognitive impairment, might be involved. They did not measure this enzyme in their studies

ENTRY AND FATE

Adsorption

The lipophilic nature of the nerve agents indicates that, as a group, they can readily penetrate the skin, lung, and gastrointestinal tract and, after entering the circulation, can be widely distributed, largely according to regional blood flow. The considerable species variation in sensitivity to these agents appears to reflect differences in the amount and distribution of nonspecific esterases that can bind the agents (Somani, 1992), although there are species differences in AChE affinity for organophosphate agents (Wang and Murphy, 1982).

Dermal

The skin does provide some degree of environmental protection, particularly against vapors. Military agents vary in the threat they pose. Tabun and sarin are rather volatile, and high concentrations (vastly higher than toxic respiratory doses) are required to produce toxicity through the skin by vapor exposure. Humans exposed to CTs of 1,000 to 1,300 mg-min/m^3 of tabun showed only a decline in serum and red cell cholinesterase. Even at a CT of 2,000 mg-min/m^3, subjects had no symptoms (Krakow and Firth, 1949). The NRC's Committee on Toxicology (NRC, 1997) cited 1951 work by McGrath in which humans were exposed to sarin vapor at CTs of 190 to 1,010 mg-min/m^3 without lowering blood enzyme levels. Exposure to levels of 1,225 to 1,850 mg-min/m^3 resulted in declines of ChE from 31 to 90 percent. No illness occurred, but two of nine subjects showed sweating. The committee considered 1,200mg-min/m3 to be a threshold effect ECT_{50} exposure.

Liquid agents applied to the skin are readily absorbed (Blank et al., 1957), but for volatile agents, such as sarin, evaporation reduces the amount of agent available for absorption (Grob et al., 1953). Applying 5 mg/kg of tabun to the

skin of volunteers produced no illness but resulted in a 30-percent fall in AChE and notable local sweating (Freeman et al., 1954).

Chlorpyrifos, a pesticide of some Gulf War interest, is very poorly absorbed through the skin (Nolan et al., 1984). Some agents of intermittent volatility (e.g., soman and cyclosarin) present a greater hazard, and VX presents the greatest percutaneous hazard (Sim, 1962).

People show distinct regional differences in skin absorption; for VX, the highest rates are on the head and neck (Sim, 1962). Moisture, heat, and abrasions can increase agent transfer, while the total area exposed is important (Blank et al., 1957). The dermal toxic dose for many agents is considerably higher than for parenteral or respiratory exposure, reflecting not only evaporative and mechanical losses but also the ability of the skin and underlying tissues to bind agents and to inactivate them enzymatically (Fredriksson, 1969). For example, the skin dose of VX required to reduce red-cell AChE by 50 percent in humans is 32 µg/kg, while the intravenous effect is attained with 1 µg/kg. VX, on the other hand, is not hydrolyzed efficiently in the skin.

Ocular

Agents can be readily absorbed from the conjunctival sac and the eye (Grob and Harvey, 1958). Theoretically, dangerous amounts of agent could be absorbed as droplets by this route, but no data suggesting this is likely. The marked local effects of miosis, dim vision, impaired night vision, headache, lethargy, and impaired accommodation are predominant features of eye toxicity.

Respiratory

Vapors and aerosols are well absorbed from the lung. Oberst et al. (1959, 1968) demonstrated that humans exposed at rest to doses of sarin retained 89 percent of the inspired agent, less (79.5 percent) if exercising. The authors noted that CTs were not highly reliable indicators of toxicity. CTs ranging from 7 to 9.7 mg-min/m^3 (exercising men) and 33 to 42.6 mg-min/m^3 of sarin all produced similar absorbed doses. Particles with adsorbed agent can also be dangerous by this route (Asset and Finklestein, 1951).

Gastrointestinal

Gastrointestinal exposure of animals has been extensively studied for organophosphate pesticides, but little has been done with nerve agents. One study suggests that gastrointestinal exposure to a nerve agent produces rapid and serious intoxication (Karakchiev, 1973). Human studies (Sidell and Groff, 1974) with 4 µg/kg of VX taken orally showed a rapid response with fall of red-cell AChE. Maximum inhibition was 70 percent at two to three hours, with mild

gastrointestinal disturbances (colic, nausea, vomiting, and diarrhea). Systemic symptoms were rapid at 20 minutes in other studies of sarin (Grob and Harvey, 1953) and VX (Sim et al., 1971).

Metabolism

Intra-arterial administration of sarin (Grob and Harvey, 1953) at 6 µg/kg showed the agent would pass the capillary bed with immediate symptoms and gradual decline of red-cell AChE to 28 percent over one hour. Smaller doses (3 to 4 µg/kg) produced no symptoms and only a minimal decline in red-cell AChE, suggesting that such lower levels may be detoxified.

Animal data show that rapidly acting agents at high dosage levels are not cleared effectively but that there are detoxification systems capable of dealing with lower levels of challenge (Somani, 1992; Fonnum and Sterri, 1981). Guinea pigs metabolize sarin at a rate of 0.013 µg/kg/min and soman at a rate of 0.009 µg/kg/min (Somani, 1992, p. 89). The rates of metabolism probably vary, with the isomers involved as in the case of soman (Benschop et al., 1981, 1984; De Bisschop et al., 1985). Carboxyesterases are important in metabolizing sarin, soman, and tabun (Walday, Aas, and Fonnum, 1991; Maxwell, 1992), but these enzymes are quantitatively much more important in rodents than humans. Nonspecific enzymes in serum and liver (aliesterases) metabolize all agents (Somani, 1992, p. 91).

Female mice are known to have plasma butyrlcholinesterase levels about twice that of matched males, with carboxyesterase levels 1.3 times those of males. The detoxifying or protective effects of these enzymes were not detectable in comparisons of the brain AChE levels in males and females three hours after intraperitoneal injection of 4 mg/kg of DFP or 0.3 mg/kg of sarin (Tuovinen et al., 1997).[8]

Some forms of paroxonase (PON1) in the serum hydrolyze sarin and soman at a high rate, breaking the P-F bond (Davies, 1996). Serum anhydride hydrolase (parathionase) is also active at least against soman (Broomfield, 1992). Variations in the abundance of these enzymes in human populations may produce variations in sensitivity to agents (Mutch et al., 1992).

The serum and some tissues contain an enzyme butyrylcholinesterase (EC 3.1.1.8), whose main biological purpose is unknown. This enzyme can hydrolyze ACh. Nerve agents and carbamates bind to this enzyme. Studies of an Israeli soldier who was hypersensitive to PB showed a variant of this enzyme that had low affinity (1/20th normal) for PB and other cholinesterases

[8]These were substantial doses, and they do not exclude a useful role in protection from lower-level exposures.

(Lowenstein-Lichtenstein et al., 1995; Schwarz et al., 1995). This suggests a role for the normal enzyme in decreasing the effects of low doses of anticholinesterase agents.

Elimination Excretion

In the case of soman, there appears to be a deposit site, probably in muscle, that does not inactivate the agent but rather stores and later releases it in toxic form. No such phenomenon has been reported for other agents, but the possibility apparently has not been evaluated. The effect lasts hours not days (Van Helden and Wolthuis, 1983; Wolthuis, Benschop, and Berends, 1981; Van Helden, Berends, and Wolthuis, 1984).

In general, the agents disappear rapidly from the blood, with rapid formation of hydrolysis products. The main metabolic product of sarin, IMPA, remains in tissues, although a great deal is eliminated rapidly in the urine, with a half-life of 3.7 hours (Fleisher et al., 1969). Cyclosarin studies show it is hydrolyzed to a similar analog with a half-life of 9.9 hours. Soman metabolism is more complex and biphasic, with half-lives of 3.6 hours and 18 hours, and soman accumulates in the lung (Shih, McMonagle, et al., 1994). Pinacolyl phosphonic acid is a major soman metabolite. IMPA was detected in the urine of Japanese sarin casualties—in one case for a week (Nakajima, Sasaki, et al., 1998; Minami et al., 1998).

Distribution

Sublethal amounts of soman injected intravenously in mice yield only trace amounts in tissues after 1 minute, with most converted to pinacolyl phosphonic acid. Studies show distribution to blood, choroid plexus, and spinal fluid at 2 minutes, with a distinct concentration in hypoglossal and vestibular nuclei, and later in the thalamus and caudate nucleus (Traub et al., 1985).

Blood flow is a key determinant in distribution of all agents (Somani, 1992, p. 79), and the higher percentage of the cardiac output going to the brain increases the risk there. Sites at which the brain is active show increased metabolic activity associated with vasodilation and increased blood flow, raising the possibility that distribution of agent might vary according to brain activity at the time of exposure (Scremin, Shih, and Corcoran, 1991; Scremin and Jenden, 1996).

Regional inhibition of AChE has been used to examine distribution effects, as has alteration of receptor properties. For example, Churchill et al. (1984) report such alteration in the olfactory bulb, hippocampus, and cortex, and such inhibition seems to be long lasting (Shipley et al., 1985). The latter regions are

important in memory, while limbic system involvement influences mood and activity. The effects show age differences in the distribution of inhibition (Shih, Penetar, et al., 1990), but the correlation with inhibited AChE and clinical findings is uncertain.

Although the carbamates, such as PB, do not bind to aliesterase (Somani, 1992), there is concern that PB might occupy other binding sites, rendering them unavailable to bind nerve agents and thus increasing the amount of agent available at sites where toxicity is manifested. However, in animal studies with VX and soman, the agent was not increased in the brains with PB pretreatment (Anderson et al., 1992).

EXPOSURE-EFFECT RELATIONSHIPS

Reports of effects vary by study and species. This section does not focus on higher-level exposures, which are less relevant to the Gulf situation. Greater attention is paid to lower-dose studies, some of which examine behavioral consequences of exposure as well as noting factors that might modulate responses to agent.

Acute Exposures, Acute Effects

There is no information on thiosarin, and the information on cyclosarin is sparse. Table 5.4 gives lethality and incapacitation estimates for the others.[9] There has also been a no-effect estimate of a CT of 1.6 mg-min/m^3 for VX (McNamara et al., 1973). However, a study cited by the Committee on Toxicology (NRC, 1997) could not identify an adverse level (no-observed-adverse-effect level) based on VX exposures ranging from 0.7 to 25 mg-min/m^3 (cited report was Bramwell et al., 1963). Lethality and incapacity estimates for cyclosarin are now available based on the extensive review of the Subcommittee on Toxicity Values for Selected Nerve and Vesicant Agents (NAS, 1997), although these are sometimes based on analogies with better-known agents. Some of their findings are included in Table 5.4. Generally, the toxicity of cyclosarin falls between that of soman and sarin (Cresthull et al., 1957), although some authors credit it as being twice as toxic as sarin (Karakchiev, 1973). Appendix B gives effect estimates for other species and modes of exposure. It is of some note that female animals show greater sensitivity to nerve agents (Callaway and Blackburn, 1954, for example), and the rate of recovery of AChE is slower (Woodard et al., 1994). A follow-up study of some Tokyo subway sarin cases

[9]See Appendix A for an explanation of CT and other dose measurements.

Table 5.4

Estimates of Nerve Agent Lethality or Incapacitation to Humans

	Skin LD_{50} (mg liquid, 70 kg man)		Respiratory LCT_{50} (mg-min/m^3)			Respiratory ICT_{50}/ Severe Effects (mg-min/m^3)	
Agent	NAS	Somani	NAS	Somani	Other	NAS	Other
Tabun	<1,500	200–1,000	<70	100–200	150–400	<50	100–300
Sarin	<1,700	100–500	<35	50–100	70–100	<25	15–75
Soman	350	50–300	<35	25–50	50–80	<25	5–25
Cyclosarin	350		<35			<25	
VX	<<5	5–15	<15	5–15	30–100	10	5–50

SOURCES: Somani (1992), p. 77; OSRD (1946), U.S. Army (1990), Karakchiev (1973), McNamara et al. (1973), Trask et al. (1959) (NRC, 1997).

found subtle neurological deficiencies in female cases (compared to control) but not in the male cases (Yokoyama, Araki, et al., 1998a).

Also of interest are fairly large-order variations in sensitivity to nerve agents at different times of the circadian cycle, as Elsmore (1981) showed in LD_{50}s of rats given soman at intervals around the clock. Agents also disrupt circadian rhythms (Mougey et al., 1985). This might mean that nerve agents or pesticides might be more toxic to troops at night than in the day, when most human studies have been done.

The U.S. Army Surgeon General has established exposure limits to a number of nerve agents for workers (eight-hour exposures) and the civilian population (MMWR, 1988). Concentration limits were established for exposures (see Table 5.5).

Such exposure limits are selected both to avoid any clinical signs and generally to provide at least an order-of-magnitude safety margin. Estimated remote cumulative doses for the Khamisiyah release appear to be higher: 0.01296 mg-min/m^3 (CIA, 1997).

Behavioral Effects

A study of 29 troops in the mid-1940s (Marrs et al., 1996) found that humans exposed to a CT of 28 mg-min/m^3 of tabun had definite symptoms; all 29 had miosis and vomiting; 26 were depressed; 22 were fatigued; and some night performance was impaired.[10] Recovery took one week, so it seems unlikely a

[10]Marrs lists the report as an unpublished Ministry of Defence report, but outside review indicates that this was a 1945 Porton Report by Curwan and Mittner (PRZ2711), which was not available for our review.

Table 5.5

Nerve Agent Exposure Limits Established by the U.S. Army Surgeon General

	General Population (72 hours, mg/m^3)	Workers (8 hours, mg/m^3)
Tabun	3×10^{-6}	1×10^{-4}
Sarin	3×10^{-6}	1×10^{-4}
VX	3×10^{-6}	1×10^{-5}

SOURCE: MMWR (1988).

NOTE: The MMWR summary did not include soman and cyclosarin. The document from the Surgeon General (DAMD17-85R0072, p. 49) on which the MMWR report was based also shows an 8-hour time-weighted average soman of 3×10^{-5} mg-min/m^3. The 8-hour time-weighted average for cyclosarin was the same as for sarin.

similar outbreak in the Gulf would have escaped medical notice. Low inhaled-dose exposure to sarin of 5 µg/kg (calculated from respiratory exposure) did not impair a variety of complex tasks.[11]

An investigation of dermally applied EA1701 (an early designator for VX) using a micrometer syringe, at several levels of exposure, found mood, thinking, and behavioral changes in 93 human volunteers exposed to VX at levels that did not produce gastrointestinal, respiratory, or muscle symptoms, although some experienced nausea. In that study, decreases in red cell AChE correlated with anxiety and decreased mental performance; the exposures were of several levels and some had no fall in AChE (Bowers et al., 1964). These volunteers are presumed to be included in the long-term follow-up study that found no long-term effects (NAS, 1985). The authors did not give the actual doses (perhaps for security reasons) but drew attention to the considerable mental effects without peripheral signs of cholinesterase inhibition.

Another study (Sidell, 1967), using intravenous VX at three levels (three subjects at the lowest, four next, and 18 at the highest), with a placebo control group (four subjects), found no significant blood pressure or heart rate changes. AChE showed 70 percent inhibition. Doses were 1.3 µg/kg, 1.4 µg/kg, and 1.5 µg/kg. There were few peripheral symptoms. Although eight were nauseated and four vomited, these symptoms took an hour to develop. Twelve subjects were dizzy or lightheaded, and nervousness was common. A number facility tests showed a significant decrease only in the 1.5-µg/kg group. Presumably, the volunteers noted above were included in the long-term follow-up study, which did not find long-term effects in volunteers (NAS, 1985), but no short-

[11]It was not possible to obtain primary sources from Marrs's references, but the text gives fairly detailed accounts.

term follow-up was included in the reports, so the duration of the effects is uncertain.

Low doses of soman and sarin (1/40th to 1/9 LD_{50}) alter rodent behavioral performance, although the animals appear well. They seemed anxious (i.e., they hesitated on some tasks and were less inquisitive), but only sarin impaired coordination and balance (Sirkka et al., 1990). Behavioral changes were also observed in rats (open field locomotion) given sign-free doses of soman (4 µg/kg) and sarin (20 µg/kg) intraperitoneally lasting over 12 hours (Nieminen et al., 1990).

Marmoset studies (Wolthuis, Groen, et al., 1995) of cholinesterase inhibitors showed little physiological response at low levels, although blood AChE was decreased. However, there was definite disruption in a number of tests (e.g., visual discrimination, eye-hand coordination, and choice-time). There were increases in no-attempt behavior (i.e., failures to respond to rewards). PB had more behavioral effects than had been expected, given that it does not usually pass the blood-brain barrier. The performance decrements took place at levels of agent without overt clinical signs. There have been many studies of animal performance in response to nerve agents, with an emphasis on soman (Hartgraves and Murphy, 1992). At 0.5 LD_{50}, soman and VX were more disruptive of performance than tabun and sarin (Mays, 1985).

Ocular Effects

The eye is sensitive to vapor or aerosols, with clinically and operationally important effects occurring at low levels (OSRD, 1946; NAS, 1997; Sim, 1956). These effects should have appeared if there were significant low-level exposures in the Gulf. Miosis refers to constriction of the pupils but is usually associated with a constellation of other problems: dim vision, pain, impaired night vision, difficulty focusing, and appearance of eye inflammation. CTs for miosis (mg-min/m^3) are 20 for tabun, 2 for sarin, 0.1 for soman, and 0.09 for VX (OSRD, 1946; Karakchiev, 1973; McNamara et al., 1973; Sim, 1956; Johns 1952). Human night vision is impaired via a retinal effect at 5 CT of sarin (Rubin and Goldberg, 1957b). The no-effect level for VX is 0.02 mg-min/m^3 (McNamara et al., 1973). The recent report of the Subcommittee on Toxicity Values for Selected Nerve and Vesicant Agents (NAS, 1997), which used multiple sources that may have been different from the above citations, determined the CT for miosis of sarin to be 0.5 mg-min/$m^{3,}$ a much lower figure than that noted above, although they noted that there were also reports of no effects at this level of exposure.

Dermal Effects

Dermal exposures generally require higher doses to generate the effects seen through other routes (see Appendix B). They are associated with slower onset

of symptoms, fewer eye and respiratory symptoms, more cardiovascular symptoms, and nervous system symptoms. Reducing blood AChE by 50 percent required total doses of 400 mg of sarin, 65 mg of soman, and 30 mg of cyclosarin (Marrs et al., 1996; note the difference in dermal effect between sarin and cyclosarin). Parenteral (e.g., intravenous) and gastrointestinal routes of exposure were reviewed but do not seem relevant to Gulf War situations. Sweating is the common marker of dermal exposure and can be rather persistent.

Combined Effects

Tabun and mustard show a marked increase in toxicity and lethality when animals are exposed to both, and serum cholinesterase recovers more slowly than when the agents are used singly (Krustanov, 1962). The recognition of possible mixed use of sarin and cyclosarin prompted study of their combined toxicity; animals did not show unique toxicity, and therapy with standard measures was satisfactory (Clement, 1994). (See the earlier discussion on treatment mentioning the resistance of cyclosarin to oxime.)

Stress and Steroid Effects

How stress influences the effects of agents has not been studied extensively. Adrenalectomy did not alter the toxicity of sarin and soman in Wistar rats. Pretreatment with ACTH, adrenal cortical extract, cortisone, prednisolone, or corticosterone did not decrease soman toxicity. Soman toxicity was significantly decreased by pretreatment with prednisolone or cortisone plus atropine, compared to atropine alone (Stabile, 1967).

Modulation by Pretreatment

Humans exposed to a CT of 5 mg-min/m^3 (30 min) of sarin after PB pretreatment had an altered miosis course with less conjunctival irritation and a shorter course of symptoms (i.e., two to three days versus seven to ten days) (Gall, 1981). This demonstrates that the clinical response of pretreated persons to low doses of agents may be modified by the pretreatment, possibly decreasing or preventing some of the signs and symptoms.

There has been concern, however, that pretreatment medications might enhance the toxicity of some agents. Physostigmine after DFP did not protect but rather enhanced toxicity (Koster, 1946).

There are limited indications that PB, not followed by treatment (e.g., with atropine or oxime), may decrease the duration and severity of symptoms and perhaps their occurrence in humans and animals exposed to low doses of an agent (e.g., sarin). Husain, Vijayaraghavan, et al. (1993) showed that sign-free PB and physostigmine pretreatments, also not followed by any treatment, pro-

vided a definite, favorable modification of the pulmonary function decreases in rats exposed to a CT of 51 mg-min/m^3 of sarin. Rats are less sensitive to sarin than humans; the LCT_{50} for the rat is about 220 (Callaway and Blackburn, 1954), and the estimated LCT_{50} for humans is about 75 mg-min/m^3 (NAS, 1997). Related studies by the same group (Vijayaraghavan et al., 1992) showed that, in rats exposed to sarin at a CT of 51 mg/m^3 aerosol, pretreatment with carbamates, PB (0.075 mg/kg intramuscular), or a "symptom free" dose of physostigmine 20 minutes before sarin exposure protected lung AChE and increased survival. Physostigmine afforded better results. No treatment was given.

Longer Exposures and Tolerance

There is less information about chronic exposures, especially with measured doses. There are no reported exposure levels in the accidental occupational exposures reviewed, some of which may have reflected low-level exposure. Hartgraves and Murphy (1992) provide a substantial review of the behavioral effects of low-dose exposures to agents, some of which were subchronic or chronic. Chronic low doses of soman impaired primate responses, but the responses were not exacerbated by physostigmine pretreatment (Blick et al., 1993).

Animals and humans exposed repeatedly to sublethal levels of anticholinesterase compounds (inhibitors, such as nerve agents, organophosphate pesticides, drugs, carbamates, and carbamate pesticides) over time (days to a week) develop a condition known as tolerance, in which further administration of the inhibitor does not produce further signs and symptoms of exposure. Animal models have also shown behavioral tolerance to sustained sublethal exposures to DFP (Costa et al., 1982; Modrow and McDonough, 1986; Russell et al., 1975; Wolthuis, Philippens, and Vanwersch, 1991; Chippendale et al., 1972). Behavioral tolerance to soman in rats was seen, although performance decrements were noted on days of soman administration (Russell, et al., 1986). Doses of 35 µg/kg were given subcutaneously three times a week for four weeks (Modrow and McDonough, 1986). Dogs exposed to 25 µg/kg/day of sarin vapor for five days were symptomatic but showed signs of developing tolerance (Cresthull et al., 1960). A large, long-term study designed to simulate occupational exposures used beagles, exposing them daily to 10 mg-min/m^3 of sarin for six months (Jacobson et al., 1959), which resulted in some illness, no direct mortality, signs of tolerance, and full recovery after the end of study.

Russell et al. (1986), showed that prolonged administration of soman (11 doses over 22 days, 35 µg/kg—0.3 log of the LD_{50}) produced few signs of toxicity, although body temperature fell initially and then showed tolerance. Hypoalgesia continued, but tolerance was shown after initial decrements for a variety of

temporal and performance activities. Brain AChE levels stabilized during the study despite continued administration of soman, implying some compensating regulatory activity (Russell, et al., 1986).

In contrast a single sublethal dose of soman in rats (100 to 150 µg/kg, intramuscular) did not produce seizures immediately but greatly altered spontaneous motor activity and test performance lasting for over 21 days. Some animals were very excitable and developed seizures when handled (Haggerty et al., 1986).

Nonspecificity

Tolerant organisms show decreased response not only to the inducing chemical but also to other anticholinesterases and cholinergic compounds such as carbachol and oxytremorine. They also show increased sensitivity to the effects of antagonists, such as atropine (Costa et al., 1982; Modrow and McDonough, 1986).

It has been shown that tolerance to the organophosphate pesticide disulfoton and the agent DFP can be induced by administering small doses that do not produce any overt signs of toxicity (Schwab and Murphy, 1981). A similar finding has been observed in humans taking the inhibitor echothiophate for glaucoma (DeRoetth et al., 1965). Tolerance has been seen in pesticide workers (Hayes, 1982) and is the probable explanation for the production and laboratory workers in the U.S. nerve agent program who had very low levels of cholinesterase but who reported no symptoms (Freeman et al., 1956; Holmes, 1959).

Extensive research has excluded increased metabolic clearance of the inhibitors as an explanation of tolerance. The uptake of choline and synthesis of ACh in the presynaptic tissues is not impaired (Costa et al., 1982).

Receptors

There are two main classes of receptors for ACh: muscarinic (with three subgroups of differing affinities) and nicotinic. Peripheral tissues, such as the gastrointestinal and pulmonary systems, are muscarinic, while skeletal muscles are nicotinic. The central nervous system contains both types of receptors, but their role is less well understood than in peripheral tissues and the autonomic nervous system. The receptors are primarily found on postsynaptic membranes, although there are some presynaptic muscarinic receptors (Costa et al., 1982).

Decreases in the abundance of both muscarinic and nicotinic receptors in response to sustained exposure to anticholinesterases has been demonstrated

and seems to be the paramount mechanism of tolerance (Costa et al., 1982; Schwab et al., 1983; Bartholomew et al., 1985). Receptor decrease has been seen in tissue cultures, as well as *in vivo*. There may be additional mechanisms distal to the synapse involved in tolerance (Schwab et al., 1983).

Muscarinic receptors have been the most studied. Their abundance is decreased by chronic exposure to anticholinesterases or direct-acting cholinergic compounds (downregulation), while the binding affinity of the receptors is not altered (Costa et al., 1982). Indications are that receptors are internalized within the cell much as ligand-bound insulin receptors are. *De novo* synthesis of new receptors is required for recovery of normal abundance of receptors. In addition to decreases in receptor abundance, the function of the remaining receptors is altered, with decreased binding of agonists and antagonists in animals tolerant of organophosphate pesticides (Schwab et al., 1983; Costa et al., 1982; Schwab et al., 1981).

Nicotinic receptors appear to be more stable, although desensitization of nicotinic cholinergic motor end plates is fairly rapid. Other nicotinic receptors are slower to decrease than muscarinic receptors, although downregulation does occur. (Buccafusco et al., 1997).

The extent of downregulation in different parts of the central nervous system varies considerably—e.g., the brain stem showed much less downregulation in muscarinic receptors of rodents than did the striatum and cortex (Bartholomew et al., 1985). A single sublethal dose of soman in rats produced a reversible decline in muscarinic receptors of the telencephalon but an irreversible decline in the pyriform cortex (Pazdernik et al., 1986). Downregulation from low levels of anticholinesterases has also been demonstrated *in vitro* (isolated synaptic membranes of the bovine caudate nuclei) (Volpe, Biagioni, and Marquis, 1985).

Carbamates also induce tolerance, although their binding to AChE is reversible. Short-acting carbamates, such as physostigmine, require sustained infusions to induce tolerance. Tolerance to neostigmine has been shown in people and animals. The mechanisms of tolerance with carbamates may be more complex than with organophosphate agents, but downregulation of muscarinic receptors has been shown with them.

Duration

For indirectly acting cholinergics (anticholinesterases), the duration of cholinesterase inhibition is the critical factor, since anticholinesterase level appears to be the ultimate regulator of sensitivity to these chemicals. Prolonged exposure to anticholinesterases produces a decline in receptor abundance (Schwab et al., 1983).

The separate RAND report on PB (Golomb, 1999) also considers receptor effects. Most studies of this compound as a pretreatment have been fairly short, many of three to five days of exposure (Gall, 1981). Prolonged use of this drug, which is fairly long acting (the oral half-life is about four hours), might induce tolerance, at least in peripheral tissues, thus decreasing the effects of nerve agents by a mechanism additional to its reversible binding to AChE.

As noted previously, there is now reason to suspect that, under severe stress conditions, PB can pass the blood brain barrier and can act centrally as well as peripherally in downregulating receptors (Friedman et al., 1996).

Tolerance May Not Be Beneficial

The decrease of muscarinic and nicotinic receptors in the brains of animals tolerant to organophosphates raises the possibility that the balance of neuronal connections might be modified, with effects on higher brain functions. Such effects, rather than being protective, might represent a pathological process (Taylor et al., 1979). Animal studies have shown correlations of reduced memory and decreased abundance of brain nicotinic receptors (Gattu and Buccafusco, 1997).

Research inspired by illnesses in Gulf War veterans (Buccafusco et al., 1997; Wickelgren, 1997) has demonstrated decreased (over 50 percent) abundance of nicotinic receptors in cortical striatal and hippocampal neurons of rats exposed to sign-free doses of DFP (0.25 mg/kg/day for 14 days). Three weeks after withdrawal of DFP, treated animals showed impaired learning of a water maze, although previously trained animals retained their maze memories. There was no recovery of hippocampal nicotinic receptors three weeks after stopping DFP. A report in *Science* (Wickelgren, 1997, p. 1404) indicates that DFP-treated rats given nicotine before the water maze test learned adequately. Related studies in nonhuman primates given DFP 0.01 mg/kg/day for 25 days did not show altered performance in a delayed-matching-to-sample task, although red-cell AChE fell to 76 percent of control. Similar results occurred with 0.015 mg/kg/day for 15 days. Impaired performance was encountered at levels of 0.02 mg/kg/day, but these animals showed mild overt toxicity (Prendergast, 1998).

Induction of C-fos

The emerging picture of how cholinesterase inhibitors rapidly induce the expression of the transcription factor for c-fos points the way for possible long-term effects and added mechanisms of tolerance. It also appears that severe stress-induced release of ACh in animal models can also induce c-fos expression. The changes in gene expression initially enhance and later inhibit neu-

ronal excitability mediated by muscarinic receptors. C-fos, an early immediate transcription factor, mediates selective regulatory effects on long-lasting activities of genes involved in ACh metabolism. This appears to create a situation in which the effects of cholinesterase inhibitors might persist long after the agents are no longer present (Kaufer et al., 1998). The role of c-fos and other intermediate early genes (IEGs), such as c-Jun, that seem to play an important role in translating stimuli into longer-term adaptive responses of cells is vast and complex and eventually may explain the longer-term effects of brief chemical exposures. A brief summary of recent information on IEGs and c-fos can be found in Appendix C.

The finding that cholinergic stimulation or stress can induce the expression of immediate early genes, such as c-fos, is not surprising in view of the variety of stimuli that activate this transforming factor. The possibility of this proto-oncogene playing a role in producing long-term effects from exposure to agents that produce cholinergic activity seems great, but the details remain to be demonstrated. This might be the mechanism by which short-term exposures produce long-term effects without killing large numbers of cells.

Although stress of various kinds increases c-fos, the regions involved vary with the stress model employed. It remains to be demonstrated which, if any, cholinergic stimuli produce effects convergent with stress responses.

Delayed Effects

The clinical manifestations of typical OPIDN begin about two weeks after exposure, with a progressive peripheral neuropathy, which can also involve the central nervous system, with axon degeneration and later demyelination. Sustained lower doses are as toxic as single exposures to larger doses, provided some threshold is crossed, with chronic dermal exposure being suspect (Cherniack et al., 1986; Hayes, 1982). Additive effects with long intervals between exposures (up to six weeks) have been demonstrated (Hayes, 1982; Davies and Holland, 1972; Abou-Donia, 1981).[12] No human case of typical delayed neurotoxicity arising from nerve agents has been reported. Sarin in repeated sublethal exposures did produce a typical neuropathy in mice (Husain, Vijayaraghavan, et al., 1993).

Sarin can produce delayed neurotoxicity in animals. However, very high levels of acute exposure (30 to 60 times LD_{50}) are required to produce the effect in hens protected from cholinesterase toxicity by treatments (PB, oximes, atropine) (Gordon et al., 1983). It was recognized that some humans with

[12]Note that a toxicity threshold would make the importance of negative studies less clear.

analogously high acute doses might survive as battle casualty treatment improved, possibly resulting in delayed neurotoxicity. But after examining sustained exposure of rodents to sarin aerosol, Husain (Husain, Vijayaraghavan, et al., 1993; Husain and Pant, 1994) questioned the impression that it took very high levels of repeated challenge with sarin to produce delayed toxicity. Husain et al. did not produce delayed neuropathy in Wistar albino rats with daily 20-minute sarin exposures for ten days (250 mg-min/m^3 exposures). However, white albino mice given 5 mg/m^3 of sarin for 20 minutes (i.e., 100 CT) daily for ten days developed classic delayed neurotoxicity (weakness, ataxia, and twitching) beginning at day 14 and confirmed by tissue pathology. The mice were not initially ill from sarin exposure and manifested no symptoms of anti-cholinesterase intoxication. The doses would be lethal in the range for humans (NRC, 1997). Since the hen has become the standard animal for OPIDN studies, there is less information about the effects in mice.

AChE levels in the brain were reduced by only 19 percent. Platelets and the spinal cord showed marked decreases in NTE levels, although less than in mipafox controls. This report has not been replicated. Rodents have generally been considered resistant to delayed neuropathy and have been used in research on the subject. The authors did not discuss why the species differed. The hen has become the standard animal for OPIDN toxicity studies with less information from mice.

Studies of the effects of the isomers of soman and sarin hinted at the possibility of nerve agents producing NTE effects at lower levels of exposure. A trend of increased inhibition of lymphocyte NTE in hens exposed to Sarin II (an isomer of sarin) suggested that longer exposures at lower levels might cause cumulative toxicity (Crowell et al., 1989). The P+ isomer of soman is a potent inhibitor of NTE, suggesting that this isomer alone could produce neuropathy at unprotected LD_{50} levels (Johnson, Read, and Benschop, 1985).

There has been considerable comment that this "low-level" exposure raises the possibility that sustained exposures in humans might result in neuropathy. The exposures in the study are at the upper range of LCT_{50} for rodents. Rodents, however, are more resistant to nerve agents than humans, and the mice in question did not require any treatment. However, the results are not congruent with earlier studies with sarin in hens or with the experience in dogs (which are not a standard animal for NTE research). Dogs do develop delayed neuropathy from DFP (Johnson, 1975). However, chronically exposing dogs to sarin vapor did not produce any neuropathy (Jacobson et al., 1959). The main weight of information makes it difficult to attribute delayed neuropathy to sarin or cyclosarin, given the very low levels calculated for the Khamisiyah release.

The studies of Gordon et al. (1983) demonstrated that soman and tabun did not produce delayed neuropathy at doses 38 times the LD_{50} of soman and 82 times

the LD_{50} of tabun. In these studies, animals were provided the appropriate chemical therapy to enhance survivability following supra doses of agent. The same studies looked at the molar concentrations of agents required to inhibit *in vitro* 50 percent of the two enzymes, NTE and AChE, and calculated the ratios of the two (Table 5.6). The presumption was that the larger the number, the greater the likelihood of encountering delayed neurotoxicity from the agent in question. The results of other studies are summarized in Table 5.7.

A measure of the complexity and difficulty of this field is demonstrated by the Lenz et al. (1996) finding that sustained infusion of high daily doses (57 µg/kg/day) of VX in rats not provided chemical therapy reduced brain NTE by 90 percent at 14 days. No study of pathology or clinical response was reported. VX was not previously thought to be capable of significant NTE affects.

Severity-Sequelae Relationships

It is uncertain whether sequelae always correlate with severity at onset, although it seems intuitively obvious. Holmes (1959) reviewed the experiences of a group of workers exposed to sarin at various levels (although none so severely as to suffer seizures). He found that the more seriously exposed were ill longer. However, Stephens et al. (1996), in a study of groups exposed to organophosphate pesticides, found no correlation between acute exposure effects and the severity of performance shortfalls in later neuropsychological testing. In the reports of accidental cases, there are instances of patients with mild initial symptoms who had rather protracted later symptoms (Gaon and Werne, 1955; Brody and Gammill, 1954; Craig and Freeman, 1953).

Table 5.6

Molar Concentration of Agent Required to Inhibit Half of Enzyme Activity

	NTE	AChE	AChE/NTE
DFP	9.3×10^{-7}	1.05×10^{-6}	1.1300
Sarin	3.38×10^{-7}	1.9×10^{-9}	0.0056
Soman	3.77×10^{-7}	4.6×10^{-10}	0.0012
Tabun	6.65×10^{-6}	3.5×10^{-9}	0.0005
VX	2.5×10^{-4}	3.6×10^{-10}	10^{-6}

SOURCE: Reprinted by permission from *Archives of Taxicology*, Gordon et al. (1983), pp. 71–82. ©1983 Springer-Verlag, Berlin, Germany.

NOTE: Cyclosarin was not studied.

Table 5.7

Results of Other Delayed-Neuropathy Studies

Tabun (GA)	A 90-day study in hens at maximum tolerated dose (plus atropine) did not demonstrate delayed neuropathy (Willems et al. 1984).
Soman (GD)	To date, only repeated doses on the order of 150 times the LD_{50} have produced delayed neuropathy (Gordon et al., 1983; Willems, Nicaise, and De Bisschop, 1984). In a 90-day subchronic study at daily doses of GD insufficient to produce clinical signs, no delayed clinical or histological neuropathy resulted (Hayward et al., 1990).
Cyclosarin (GF)	GF has not been studied as extensively as the other agents. Vranken, De Bisschop, and Willems (1982) demonstrated that GF *in vitro* is a very potent inhibitor of NTE, but at doses where some lethality was encountered, no neuropathy occurred (Willems, et al., 1983).
VX	Most *in vitro* and *in vivo* studies fail to suggest that VX has any delayed neuropathic potential (Gordon et al., 1983; Vranken, De Bisschop, and Willems, 1982; Willems, Nicaise, and De Bisschop, 1983). However, Lenz, Maxwell, and Austin, (1996) raises some doubts about this conclusion.
Thiosarin	There is no information about this agent.

CLINICAL FINDINGS

A diligent effort to locate data about human clinical experience from exposures to nerve agents, while examining organophosphate pesticide experience for comparison, produced the following reports providing descriptions and consequences of exposures:

1. **Books and manuals:** NATO (1973), Grob (1956), OSRD (1946), Marrs et al. (1996), SIPRI (1971), SIPRI (1973), Lohs (1975), Karakchiev (1973), SIPRI (1976); Boskovic and Kusic (1980); Marrs et al., 1996); Sidell, Takafuji, and Franz (1997); Somani (1992)
2. **Organophosphate pesticide data:** Hayes (1982)
3. **Reviews:** IOM (1997), Jamal (1995b), Karczmar (1984), AFEB (1994), Grasso (1984), Clark (1971), Lotti (1995), Grob and Harvey (1953)
4. **Reports of intentional poisonings, Iran and Japan:** UN (1984); Perrorta 1996); Nozaki and Aikawa (1995); Nozaki et al. (1995); Okumura et al. (1996); Yokoyama, Ogura, et al. (1995); Yokoyama, Yamada, et al. (1996); Yokoyama, Araki, et al. (1998a, 1998b); Masuda et al. (1995); Hatta et al. (1996); Suzuki et al. (1995); Yasuda et al. (1996); Kato and Hamanaka (1996); Nohara and Segawa (1996); Inoue (1995); Morita et al. (1995); Ohtomi et al. (1996); Nakajima, Sato, et al (1997); Nakajima, Ohta, et al. (1998); Suzuki (1995); Murata et al. (1997)

5. **Reports of accidental and occupational exposures to nerve agents and pesticides:** Kaplan et al. (1993), Savage et al. (1988), Gershon and Shaw (1961), Whorton and Obrinsky (1983), Korsak and Sato (1977), Sim et al. (1971), Metcalf and Holmes (1969), Stephens et al. (1996), Tabershaw and Cooper (1966), Dille and Smith (1964), Seed (1952), Callaway (1950), Duffy and Burchfiel (1980), Cullen (1987), Sparks et al. (1994), Bell et al. (1992), Burchfiel and Duffy (1982), Holmes (1959), Callaway and Blackburn (1954), Sidell (1973), Senanayake and Karalliedde (1987), Sidell (1974), Brody and Gammill (1954), Freeman et al. (1956), LaBlanc et al. (1986), Craig et al. (1959), Finesinger et al. (1950), Vale and Scott (1974), Namba et al. (1971), Coombs and Freeman (1954), Richter et al. (1986), Kundiev et al. (1986), Rengstorff (1994), Cadigan and Chipman (1979), Craig and Freeman (1953), Gaon and Werne (1955)
6. **Experimental studies of human exposures:** Marrs et al. (1996); Sim (1956); Sim (1962); Sidell (1967); Rubin, Krop, and Goldberg (1957); Freeman et al. (1954); Oberst et al. (1959); Oberst et al. (1968); Grob and Harvey (1953); Cresthull et al. (1963); Sim et al. (1964); Bowers et al. (1964); Krachow (1947); Neitlich (1965); Rubin and Goldberg (1957a, 1957b); Grob and Johns (1958); Grob and Harvey (1958); Grob et al. (1947); Sidell and Groff (1974); Craig et al. (1959); Wilson (1954); Burchfiel (1976); Oken and Chiappa (1986); Freeman et al. (1952).

The reports of sarin and tabun accidents from the U.S. test and production efforts in the 1950s were especially helpful for this review, because many provided case descriptions and included at least short-term follow-up (Holmes, 1959; Craig and Freeman, 1953; Brody and Gammill, 1954; Gaon and Werne, 1955; Finesinger et al., 1950). Longer-term follow-up of sarin workers and pesticide workers has been difficult to locate, but some reports are available. Research subjects also had short periods of follow-up but were the subject of long-term review by the NAS in 1985. Experimentation on human subjects stopped at Edgewood Arsenal in 1975, with 1,300 having been exposed to anticholinesterase chemicals. Most studies with nerve agents had begun after 1954, with few after the mid-1960s (NAS, 1982). So, follow-up was in the 20- to 30-year range (NAS, 1985). The recent experience in Japan, where sarin was released in urban areas, provided information from well-equipped, well-staffed hospitals, with follow-up information for at least three months, with limited longer (six- to eight-month) reports.

This section concentrates on ocular, dermal, and respiratory exposure routes, emphasizing lower levels of exposure and clinical severity and information about long-term consequences and any patterns of illness similar to illnesses in Gulf War veterans. Earlier case reports suggest that clinicians did not expect long-term symptoms to arise from very mild exposures and sometimes consid-

ered alternate explanations for such patients when encountered (e.g., chronic anxiety). There is not much information about repeated or sustained exposures.

The amount of information about specific agents is uneven. There is much information about human exposures to sarin, but much less for tabun, soman, and VX. The only information available on human experience with cyclosarin is from secondary sources. The 1982 NAS review indicated that 27 volunteers were exposed to cyclosarin and that there were apparently both some sensory and oxime treatment studies. No information about thiosarin is available.

For nerve agents and pesticides, it is not always easy for the clinician to determine if an exposure has occurred, and AChE levels correlate poorly with the clinical findings. Holmes (1959) stated, with respect to a sarin production facility, that

> The examiner frequently asks himself the question "Is this a true exposure?" If so how serious is it? Except possibly for miosis, there seems to be no single symptom which occurs in every exposure. Only in the more severe exposures was miosis present in every instance. It is apparent that in milder exposures a single symptom related to a system occurs. When this happens it raises a question as to whether a particular symptom is a result of exposure or represents a symptom related to some other medical problem, such as a cold etc. When several symptoms related to a system occur, there is little doubt in the examiners mind both that this is a true exposure and in all probability a fairly severe exposure. When there is a scattering of symptoms related to different systems then the question arises if an exposure has occurred. Correlation with acetylcholinesterase is unreliable—in many instances a person is judged to have a mild exposure when the red cell acetylcholinesterase shows a greater drop than expected.

Acute Effects

Table 5.8 summarizes signs and symptoms arising from nerve agent exposure by several routes. No clinical differences are expected between various nerve agents. The clinical signs and symptoms from other organophosphate pesticide exposure are also quite similar (Hayes 1982; Namba et al., 1971). Table 5.9 classifies the severity of poisoning and is the basis of classifications used through this report. The emergency department of a Japanese hospital in Tokyo (Okumura et al., 1996) has summarized its experience with 640 victims of the subway attack. The department treated and released 82 percent (528) of the cases. The detailed findings in this group have not been reported, but these cases were considered to have been full recoveries. Of the 111 patients (17.3 percent of those in emergency department) admitted, 107 were considered moderate cases (16.7 percent) and four were considered severe. One of these

Table 5.8

Signs and Symptoms Following Short-Term Nerve Agent Exposure

Site of Action	Signs and Symptoms
Ciliary body	Frontal headache, eye pain on focusing, blurred vision
Conjunctivae	Hyperemia
Nasal mucous membranes	Rhinorrhea, hyperemia, but this may also be present after systemic absorption
	Following systemic absorption of liquid and prolonged vapor exposure
Bronchial tree	Tightness in chest sometimes with prolonged wheezing, expiration suggestive of bronchoconstriction or increased secretion, dyspnea, slight pain in chest, increased bronchial secretion, cough, pulmonary edema, cyanosis
Gastrointestinal	Anorexia, nausea, vomiting, abdominal cramps, epigastric and substernal tightness (cardiospasm) with "heartburn" and eructation, diarrhea, tenesmus, involuntary defecation
Sweat glands	Increased sweating
Salivary glands	Increased salivation
Lachrymal glands	Increased lachrymation
Heart	Bradycardia
Pupils	Slight miosis, sometimes unequal, later maximal miosis (pinpoint pupils); sometimes mydriasis is observed
Bladder	Frequent, involuntary microurination
Striated muscle	Easy fatigue, mild weakness, muscular twitching, fasciculation cramps, generalized weakness including muscles of respiration with dyspnea and cyanosis
Sympathetic ganglia	Pallor, occasional elevation of blood pressure
Central nervous system	Ataxia, generalized weakness, coma with absence of reflexes, Cheyne-Stokes respiration, convulsions, depression of respiratory and circulatory centers resulting in dyspnea and fall in blood pressure; emotional effects very often occur

SOURCE: NATO (1973).

died. Table 5.10 summarizes the clinical findings in the moderate and severe cases. There were two deaths, one in the emergency department and one later and 638 patients were considered to have made a full recovery. The moderate cases were hospitalized for 2.4 days (mean).

Table 5.11 summarizes the mild cases encountered in the workers accidentally exposed to "G" agents (tabun and sarin) studied by Craig and Freeman (1953). These cases show a lesser prevalence of eye, gastrointestinal, and nervous system findings but more rhinorrhea than in the Japanese cases, but the Japanese cases were a single event, while Craig and Freeman summarized multiple events. Their table is somewhat misleading in that, as in the Japanese cases, miosis was the most consistent finding (48 of their 53 cases). Most of their cases recovered rapidly, 78 percent within two days, most of the remaining ten cases within one week, with one case still symptomatic at 20 days.

Table 5.9

Severity Classifications for Organophosphate Pesticide Poisoning

Term	Description
Latent	No clinical manifestations
	Serum cholinesterase activity is 50 to 90 percent of normal
Mild	Fatigue, headache, dizziness, nausea and vomiting, increased salivation and sweating, chest tightness, abdominal cramps or diarrhea, can still walk, numbness of extremities
	Serum cholinesterase activity is 20 to 50 percent of normal
Moderate	Unable to walk, generalized weakness, difficulty talking and fasciculations, in addition to the symptoms but more miosis associated with mild poisoning
	Serum cholinesterase activity is 10 to 20 percent of normal
Severe	Unconsciousness, loss of pupillary light reflex, fasciculations, flaccid paralysis, moist rales in lungs, seizures, respiratory difficulties and cyanosis, secretion from mouth and nose
	Serum cholinesterase activity is less than 10 percent of normal

SOURCE: Modified from Namba et al. (1971); AD Little (1986), Ch. 5.

NOTE: Namba's classification, developed from experience with parathion and methyl parathion poisoning, has been adopted for classification of nerve agent exposures.

Commonly, cases are stratified clinically according to severity, as follows:

1. **latent:** exposed but asymptomatic so far
2. **mild:** distinct symptoms but ambulatory
3. **moderate:** unable to walk, distinct symptoms but conscious, able to sit
4. **severe:** seizures, coma, prostration.

The following subsections discuss these in reverse order (omitting the latent stage). A discussion of delayed effects follows.

Severe Intoxications. Several severe intoxications will be described, from accidents and the incidents in Japan to give a clinical picture of the problems that confronted military planners in the Gulf and to describe some of the sequelae of severe intoxications. Sidell (1973, 1974) reported two severe intoxications requiring hospitalization. The first was a 33-year-old man who sustained an accidental combined respiratory, cutaneous, and mucosal exposure to soman (less than 1 ml). He immediately decontaminated himself, was asymptomatic on arrival at the emergency room about five minutes later, but then collapsed. He was cyanotic with labored breathing, had a blood pressure of 180/80 mm Hg, and had a heart rate of 150/min. His conjunctivae were very inflamed; hehad marked oral and nasal secretions and widespread fasciculations. He was given intravenous atropine and 2-pyridine aldoxime methiodide (2-PAM). Cyanosis worsened, but he became conscious about 30 minutes later. Fascicu-

Table 5.10

Signs and Symptoms in Patients with Moderate to Severe Sarin Exposure

		Patients	
	Sign or Symptom	Number	% (n=111)
Eye	Miosis	110	99.0
	Eye pain	50	45.0
	Blurred vision	44	39.6
	Dim vision	42	37.8
	Conjunctival injection	30	27.0
	Tearing	10	9.0
Chest	Dyspnea	70	63.1
	Cough	38	34.2
	Chest oppression	29	26.1
	Wheezing	7	6.3
	Tachypnea	28	31.8[a]
Gastrointestinal tract	Nausea	67	60.4
	Vomiting	41	36.9
	Diarrhea	6	5.4
Neurologic	Headache	83	74.8
	Weakness	41	36.9
	Fasciculations	26	23.4
	Numbness of extremities	21	18.9
	Decrease of consciousness level	19	17.1
	Vertigo and dizziness	9	8.1
	Convulsion	3	2.7
Ear, nose and throat	Running nose	28	25.2
	Sneezing	5	9.0
Psychological	Agitation	37	33.3

SOURCE: Okumura et al. (1996). ©1996 American College of Emergency Physicians (ACEP). Reprinted by permission.

[a]n=88.

lations continued, and he was restless, with nausea and vomiting, and electrocardiogram changes showed sinus tachycardia and then atrial fibrillation for 20 hours. His physical condition improved more rapidly than his psychiatric condition. He was observed to be depressed and withdrawn and had bad dreams that improved with scopolamine treatment. He had difficulty calculating. AChE remained low, but other laboratory tests were normal. By six weeks, he was back to his premorbid level and was doing well at six months.

The second was a 52-year-old man whose mask malfunctioned in a sarin-filled room. He noted respiratory difficulty; on arrival at the emergency room 5 to 10 minutes later, he was cyanotic and having seizures. He was given intravenous atropine and oxime and required respiratory assistance for apnea. Fasciculations were prominent, and he had marked wheezing, developed an S4 gallop, and later had electrocardiogram changes typical of ischemia. He resumed

Table 5.11

Incidence of Symptoms in Workers Accidentally Exposed to Tabun and Sarin (mild cases)

Symptoms	Number of Workers	% Exposed Workers
Respiratory symptoms	41	77
Pressure sensation	35	
Localization not recorded	17	
Throat	2	
High sternum	3	
mid sternum	9	
low sternum	3	
not localized	1	
Cough	20	
Unproductive	15	
Productive	5	
Wheezing	9	
Inability to obtain a satisfactorily full inspiration	8	
Increased exertional dyspnea	7	
Dyspnea at rest	1	
Rhinorrhea	31	58
Eye Symptoms	29	55
Dim vision	24	
Impaired accommodation	13	
Pain on accommodation	6	
Central Nervous System Symptoms	27	51
Headache	17	
Headache as only CNS symptom	7	
Disturbed sleep	13	
Mood change	12	
Easily fatigued	10	
Increased perspiration	3	
Dizziness	2	
Gastrointestinal symptoms	14	26
Anorexia and/or nausea	9	
Increased GI activity	6	
Diarrheal stool	6	
Vomiting	2	
Miscellaneous		
Unpleasant taste to tobacco	10	
Poor driving	6	

SOURCE: Craig and Freeman (1953).

breathing one hour later. This patient was more alert at three hours and was able to ambulate at nine hours. He was rehospitalized four months later because of fatigue and dyspnea on exertion and was diagnosed with depression at six months. He died of myocardial infarction 18 months after exposure (Sidell, 1973, 1974).

There were two major sarin events in Japan, the first at Matsumoto in June 1994 (seven deaths and about 600 persons poisoned). The second was in Tokyo in March 1995 when sarin was released into the subway (11 died and 5,000 were poisoned) (Morita et al., 1995; Okumura et al., 1996). A third event was a man sprayed with VX (Nozaki and Aikawa, 1995).

The Japanese cases were well-documented. Some patients were comatose on admission, with miosis, seizures, fasciculations, flushing, tachycardia, hypotension, and respiratory distress; hypoxia was common. Many required intubation. Creatine kinase and glucose levels were elevated; many patients were acidotic at pH 6.8. Reports on secretions varied (Suzuki et al., 1995; Nozaki and Aikawa, 1995). Many recovered well, but some reported dysesthesias. One person had retrograde amnesia for 70 days (Hatta et al., 1996), and another was delirious and had hallucinations for over one week (Inoue, 1995). There were few deaths following hospitalization, but one man remained in a vegetative state in Matsumoto six months later.

The VX patient initially presented with blurred vision; seizures, fasciculation, and sweating followed. There was no miosis. The patient became cyanotic and was on a respirator for several days. He required atropine drip and intravenous diazepam. He was released after 15 days with brachial plexus neuropathy and antegrade and retrograde amnesia. Unlike the sarin cases, bradycardia had been prominent (Nozaki and Aikawa, 1995).

Moderate Exposures. At the hospital receiving the largest number of patients from the Japanese subway attack, 111 were categorized as severely or moderately injured (4 severe and 107 moderate) on admission (see Table 5.10). In these patients, miosis (99 percent) and headache (75 percent) were the most frequent symptoms, followed by dyspnea (63 percent) and nausea (60 percent); bradycardia was uncommon. Even at discharge, headache and eye pain were common. All but one made full recoveries (one severe case died), although 37 (33 percent of the 111) had acute stress disorders and four were diagnosed with PTSD at three months (Okumura et al., 1996). Of 213 patients seen initially at another Tokyo hospital, none had complaints at three-month follow-up (Yokoyama et al., 1996), similar to other Japanese reports.

In a three-week follow-up of some 117 mild and moderate cases in Matsumoto (Morita et al., 1995), the initial symptoms included rhinorrhea (78), headache (53), dark vision (52), sneezing (24), fatigability (18), dizziness (17), and nausea (14). Others reported diplopia, dysesthesia, vomiting, dysphagia, increased tearing, or gait disturbances. Most of these symptoms cleared by three weeks. At six months, five people visited hospitals regularly, with diverse complaints. One man with no history of lung disease was mildly hypoxic, and another had low-grade fevers.

Grob et al. (1953) reported a moderately severe reaction to percutaneous sarin in a volunteer. Illness was delayed several hours after exposure, lacked immediate eye or respiratory findings, and ran a protracted course with waves of recurring symptoms over four to five days. The dose was about 0.18 mg/kg through abraded skin. After 2-3/4 hours, there was local sweating; at 5-3/4 hours, the patient experienced general sweating, giddiness, and abdominal cramps. Blood pressure rose, and he was given atropine. One hour later, nausea, sweating, generalized weakness, and fatigability existed. He was short of breath with abdominal cramps but no wheezing. Maximum symptoms occurred at 10 to 11 hours, with dilated pupils and decreased vital capacity. He had mild symptoms with atropine at 13 hours, was fatigued and weak at 21 hours, and had insomnia and nightmares at 40 hours, finally recovering over the ensuing days.

Mild Exposures. *Respiratory.* Review of the inhalation exposures of sarin, tabun and some V-agent accidents supports the description in Wilson (1954) of mild intoxication, of symptoms that develop rapidly and then evolve over time:

> The chief effects consist of a feeling of constriction in the throat and chest, a tendency to cough, and eyes that quickly become red and painful with contracted pupils such that the subject finds it painful to focus on near objects. A severe and persistent frontal headache usually follows, and he becomes dejected and not inclined to bother to do anything unless he must. At night he is restless and has difficulty getting to sleep, and when he does has vivid dreams and nightmares; these symptoms may last 3–5 days. With larger doses, there is anorexia, nausea, vomiting, abdominal pain, salivation and diarrhea. There is sweating and generalized muscle weakness and fasciculations. Psychological changes include restlessness, irritability and insomnia. The appearance of the symptoms bears no relation to the plasma cholinesterase activity.

The occupational experience related to sarin production and testing was extensive. Holmes (1959) analyzed 991 cases in two groups, stratified by four levels of red-cell cholinesterase levels, with initial and follow-up examinations. All but a few cases were mild, and there was little treatment. These cases did not have long-term follow-up of the whole group, but cases were followed enough to determine that 10 percent had symptoms lasting two or more weeks. Appendix B includes information from this extensive report.

Several other reports also shed light on this issue: Brody and Gammill (1954), 75 cases; Gaon and Werne (1955), 244 persons; Craig and Freeman (1953), 53 cases; and Finesinger et al. (1950), 40 cases. Sarin was the primary agent to which people were exposed, but Freeman also reported four tabun exposures and two combined tabun-and-sarin exposures. The reports arose from medical examinations of exposed persons and later follow-up exams. The symptoms are those shown in Table 5.11 (Brody and Gammill, 1954; Craig and Freeman, 1953, had similar findings). Data from Holmes (1959) suggest a tendency for

persons with higher percentages of cholinesterase inhibition to have more symptoms and longer periods of illness. Gaon and Werne (1955) could not make such an assertion; their cases, which lasted over three weeks, showed 47 percent with no significant reduction in enzyme level. In a series of 182 cases, they reported that 106 (58.2 percent) had recovered in three days, 34 (18.6 percent) in one week, 19 (10.4 percent) in two weeks, but 23 (12.6 percent) took three weeks or more. They did not describe their follow-up process but documented two cases with persisting symptoms 10 to 11 months after exposure (recurring headaches, dizzy spells, fatigue, syncope, and weakness for one; memory problems, inattention, and fatigue in the second).

Table 5.11 gives an idea of the prevalence of symptoms, with eye problems being acutely the most prevalent, as was noted previously. Note the considerable percentages of symptoms related to the central nervous system, including mood changes, thinking problems, and fatigue.

Although most cases resolved within three days, some 12 percent of Gaon's cases persisted for three weeks or more (Gaon and Werne, 1955). Many exposed patients did not attribute their symptoms to exposure but rather to colds.[13] Some patients who had asymptomatic miosis and/or depressed AChE levels experienced no symptoms, while some obviously exposed and symptomatic individuals had little decrease in AChE levels.[14]

In an effort to understand factors related to accidental exposures, it was noted that accidents seemed more common in colder weather; the authors attributed this to workers seeking shelter from the cold in heated shacks, where agents trapped on clothing could evaporate in a closed space, causing some sustained exposures (Brody and Gammill, 1954). The specific symptoms in patients with prolonged symptoms were not reported, which was also the circumstance with Holmes (1959). He did not report beyond "two weeks or more." His smaller group of 156 cases noted 10.9 percent lasting two weeks or more, while in his larger group of 635 cases, 20 percent were symptomatic for three weeks or more.

Those recovering were prone to motor vehicle and other accidents. It became the practice to forbid driving or night work for several weeks. Speaking clearly, thinking, and remembering were significant problems for some, lasting for weeks. Some individuals experienced an initial euphoria or giddiness; emo-

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[13]These patients came to attention because their supervisors referred them; because other findings had been noted for them, such as miosis and abnormal blood tests; or because they came from settings in which exposures were suspected, e.g., coworkers were obviously sick.

[14]The authors of these early papers did not mention the possibility of tolerance arising from repeated exposures, altering the clinical response to further exposures. One would expect tolerant persons to have depressed AChE levels.

tional problems and irritability with family members and supervisors were documented frequently (Gaon and Werne, 1955). Some individuals became less careful and reliable and were thought to be "acting silly," which was out of character. Protracted fatigue and weakness were common, lasting two months in some cases. It was common for workers to report that the taste of cigarettes was lost or unpleasant. One soldier thought smoking worsened his weakness (Gaon and Werne, 1955).

Brody and Gammill (1954) frequently noted a distaste for cigarettes in their 75 cases. They also provide individual case descriptions, such as that of a 23-year-old man who was wearing protective equipment, but not a hood, who collected samples after an aircraft spraying flight of sarin on November 10, 1953. He was able to detect the odor of sarin and tightened his mask, but he developed a frontal headache and rhinorrhea. Four hours later, he had pinpoint pupils, photophobia, headache, and eye pain. Mild substernal pressure and dyspnea on exertion were noted. He was mildly ataxic and reported that his joints felt stiff. He took oral atropine and was continued in work status.

That night, he was restless; the headache continued, and he awoke from several nightmares. On arising, he was disoriented and had numb legs. His nausea quickly improved. His night vision was poor. He continued to work despite continued headache.

On November 12, he had headache, small pupils, and trouble reading and focusing. That night, he woke every two to three hours. He reported that 0.4 mg of oral atropine helped with the symptoms. He remained somewhat unsteady. He worsened at work during the day: The rhinorrhea increased; he vomited after lunch and then started diarrhea with 12 loose stools. He developed a cough productive of thick mucous.

That evening, he had vertigo and nearly fell. He took atropine, but sleep was frequently interrupted. He felt confused and numb all over. The next day, his "memory was no good," at times blank—he said things he did not remember saying. His joints were stiff, and he developed heartburn and belching. He vomited a few times. He felt very depressed—"nothing mattered any more." He gradually improved and recovered after several days. His ChE level was initially about 30 percent of normal.[15]

Gaon and Werne (1955) use one patient to illustrate late development of symptoms. The patient had two sarin exposures; symptoms of the first cleared in one day, and symptoms of the second cleared in three days. Eight months later, the patient complained of absentmindedness, an example being a near

[15]This case, although technically "mild," appears fairly incapacitating for many types of work.

accident when he failed to look both ways while driving across railroad tracks. He complained of difficulty with arithmetic and forgot lighted cigarettes around the house. He felt "lazy" and "draggy." He stated that his potency was impaired and his legs were weak. In several other cases, physicians tended to be skeptical of the relation to sarin exposure. Used to exposures in which most symptoms were over in a few days, they had difficulty accepting the possibility of long-term effects arising from mild exposures.

Two experimental studies of respiratory exposure of humans to measured low-level amounts of sarin appear relevant to understanding low-level effects (Oberst et al., 1959; Freeman et al., 1952). In these, the absorbed doses were in the range of decimals to a few micrograms per kilogram. Since the minute volume of respiration is proportional to the degree of physical activity, the amount of absorbed nerve agent (at a given concentration) is also proportional to the minute volume.

In a large study (141 subjects), Oberst et al. (1959) studied inhalation exposures of sarin vapor at varying levels for 2 minutes, in men at rest or exercising. The amount of sarin absorbed and the inhibition of red-cell AChE were measured. The amounts of sarin retained ranged from 0.1 to 4.9 µg/kg. At rest, 87 percent of the sarin inhaled was retained; minute volumes averaged 7.9 l/min. During exercise, 79.5 percent of the sarin was retained; minute volumes averaged 42.9 l/min. The degree of inhibition of erythrocyte AChE was proportional to the retained dose, with 3.8 to 4.2 µg/kg required to inhibit 50 percent of the enzyme activity. Absorbed doses of 0.1, 0.2, and 0.3 µg/kg showed enzyme inhibitions of 3, 2, and 4 percent, respectively. Absorbed doses of 0.5 µg/kg showed inhibitions ranging from 0 to 25 percent, with the line of least square regression showing 8 percent inhibition. Absorbed doses of 1 µg/kg produced inhibition of 0 to 54 percent, with a mean of 14 percent. CT exposures ranged from 2.4 to 46.2 mg-min/m^3. The authors note the unreliability of CTs, since CTs of 33 to 46 in resting men and 7 to 9.7 in exercising men produced similar absorbed doses. Comparing animal enzyme and lethality data, they estimated human LD_{50} to be in the range of 11.9 to 26.2 µg/kg. Unfortunately, they did not describe any of the clinical responses of the subjects to these measured levels of absorbed sarin. The reviewer notes that, although the group data were consistent, there was considerable individual variation in the degree of inhibition from a given dose of sarin. How much of the variation reflects differences within the lungs (mucous trapping, tissue absorption) and how much reflects differences in detoxifying metabolism are not known. Given the paucity of data about clinical effects from measured, documented human exposures, it might be worthwhile to see whether the medical records of this large number of subjects, which we believe to still be on file at Edgewood Arsenal, contain clinical observations about their response to defined dosages of sarin.

Fortunately, the second study (Freeman et al., 1952), although smaller, obtained data on signs and symptoms, neuromuscular function, EKGs, pulmonary function, and red-cell and serum AChE levels arising in controls (six), men at rest (eight), and men exercising (nine). The latter two groups breathed sarin vapor for 2 minutes via a tube in the mouth and without eye exposure. The amount of sarin retained was measured. The total absorbed dose in the resting group ranged from 0.08 to 0.16 µg/kg (average 0.12 µg/kg). The exercise group ranged from 0.22 to 0.99 µg/kg (average 0.56 µg/kg). There were no significant differences before and after in the three groups in ChE levels, hand grip and fatigue, vital capacities, maximum breathing capacity, and EKGs. (Two exposed subjects showed declines in ChE levels of about 10 percent.) The control group had no signs, but one subject experienced cough and a sense of incomplete inspiration, which was not present 24 hours later. Of the exposed resting group, two developed signs (transient rhonchi and inspiratory wheezing on examination of the chest), while six (including the two above) noted cough, chest pressure, and incomplete inspiration (doses ranged from 0.08 to 0.16 µg/kg). All these symptoms had cleared 24 hours later, but one subject (0.13 dose) reported disturbed sleep. Of the exercise group, three developed signs (inspiratory wheezing, scattered rales, and rhonchi) at 0.22 to 0.71 µg/kg, while five developed cough, chest pressure, and incomplete inspiration (0.22 to 0.99 µg/kg). At 24 hours, two had continued chest pressure, and one of these had developed headache. The authors did not report CTs, which the reviewer estimates to represent levels of 0.5 to 0.6 mg-min/m^3. Individual variation is again noted, with some exposed persons reporting no symptoms. The levels of exposure are slightly above those known to produce miosis and are at levels that could be measured by standard detectors.

Dermal. Low-level dermal exposures to G and V agents are documented. Some exposed persons are asymptomatic but have depressed AChE levels (Freeman et al., 1956). In general, dermal exposure produces symptoms more slowly, with eye and respiratory symptoms occurring later. Results from the literature are given in Table 5.12. Note that the cyclosarin result suggests it represents a serious cutaneous hazard (Marrs et al., 1996).

In 33 of 40 subjects exposed dermally to VX, AChE fell to 50 percent from doses of 5 to 20 µg/kg (Sim, 1962). Signs and symptoms appeared in 28 subjects, and eight required treatment. Local signs were sweating, erythema, and itching; systemic signs were weakness, fasciculations, dizziness, headache, abdominal cramps, vomiting, and diarrhea. One patient had orthostatic hypotension.

In a V-agent accident (Freeman et al., 1956), a chemist had bilateral forearm exposure. She developed a headache that night, and had unusual sweating of forearms the next day. Later that day, she developed chest pressure, which

Table 5.12

Signs and Symptoms of Dermal Exposures to Nerve Agents

Tabun vapor, 2,000 CT	No symptoms and fall in AChE in masked subjects (Krakow and Fuhr, 1949)
Tabun (5 mg/kg) liquid, 400 mg (total dose)	Produced local sweating and a fall in AChE by 30 percent, with sweating lasting 8 to 14 days for most subjects, but one had sweating for 95 days (Freeman et al., 1954)
Sarin liquid, nonpersistent application 6 mg (total dose)	No symptoms, no fall in AChE (Grob and Harvey, 1953)
Sarin liquid, sustained exposure, 20 mg (total dose)	No symptoms, fall in AChE by 23 percent (Grob and Harvey, 1953)
Soman liquid, 35–75 µg/kg	Study I—There was some vapor exposure with chest tightness and miosis—cleared in 3 hours Symptoms began at 35 µg/kg. Testing, which lasted 3 minutes, stopped at 75 µg/kg (Neitlich, 1965)
	Study II—2–8 mg/man produced local sweating, slight fall in AChE (Neitlich, 1965)
Soman liquid, 65 mg (total dose)	Sweating, fall in blood AChE by 50 percent (Mumford, 1950, in Marrs, Maynard, and Sidell, 1996)
Cyclosarin liquid, 30 mg (total dose)	Sweating, fall in blood AChE by 50 percent (Mumford, 1950, in Marrs, Maynard, and Sidell, 1996)
VX vapor, exposure of arms, 28–681 CT	No symptoms, AChE maximum inhibition 43 percent (Marrs, Maynard, and Sidell, 1996)

lasted three days. She became extremely fatigued but continued to work; the sweating lasted 36 hours. She had previously been exposed to sarin, which had made her "slap happy"; her experience with the V agent produced an emotional tenseness that she found unpleasant.

Ocular. Most respiratory exposures involve the eye, and vice versa. The clinical effects of the various agents are similar, although their potencies differ. V agents produce miosis, headache, dim vision, impaired accommodation and conjunctivitis (Freeman et al., 1956) quite similar to tabun (OSRD, 1946) or sarin. Grob and Harvey (1958) instilled 0.3 µg of sarin into the eye producing miosis lasting 60 hours. There were local symptoms of pressure.

Miosis was very common in Japanese victims, with dim vision, eye pain and conjunctival injections (inflamed appearance) in 40 to 80 percent (Kato and Hamanaka, 1996; Nohara and Segawa, 1996). Discomfort with accommodation, due to ciliary spasm, occurred in 15 percent. Rengstorff (1994) studied two sarin accident exposures, and found little effect on vision, despite miosis. Japanese clinicians have reported a variety of abnormalities ranging from blurred discs to field defects. Rubin and Goldberg (1957a, 1957b) showed that night vision is impaired by sarin by a central mechanism, corresponding to the

experience with U.S. accident cases. Narrowed field of vision and photophobia were found in dose-effect studies of sarin in producing miosis (Sim, 1956).

Intermediate Syndrome. An acute neurotoxic syndrome can follow the cholinergic period of organophosphate pesticide poisoning. The syndrome includes paralysis of limb and respiratory muscles and cranial nerves and occasional peripheral neuropathy, which seems different from classic delayed polyneuropathy. This intermediate syndrome has only been reported after substantial organophosphate pesticide exposures (Senanayake and Karalliedde, 1987) and has not been recognized with human or experimental nerve agent exposure, not even in the large Japanese experience (Ohtomi et al., 1996).

Long-Term Effects

It was to be expected that there would be interest in possible long-term effects from such highly toxic chemicals as nerve agents. The view of early experts in the field of nerve agent and organophosphate pesticide toxicity was that "recovery from moderate intoxications from nerve gas has always been complete" (Grob and Harvey, 1953). Long-term effects were only expected after severe intoxications, especially in cases experiencing severe hypoxia that was known to have damaged the brain. Soviet authors also considered that prolonged sequelae, such as vegetative-asthenic and extrapyramidal syndromes or toxic encephalopathy, were only expected after severe exposures (Karakchiev, 1973).

In occupationally exposed sarin workers, the duration of short-term symptoms, measured in days and weeks, showed some rough correlation with severity of initial symptoms and degree of cholinesterase inhibition, but exceptions were common (Holmes, 1959).

Other investigators, such as Gaon and Werne (1955) and Craig and Freeman (1953) were diligent in documenting the duration of signs and symptoms after exposures, with most follow-up reports ending at three weeks.

This diligence is part of the reason that Sidell and Hurst (1997) discounted the idea that long-term effects would be overlooked, citing alertness of supervisors in referring workers to medical care who did not seem "right."

Volunteer Follow-Up. NAS-NRC undertook a follow-up study of volunteers exposed to many different chemicals in studies at Edgewood Arsenal. NRC (1982) reviewed the known effects of "anticholinesterase compounds," including nerve agents (and some organophosphate pesticides) and then reviewed in detail the clinical records of 15 percent (219 cases) of the volunteers exposed to these agents. Of the 1,406 volunteers, 246 were exposed to sarin, 740 to VX, 26 to tabun, 21 to cyclosarin, 83 to soman, 11 to DFP, 32 to EA3148, 27 to PB, and 10 to malathion. Some volunteers received treatments, others not.

The 1982 panel concluded that, in the doses used, there was no evidence of long-term effects from the compounds surveyed, but noted that the survey under way might add further information. They suggested it would be possible to conduct studies of electroencephalograms (EEGs) in volunteers and controls to look for the changes reported by Duffy and Burchfiel (1980).

The final report (NAS, 1985) contacted volunteers about their health status, reviewed morbidity data from hospitalizations (including hospitalizations for mental illness), and reviewed overall mortality, including cause of death. Results were also analyzed in a stratified manner by class of agent and by specific agents. No unusual pattern of mortality or morbidity was identified and there were no indications of adverse long-term effects in the volunteers exposed to anticholinesterase agents.

Iranian clinicians have documented later consequences of mustard exposures in Iranian casualties of the war with Iraq, but apparently have not published reports of long-term health problems in their casualties from nerve agents.[16]

Limitations of Follow-Up Studies. While the above information supports the conventional view that long-term effects are not to be expected from nerve agent exposures except in the most severe intoxications and that there is little reason to be concerned about long-term health effects from lesser exposures, the situation is, regrettably, not so clear cut. Other medical and scientific personnel have expressed concern about possible long-term health effects of nerve agent exposure while also drawing on the larger human experience with organophosphate pesticides (Lohs, 1975; Boskovic and Kusic, 1980; Cadigan and Chipman, 1979).

Lohs (1975) noted there was little information about the effects of nerve agents, but considered that the chemical and toxicological effects of organophosphate pesticides made comparisons valid. He drew attention to the problem of evaluating long-term effects in trying to determine whether the patient had a history of acute poisoning or whether a subacute course of poisoning had been brought on by imperceptible doses.

The U.S. occupational and accidental exposure reports did not discuss the possibility of long-term health effects arising from subacute exposures. Although it was common to encounter workers with depressed levels of cholinesterase who did not seem ill, no follow-up studies of such workers has appeared.[17]

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[16]Although patients take pyridostigmine for prolonged periods for myasthenia gravis and although anticholinesterase drugs are used in the treatment of Alzheimer's disease, we did not look at follow-up studies on these conditions, believing that the effort would be beyond the scope of this review.

[17]Longer-term studies were confined to workers with symptomatic exposures (Metcalf and Holmes, 1969).

Lohs (1975) cites and quotes Spiegelberg's studies of German World War II workers involved in nerve agent production. Lohs indicates that there were long-term effects in these workers, although the nature of their exposures or other exposures to organophosphate pesticides is not discussed (see the later discussion on longer-term psychological effects). One group had indications of autonomic dysfunction and decreased libido intolerance to alcohol, nicotine, and medications. A second clinical group had depression, syncope, and indications of neurological dysfunction. According to Lohs, Spiegelberg noted that some persons exposed to nerve agents recovered completely.[18]

Japanese Follow-Up Reports. Several Japanese reports claim patients are free from sequelae at three months (Ohtomi et al., 1996; Yokoyama, Araki, et al., 1998a), but Morita et al. (1995) identified a small number with problems at six months. Reports are now emerging of specialized follow-up studies in small groups of Japanese exposed to sarin. Yokoyama et al. (1998a) reported computerized static posturography studies in 18 exposed persons from the 1995 subway attack (nine men and nine women) and 53 matched controls. The exposed had reduced serum cholinesterase levels on the day of exposure, and their clinical findings (mild to moderate) were documented. They had recovered rapidly and, at the time of study (six to eight months later) were asymptomatic. Women subjects showed significant differences in eye open anterior sway and area of sway, which was interpreted as indicating delayed vestibular-cerebellar effects (Murata et al., 1997). These findings corresponded to similar findings in organophosphate pesticide workers (Sack et al., 1993).

The authors also referred to other work they had done in clinically recovered cases six to eight months later (Yokoyama, Araki et al., 1998b) who showed significant declines in neurobehavioral testing. The study compared 18 currently asymptomatic individuals, who had been exposed to sarin six months previously in the subway attack, with controls matched for age and gender. PTSD checklist scores were high for the sarin group, but the scores did not correlate with the neurological test findings. Brain stem auditory evoked potentials and visual evoked potentials showed prolonged latency in the sarin group. Electocardiographic R-R interval variability was different in the sarin group and correlated with AChE levels determined immediately after the attack. The authors concluded the neurotoxic effects of sarin, not PTSD, accounted for the differences (Yokoyama et al., 1998b).

Two further Japanese studies have looked for longer-term effects in patients and workers exposed in the Matsumoto event, where it is now estimated 12

[18]Because the primary documents are currently unavailable, this information should be viewed with caution. Note that the exposures are vague in Lohs (1975), and there is no mention of control subjects.

liters of sarin were released from a point source in a housing area at night (Nakajima et al., 1997; Nakajima, Ohta, et al., 1998). Nakajima and colleagues conducted a survey of all the inhabitants in an area 1 km downwind from the release site and 850 m wide. They contacted 2,052 people, of whom 1,743 responded to the survey. Those with symptoms were followed at four months and one year; 471 sarin victims were identified. Muscarinic symptoms were common to all victims, but nicotinic signs were confined to the most severely injured. Three weeks after the intoxication, 129 patients still had symptoms, such as dysesthesia of extremities. Some victims were experiencing asthenopia. The prevalence of that symptom had increased at four months. Although victims generally felt that the symptoms had decreased over the year, some were still troubled by eye complaints.[19]

A second study was smaller and followed the effects on rescue workers who had been involved in the event at different times after the release. Of 52 workers, 18 experienced some symptoms of sarin exposure. These were generally the earliest-arriving workers. The only worker who required hospitalization was one of the first who had been very active. The symptoms encountered were typical: eye pain, dimmed vision, narrowed visual field, nausea, vomiting, headache, sore throat, fatigue and dyspnea. On examination three weeks later, no worker had abnormal physical or neurological findings. In one-year follow-up, no rescuer had symptoms, unlike the residents: Seven residents were killed; 76 percent of the residents had symptoms; 28 percent were admitted to the hospital; and 21 percent consulted physicians (Nakajima, Sato et al., 1997).

There are examples of acute cerebellar signs in sarin exposure (Brody and Gammill, 1954; Gaon and Werne, 1955). Some animal data indicate that females are more sensitive to nerve agents (Callaway and Blackburn, 1954; Woodard et al., 1994) and recover more slowly.[20]

In summary, the Japanese studies, which primarily follow up moderately severe cases from the Tokyo attack, show victims who appear to be well but have subtle neurological changes detectable by special tests. PTSD has also appeared in victims of the attack and complicates evaluation of nerve agent effects. Long-term follow-up of mild cases has not been reported. Some were apparently included in the survey of cases after the Matsumoto attack, but it is unclear how many of the mild group were among those with illness at six months and one year. Overall, the vast majority of Japanese cases have made clinical recoveries, according to reports available.

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[19]Asthenopia is a set of eye symptoms: blurred vision, eye fatigue, discomfort, lachrymation, and headache. Similar prolonged symptoms have been reported after organophosphate pesticide poisoning (Tabershaw and Cooper, 1966).

[20]It should be remembered that the cerebellum has important cortical projections and has functions beyond balance and coordination.

Focused Occupational Studies. Metcalf and Holmes (1969) reported follow-ups of two different groups of workers exposed previously to sarin and compared them with controls who were not exposed to chemicals. The authors did not describe how they selected their cases. In their 1952 study of 52 workers and 22 controls, the workers reported a high prevalence of aches and pain, fatigue, and drowsiness. Again in 1969 using a control group, workers who had not been exposed to sarin in the preceding year showed disturbed memory and soft neurological findings, suggesting incoordination. Their EEGs differed from the controls, although they were not clearly pathological.

A somewhat controversial study of 77 workers exposed to sarin more than a year previously and 16 controls found differences in the automated EEG interpretations of the two groups without readily diagnosed pathology (Duffy and Burchfiel, 1980).

Effects of Assumptions on Observations. Some accident reports document cases with substantial health effects—especially on memory and thinking, with fatigue prominent at 10 to 11 months after mild exposures (Gaon and Werne, 1955). In other reports (Brody and Gammill, 1954), it was clear that investigators had difficulty accepting the idea that mental problems four months after exposure could be related to the exposure. Gaon's use of a "possible" late effect also reflects this doubt. It seems possible that some long-term effects of nerve agent exposure may have gone unrecognized because clinicians' preconception that they did not occur.

The review indicates there is some possibility and evidence of long-term health effects arising from mild symptomatic exposures to nerve agents. The relationship to exposure levels or repeated exposures is unknown. The long-term effects, if any, of cumulative subacute clinically inapparent exposures have not been studied with respect to nerve agents. Single low-dose exposures of volunteers have not resulted in long-term problems. It has been shown that longer-term (months) decrements in performance on tests of mental function can occur from clinically inapparent organophosphate pesticide exposure (Stephens et al., 1996).

Delayed Neuropathy. The organophosphate compounds indirectly referred to are TOCP and leptophos, which are inhibitory to esterases, including AChE. These chemicals were associated with massive outbreaks of delayed neuropathy.[21] The organophosphate TOCP was recognized as causing delayed neuropathy ("ginger jake paralysis") by classic research of Smith et al. (1930),

[21]Recent review comments note that Smith et al. (1930, U.S. Public Health Service 45, pp. 2,509–2,529) preferred the classic research, which identified the basis for large outbreaks of neuropathy. We have been unable to obtain this paper.

who were concerned with the etiology of a peculiar form of paralysis afflicting large numbers of the population. During the 1970s, the highly publicized problem with OPIDN had focused on the insecticide leptophos, which brought to the forefront the health risks concerning insecticide manufacture in use. It was also appreciated that DFP, an anticholinesterase developed in World War II (OSRD, 1946), could produce a delayed neuropathy. There was understandable concern that other agents might have neurotoxic effects, although no delayed neuropathy was recognized in the approximately 900 occupational exposures to sarin (Holmes, 1959). There was no indication of delayed neuropathy in the NAS follow-up studies of volunteers exposed to agents (NAS, 1985).

Later experience with pesticides showed that some organophosphate pesticides, such as mipafox and leptophos, could also produce a delayed neuropathy. The leptophos experience is interesting in that the typical delayed neuropathy was not immediately evident; the pattern was a peripheral one, involving legs more than arms and sparing higher brain functions. In the case of leptophos, workers were diagnosed as having multiple sclerosis, encephalitis, or psychiatric disorders (Abou-Donia, 1981). Hayes (1982) reviewed this disorder in the case of TOCP and found in long-term follow-up that some of those exposed had dementia, although other factors could not be excluded (Hayes, 1982; Johnson, 1975; Abou-Donia, 1981). Kaplan et al. (1993) also found delayed-onset neuropathy among eight persons who had minimal or no acute symptoms after household exposure to the pesticide chlorpyrifos, generally considered to have low potential for neuropathy.

There is no established therapy for delayed neuropathy (Hayes, 1982). Oximes do not help (Johnson, 1975). Phenylnicotinamide may compete for binding sites and has shown some promise in TOCP-poisoned hens (Lotti, 1991).

Longer-Term Psychological Effects. One study (Coombs and Freeman, 1954) evaluated workers soon after accidental exposure to sarin and then three to six months later, when recovery was assumed to be complete. The researchers used a battery of intellectual performance tests (Wechsler Bellevue, nonverbal, and block design) to evaluate high-exposure and low-exposure groups. The mildly intoxicated "higher" group showed consistent declines in subtest scores for comparison and similarities on Wechsler tests, but the lower level group was normal. Both groups were normal on later testing (Coombs and Freeman, 1954). The long-term NAS follow-up study (after 20 or more years) of U.S. volunteers exposed to nerve agents did not find evidence of long-term health effects (NAS, 1985).

The SIPRI paper (Lohs, 1975) on long-term effects of nerve agents referred to Spiegelberg's studies of German nerve agent production workers many years after exposure. Spiegelberg's studies reported psychiatric syndromes consisting

of lowered vitality and drive; defective autonomic regulation (headaches, gastrointestinal symptoms, and cardiovascular symptoms); intolerance to medicines, nicotine, and alcohol; premature aging; depressive symptoms; syncopal attacks; slight to moderate amnesia and dementia; and extrapyramidal neurological changes. No control group was described.

Beginning in 1952 and continuing into the late 1960s, Metcalf and Holmes (1969) of the University of Colorado Medical Center were involved in long-term evaluations of workers exposed to sarin. The sarin production facility did not use modern practices of occupational health surveillance. It does not seem to have occurred to anyone to include in the long-term studies persons from that workplace who did not report acute symptoms. These workers had symptomatic accidental acute exposures previously recorded. Initially, these were shorter term studies during recovery, but later, longer-term (months to years) studies concentrated on psychiatric and neurological effects, performance on behavioral tests, and EEGs. In 1952 (summarized in the 1969 report), 52 exposed workers and 22 controls had psychiatric interviews and evaluations. Muscle aches and pains, drowsiness, and fatigability were significant problems in the exposed group. The 1969 study evaluated men previously exposed to sarin. They had psychiatric interviews, neurological examinations, psychological tests and EEGs. The exposed group showed disturbed memory and difficulty in maintaining alertness and attention. Neurological findings were soft, with an impression of minor coordination deficits. Interviews showed higher numbers of the exposed patients were nervous or irritable or had changes in memory, decreases in libido, changes in sleep habits, or increased fatigue. The nature, time, and amount of exposure, along with the initial symptoms, were not reported. The authors had a control group but did not study workers from the sarin production facility who had not reported symptoms. However, none had exposure within the year. Post-hyperventilation EEGs differed from those of controls, and night EEGs showed many with a narcoleptic pattern. The authors concluded that the collective deficits indicated the deep midbrain effects of organophosphate pesticides.

In a study of 77 workers previously exposed to sarin (none in the year preceding the study) (Duffy and Burchfiel, 1980; Burchfiel and Duffy, 1982), 41 had three or more exposure episodes in the previous six years. There were 38 controls. Inspection of EEGs by blinded interpreters could not distinguish the groups. Spectral analysis by computer, however, showed significantly higher beta activity, lower alpha, and more rapid-eye-motion (REM) sleep in the exposed group.

In another study (Sim et al., 1971; Burchfiel, 1976), rhesus monkeys were injected with enough sarin for serious intoxication or for asymptomatic levels.

EEGs taken a year later were different from those at the time of injection for both groups, although more so with the higher-dose group.[22] The clinical significance of these two studies is uncertain, but it seems clear that doses of sarin that did not produce overt illness in the animals over several days of exposure (1 µg/kg for ten days) can produce long-term changes in EEGs.[23]

Karczmar (1984) reviewed acute and long-term consequences of organophosphate toxicity, summarizing the short- and long-term mental effects of nerve and organophosphate pesticide agents, and reviewed the follow-up studies conducted on sarin workers. The key long-term effects were affect (i.e., mood) and memory deficits, exacerbation of preexisting psychiatric problems, sleep disorders, insomnia, hallucinations and delusions, and psychotic and paranoid reactions. Most of the studies Karczmar reviewed pertained to pesticide experience.

Organophosphate Pesticide Experience. Other studies of organophosphate pesticides provide further insight into neurological, psychiatric, and psychological long-term effects of this class of chemical, including nerve agents. Workers with sustained organophosphate exposure but without toxic symptoms showed definite impairment in trail-making tests and visual gestalt tests, indicating left-frontal-lobe dysfunction. Abnormal EEG changes were also observed (Korsak and Sato, 1977).

Twenty-two farm workers became ill when exposed at mild to moderate severity to two organophosphate pesticides in combination. Nineteen were followed for over four months. Blurred vision lasted over two months, and anxiety that began at three weeks continued. Weakness cleared by two months, but it took two to three months for most to improve (Whorton and Obrinsky, 1983).

Savage et al. (1982, 1988), working from cases of documented organophosphate exposures in a registry, performed hearing, eye, clinical, laboratory, EEG, neurological, and psychological tests on exposed persons and controls. Exposed persons showed larger decreases in memory and abstraction and had more mood changes than controls. On psychological testing, the exposed group had Halstead-Reitan scores in the range seen with cerebral injury.

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[22]There has been some critique of the primate model, which used implanted electrodes, but the control animals were similarly treated. The method of EEG analysis has also been criticized and rebutted (Oken and Chiappa, 1986). In a 1988 Federal Registry notice, the Centers for Disease Control found the changes to be not clinically significant. The findings need not indicate illness, but long-term rearrangements of brain electrical activity after low-dose exposure to nerve agent indicate of an effect that requires further attention.)

[23]Having an abnormal EEG does not constitute a disease process. However, the fact that a rather modest exposure to a nerve agent can produce what seems to be permanent rearrangement of rhythmic activity in the brain is a substantial effect. It suggests that other long-term neurological and behavioral complaints attributed to low agent exposure must be taken seriously.

Other investigators have identified similar changes in neurologic status or mental health of those exposed to organophosphate pesticides. Among the more interesting:

- several cases each of gastrointestinal problems, headaches, chest pain, and nervousness, plus three motor vehicle deaths among 235 persons exposed to organophosphate pesticides within the previous three years (Tabershaw and Cooper, 1966)
- a distinct decrement in performance on syntactic-reasoning and symbol-digit substitution tests among sheep dippers exposed to organophosphate pesticides; there was no correlation between test performance and prior symptoms of exposure (Stephens et al., 1996).

Jamal (1995b) has reviewed and summarized studies of organophosphate (including sarin) exposure (see Tables 5.13 and 5.14). Most of the clinical experience with nerve agents arises from exposures whose actual levels were unknown and not measured, arising from accidental exposures, from occupational exposures and from human volunteer studies. (Jamal did not review any actual attack experience.)

CLINICAL ASPECTS OF EXPOSURE

Asymptomatic Exposures

It is possible to have exposures that do not produce symptoms but that can lower serum and blood cholinesterase levels greatly (Holmes, 1959; Gaon and Werne, 1955; Freeman et al., 1956). No longer-term follow-ups on such persons are available, although papers reporting the measures of return of enzyme levels mention no problems. The general experience with the occupational exposures was that cholinesterase levels did not correlate well with clinical findings (Craig and Freeman 1953; Gaon and Werne, 1955).

Acute Effects

The effects of acute exposure are sometimes incorrectly interpreted as being due to other common health problems, such as respiratory infection, allergy, asthma, flu, or gastroenteritis, as was the case for workers who knew they were around nerve agents (Gaon and Werne, 1955; Craig and Freeman, 1953).

Acute effects vary by route of exposure. Vapor and aerosols produce eye and upper and lower respiratory symptoms, followed by other symptoms of mental confusion, gastrointestinal distress, and neuromuscular findings with weakness. At lower levels of exposure, it may take one hour from exposure for symp-

Table 5.13

Summary of Published Work on Chronic Effects of Long-Term Exposure to Small or Subclinical Organophosphate Quantities

Exposed Groups	Result[a]	Controls	Agent	Source
Workers Scientists	Psychiatric +ve Anecdote[b]	None	Organophosphate insecticide	Gershon and Shaw (1961)
Workers	Major psychiatric +ve Anecdote[b]	None	Organophosphate insecticide	Dille and Smith (1964)
Workers	EEG +ve Psychology test +ve	High/low	Organophosphate insecticide	Metcalf and Holmes (1969)
Sprayers	Behavior –ve	Nonexposed	Organophosphate insecticide	Rodnitzky (1975)
Farmers	Behavior –ve	Matched (small number)	Organophosphate insecticide	
Primates	EEG +ve	Control animal and preexposure EEG	Sarin	Burchfiel (1976)
Sprayers	Anxiety +ve	Nonexposed	Organophosphate insecticide	Levin et al. (1976)
Farmers	Anxiety +ve	Nonexposed		
Workers	EEG +ve Psychology +ve	None	Organophosphate insecticide	Korsak and Sato (1977)
Workers	EEG +ve Multivariate analysis	Nonexposed	Sarin	Burchfiel and Duffy (1982)
Rhesus monkeys	EEG +ve	Nonexposed		Duffy and Burchfiel (1980)
Workers	Psychometric tests –ve	Nonexposed	Diazinon	Maizlish et al. (1987)
Mice	End plate potential Jitter +ve		Mipafox	Kelly et al. (1994)

SOURCE: Reprinted with permission from Jamal (1995b)

[a]+ve and –ve are standard expressions used in toxicology to mean positive and negative, respectively.

[b]Reports from scientists working with organophosphate pesticides; not examined in detail.

Table 5.14

Summary of Published Work on Delayed Effects of Acute or Symptomatic Organophosphate Intoxications

Exposed Groups	Result[a]	Controls	Agent	Reference
Workers	EEG +ve Anecdote	None	Organophosphate insecticide, sarin, DFP	Holmes and Gaon (1956)
Workers	Psychiatric +ve Visual +ve etc.	None	Organophosphate insecticide	Tabershaw and Cooper (1966)
Rhesus monkeys	EEG +ve	Controls matched	Sarin	Burchfiel (1976)
Workers	EEG +ve Behavior +ve	Low-dose above	Organophosphate insecticide	Korsak and Sato (1977)
Workers	EEG +ve Psychiatric +ve	None	Organophosphate insecticide	Hirshberg and Herman (1984)
Workers	EEG –ve Psychometric test +ve	Controls matched cohort	Organophosphate insecticide	Savage et al. (1988)
Rats	Neuronal necrosis	Untreated	Fenthion	Veronesi et al. (1990)[b]
Workers	Psychometric test +ve	Controls matched	Organophosphate insecticide	Rosenstock et al. (1991)
Mixed	Neurobehavioral +ve Neurological +ve Neurophysiological +ve	Controls	Organophosphate insecticide	Steenland et al. (1994)

SOURCE: Reprinted with permission from Jamal (1995b), p. 89.

[a]NOTE: +ve, positive result; –ve, negative result.

[b]Repeated dosing for 2 or 10 months.

toms to develop (Craig and Freeman, 1953). Dermal exposures are slow in onset and only show eye and respiratory effects late in their course, with mental, gastrointestinal, neuromuscular, and circulatory effects preceding them (Bowers et al., 1964; Sim, 1962).

Eye effects of miosis (difficult and painful accommodation, blurred vision, dim vision, impaired night vision, and dilation of blood vessels in the conjunctivae with an associated headache) are the most consistently observed effects of vapor or aerosol exposures to nerve agents and, at very low levels of exposure, may be the only finding (Craig and Freeman, 1953; Holmes, 1959; Okumura, 1996).[24] In a combat environment, it would be surprising if impaired vision and night vision would go unnoticed and unreported, even if some of the other findings might be misdiagnosed. How much PB might modify the response of the eye to nerve agent is not known. In one study, standard pretreatment with PB for three days in volunteers did not prevent miosis from a CT of 5 mg-min/m^3 sarin but did decrease the severity and duration of the effect (Gall, 1981).

Respiratory effects—upper effects of rhinorrhea and lower effects of chest tightness, coughing, and wheezing—are very common but less specific to nerve agents than are the eye effects. There are no human data on how PB might alter respiratory clinical responses to nerve agents, although some animal data suggest some decrease in effect after PB pretreatment (Husain, Kumar, et al., 1993).

Gastrointestinal effects—nausea, vomiting, abdominal cramps, and diarrhea—occur later than respiratory effects. Excessive salivation is uncommon, except in higher exposures. A common report following exposures was a loss of taste or distaste for cigarette smoking (Holmes, 1959; Gaon and Werne, 1955).

Central nervous system effects of confusion, dizziness, difficulty thinking, and incoordination followed by muscle fasciculations and weakness are common and evolve over time but are present early. Less common are indications of peripheral nerve involvement with numbness and tingling of the hands and feet (Holmes, 1959; Morita et al., 1995). Since PB does not normally reach the brain, no protective effect on central effects of agents is expected from pretreatment.

Mental effects, with impaired thinking and memory, anxiety, and nervousness, have been documented in cases of dermal exposure in which the volunteers experienced no other systemic symptoms or signs (Bowers et al., 1964). Mental effects observed in exposed workers included altered behavior, impaired work performance, irritability, sleep disorders with vivid dreams, and frequent acci-

[24] Actual measures of low exposures have been uncommon; miosis without other complaints was often noted in production workers.

dents at work and driving (Gaon and Werne, 1955; Craig and Freeman, 1953; Brody and Gammill, 1954). As Craig and Freeman (1953) noted "inhalation of sarin may effect psychological disturbances of serious consequences for both the individual and those dependent on their judgment."[25]

With dermal exposures, there are skin signs, with excessive sweating, which may be prolonged for many days. Itching and erythema can occur (Sim, 1962; Freeman et al., 1954).

Recovery

The general experience has been that recovery from mild exposures is rapid, although in some studies some 20 percent had continued symptoms at or beyond two weeks, and 10 percent had them at or beyond three weeks (Holmes, 1959; Gaon and Werne, 1955). As will be discussed in longer-term effects, there were examples of much longer periods of symptoms (Gaon and Werne, 1955). Rapid resolution of moderately severe exposures was also the experience in Japan (Okumura et al., 1996). The milder cases in Japan do not seem to have been included in follow-up studies.[26] The muscle weakness encountered in some of Gaon and Werne's cases (1955) may be related to the disorder of myoneural junctions seen with anticholinesterase poisoning in animals (Gupta et al., 1987a, 1987b), but as with the animals is associated with recovery after exposure stops.

SUMMARY OF ACUTE EFFECTS

The conventional view that recovery from mild acute exposures is to be expected is further supported by the NRC follow-up studies (NAS, 1982; NAS, 1985), which found no adverse health effects in volunteers exposed to nerve agents.

Neuropathy Target Esterase Effects (Delayed Neuropathy)

No typical syndrome of delayed neurotoxicity of the type related to NTE has been described as occurring after nerve agent exposure, involving at least 7,000 persons. Animal studies have required very high acute exposures or repeated exposures to produce any neuropathy and could not produce the effect with some agents, such as VX (Gordon et al., 1983). The function of NTE is not known, and it is found in both neural (brain, spinal column, and sciatic nerve)

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[25]There is also a considerable literature on mental and neurological effects of organophosphate pesticides, e.g., Gershon and Shaw (1961), Tabershaw and Cooper (1966), Stephens et al. (1996), and Korsak and Sato (1977).

[26]Also note other comments about the Japanese experience later, in "Longer-Term Effects."

and nonneural (lymphocytes, platelets) tissue. Higher brain functions are not affected in typical organophosphate delayed neuropathy. Other organophosphate chemicals have produced some atypical syndromes, however. Workers whose toxicity was from leptophos had a variety of diagnoses—mental illness, multiple sclerosis, encephalopathy—made before the nature of their toxicity was recognized (Abou-Donia, 1981). A family that developed neuropathy after exposure to chlorpyrifos (normally not though highly likely to produce neuropathy) also had a variety of mental symptoms (Kaplan et al., 1993). Follow-up of TOCP cases (Hayes, 1982) showed some with dementia and other mental changes, raising questions about the possibility of atypical syndromes.

Epidemiology

In analyzing epidemiological data for indications of low-level nerve agent exposure, the conditions that were commonly mistaken for the agent effects, such as respiratory infections, allergies, and gastroenteritis, should be kept in mind. Two organophosphate pesticide experiences are instructive. There were significant increases in outpatient clinic visits on an Israeli collective farm for eye, respiratory, and headache complaints on days when organophosphate pesticides were sprayed nearby (Richter et al., 1986). A generally higher morbidity for many different complaints has been reported for organophosphate pesticide-exposed greenhouse workers than for controls (Kundiev, Krasnyuk, and Viter, 1986).

Accidents

Changes in management and operational assignments evolved after the early observations that sarin workers with mild intoxications after release to duty were prone to industrial and vehicular accidents (Gaon and Werne, 1955; Brody and Gammill, 1954), although no statistical studies were done on the prevalence and duration of the problem. It became the practice to take exposed workers off night work and driving for several weeks. No studies were available that correlate farm-worker accidents with organophosphate pesticide exposure. However, in their 1966 follow-up study, Tabershaw and Cooper noted without comment that three cases were lost to follow-up by reason of death from motor vehicle accident, and clinical notes show that several others were injured in motor vehicle accidents. No data were available with which to compare this experience.

Many factors no doubt contributed to accidents during the Persian Gulf War and to the motor vehicle accidents that accounted for increased mortality in returned veterans (Kang and Bullman, 1996). Other studies of the motor vehicle accidents are under way, and the findings noted above might be kept in mind as another factor for analysis.

Tolerance

Tolerance to anticholinesterases occurs in humans but has not been the subject of follow-up studies (Hayes, 1982). Some of the nerve agent workers with very low cholinesterase levels who reported no symptoms were perhaps tolerant (Freeman et al., 1956). Tolerant persons do not show typical signs and symptoms when exposed to agents at lower levels, which would otherwise be symptomatic. Tolerance is not specific for particular agents but rather a tolerance for anticholinesterases in general, so that persons made tolerant to organophosphate pesticides would be expected to be tolerant of low doses of nerve agents as well. There is no information on whether there were "tolerant" personnel in the Persian Gulf theater. Individuals potentially could have become tolerant from unauthorized use of "flea collars" containing chlorpyrifos. Other than reduced response to further exposure to anticholinesterases, tolerant persons are expected to show increased sensitivity to atropinelike anticholinergic drugs.

LONGER-TERM EFFECTS (FOUR MONTHS OR MORE)

The possibility of long-term effects from severe exposures is well accepted, although not inevitable (Sidell, 1974; Morita et al., 1995). It seems impossible that severe cases would have gone unreported during the Gulf War.

Six-month follow-up studies of one-moderate-exposure and many-mild-exposure rescue workers in Japan did not reveal any ill health (Nakajima, Sato, et al., 1997). Surveys of exposed residents of Matsumoto, Japan, did note some ill health in those who had mild and moderate exposures at six months, with fewer cases ill at one year (Nakajima, Ohta, et al., 1998).

At six months, a small group of Tokyo victims that had moderately severe intoxications seemed well but had subtle changes on special tests, woman showing more effects than men (Yokoyama, Araki, et al., 1998a).

There are at least three reports of workers with mild exposures (or two in one case) who had problems with memory fatigue, concentration, and irritability four to ten months after their exposures (Gaon and Werne, 1955; Brody and Gammill, 1954). Because at the time it was commonly assumed that recovery from nerve agents was rapid, there was a possibility of underdiagnosis of long-term problems after exposure, since such effects were not thought possible. Some clinical notes of that period reflect doubt that nerve agents were related to the mental symptoms reported.

Metcalf and Holmes (1969) reported on two different groups of sarin workers compared to controls. In the second group, no worker had been exposed to sarin within the year, although some workers had multiple exposures in the past. Compared to controls, this group reported poorer health more mental and emotional problems and had neurological findings suggesting poor coor-

dination. There were nonspecific EEG changes that were greater than in controls. Burchfiel and Duffy (1980) studied workers who had not been exposed to sarin in the previous year, comparing them to controls. They found more abnormal but nonspecific EEG changes in the exposed group.

Lohs (1975) reports studies by Spiegelberg on German World War II nerve agent production workers in follow-up after the war. Exposures are not defined, but this group was said to be in poor health with several clusters of disorder suggesting autonomic dysfunction, depression with intolerance to nicotine alcohol and medicines. The primary report is unavailable, and there may have been many confounding variables.

Organophosphate pesticide follow-up studies of several years' duration to several different chemicals at undefined levels have found health and mental function changes in formerly intoxicated patients (Tabershaw and Cooper, 1966; Savage et al., 1988).

It remains unknown whether unrecognized exposures can cause long-term effects, since no long-term follow-up of asymptomatic persons with low cholinesterase levels from agent exposure has been done. Likewise, no survey or study of production workers who did not report symptomatic exposures has been done. It is unwise to assume that there can be no long-term effects from unrecognized exposures. The organophosphate pesticide study of Stephens et al. (1996) documented exposures in sheep farmers, but mental performance decrements in formal tests conducted several months later showed equal degradations in farm workers who reported no signs and symptoms as in those who did.

"UNRECOGNIZED" EXPOSURES

There are several different categories of unrecognized exposures, with differing prospects for having long-term effects:

- **Acute exposure at a very low level in the "no effect" range, at which detoxification mechanisms eliminate agents, and little inhibition of cholinesterase occurs**—This may have been the situation with the very low levels of agent exposure projected from the Khamisiyah release—with no expectation of long-term effects. Subacute exposures are levels that do not produce overt symptoms but that over time produce substantial decrements in cholinesterase levels, more from repeated small exposures than a single exposure. Such exposures might result in the condition of tolerance.
- **Individuals with low cholinesterase levels but without symptoms**—It is not known whether such persons have long-term consequences, since the matter has not been studied. The report of sheep farm workers exposed to organophosphate pesticides (Stephens et al., 1996) indicates that it is pos-

sible to have substantial mental performance decrements after exposures that were not clinically apparent, some months after exposure. It may be possible to have long-term effects from this "unrecognized" category of exposures. It is believed that this group, which may arise from more sustained low-level exposures, could not have existed in the Gulf War, since exposures were all thought to be acute. However, agents trapped on dust or clothing, brought into shelters or vehicles, and released in closed spaces might provide some longer exposure opportunities (Gaon and Werne, 1955; Holmes, 1959; Freeman et al., 1956).

- **Individuals whose clinical response to an otherwise notable exposure to a nerve agent is modified by tolerance developed from sustained exposures to another anticholinesterase chemical (e.g., chlorpyrifos from flea collars)—**There have been no follow-up studies of tolerant persons. The possibility of long-term effects remains an open issue.
- **Individuals whose clinical response to nerve agents is modified by pretreatment with PB—**It is known that the severity and duration of mild exposures of the eye are diminished by pretreatment (Gall, 1981), but it is not known how much benefit may arise in decreasing other signs and symptoms. The matter has not been reported in low-level studies of animals, and human trials are unlikely.
- **Individuals exposed to amounts of nerve agent that produce symptoms and signs that are misidentified as arising from common illnesses, as has been noted in occupational settings—**It may be unlikely that there are long-term effects from mild or unrecognized exposures, but there is reason to think that such effects are possible, with some evidence that long-term effects have occurred (Gaon and Werne, 1955; Holmes, 1959).

PATHOLOGY AND PATHOPHYSIOLOGY

This section provides a picture of the spectrum of effects on various physiological systems following nerve agent exposure. More detailed studies from experimental animals are available, although they primarily deal with higher level exposures (McLeod, 1985; Baze, 1983; Koplovitz et al., 1992; Johanson, Anzueto, et al., 1985); however, subchronic studies have been reported, which were largely negative (Bucci et al., 1992a, 1992b, 1992c, 1992d). Myopathy and myoneural junction degeneration have been studied extensively (Gupta, 1987a, 1987b; Dettbarn, 1984; Ariens et al., 1969).

Recognition has been growing that nerve agents initiate processes with "cascade" effects that continue after direct agent effects have stopped. For example, agents induce seizures, which in turn release excitotoxic neurotransmitters that induce calcium influx into the cells, resulting in lipid peroxidation with ongoing oxidative and free radical damage to cells (Pazdernik et al.,

1996), possibly inducing apoptosis. Nerve agents can also induce massive, cardiotoxic, adrenergic outpouring (Filbert et al., 1993). However, no massive intoxications were encountered in the Gulf War, and animals with lower-level exposures or without seizures have not shown these dramatic changes (Petras, 1984).

Nervous System

This is the main site where the effects are seen. Both peripheral and central dysfunction occur at low levels of exposure (Sim, 1962; Bowers, Goodman, and Sim, 1964). Most animal studies (except for the negative subchronic studies, Bucci et al., 1992a, 1992b, 1992c, 1992d) have focused on the histopathology of higher-level intoxications. For many years, hypoxia was considered the precipitating factor in seizures and in brain injury. However, hypoxia preceding seizures has been ruled out by well-monitored primate studies (Johnson, 1985; Johnson et al., 1988; Johnson, Anzueto, et al., 1988). More-recent information gives a central role to seizures (directly caused by nerve agents) as the inciting stimulus for brain injury (Olney, 1990; Lipton and Rosenberg, 1994). When seizures are prevented, lesions are prevented or reduced (Baze, 1993; McDonough et al., 1995). However, sublethal doses of soman and sarin induced tissue changes without producing seizures, although such changes were not seen with DFP or metrazol (Kadar et al., 1992). Lesions show neuronal degeneration and necrosis with edema. Lesions in primates are concentrated in the frontal cortex, entorhinal cortex, amygdaloid complex, caudate nucleus, thalamus, and hippocampus, a distribution resembling anoxic damage (McLeod, 1985; Petras, 1984; Baze, 1993).

There is evidence of progression of injuries with encephalopathy, mineralization, encephalomalacia, and hydrocephalus in animals surviving substantial intoxications (McLeod, 1985; Wall, 1985). Animals surviving a single LD_{50} dose of sarin showed progressive neurological damage, initially in the hippocampus, pyriform cortex, and thalamus, but extending to other regions over three months (Kadar et al., 1992, 1995). Soman was more likely to produce frontal cortex damage.

The effects of nerve agents and other organophosphate compounds on sensory systems have been studied less than other systemic effects. Cutaneous peripheral receptors, but not mechanoreceptors, showed a reduction following subcutaneous soman administration 2.5 µg/kg/day for ten days in cats, or 5 µg/kg/day for five days. The studies involved the tibial nerve (Goldstein, 1985). In cats, low doses of soman in the range of 3 to 15 µg/kg intravenously were studied with respect to visual sensory performance. There was an abrupt decrease in visual evoked potential with doses above 5 µg, as well as decreases in contrast sensitivity and system gain (De Bruyn, 1991).

Nerve agents cause vascular dilation and increase cerebral blood flow in a manner that might influence their distribution (Scremin, Shih, and Corcoran, 1991). Soman, for example, causes some local constriction in cerebral flow (Sellstrom 1985). The mechanism seems independent of seizure activity (Drewes, 1985). Because of the effects on cerebral blood vessels, there has been suspicion that agents might alter the blood-brain barrier. In severe intoxications, the barrier can become more permeable, but this effect only occurs with seizures (Petrali et al., 1984; Drewes, 1985). Anticholinesterases alter the eye-blood barrier, an analogous situation, independent of seizures (Pshenichnova, 1985). Sublethal injection of soman (0.1 to 0.7 LD_{50}) permits the penetration of Sindibis virus, which usually cannot cross the blood-brain barrier (Grauer et al., 1996), indicating that soman at least might alter the blood-brain barrier without seizures. Increased permeability may make possible the entry of other toxicants, but also the entry of therapeutic agents.

A 51-year-old man was severely poisoned by sarin in the Tokyo attack and was in a vegetative state until he died some 13 months later. Neuropathological examination revealed marked nerve fiber decreases in the sural nerve and moderate decreases in the sciatic nerve. The dorsal root ganglia, dorsal roots, and posterior columns of the spinal cord were normal. It was considered that the dying back distal peripheral axonopathy was a late result of sarin intoxication (Murayama 1997; Himuro et al., 1998).[27]

Studies to identify sarin metabolites were conducted on the stored, formalin-fixed cerebellums of four fatal sarin cases from the Tokyo attack. Sarin-bound AChE was solubilized from the cerebellums, purified, and digested, and a search for sarin hydrolysis products was conducted by gas chromatography. MPA was identified, but IMPA was not and was presumed to have undergone hydrolysis during storage. This appears to be the first report of identifying nerve agent metabolites in formalin-fixed tissues (Matsuda et al., 1998).

Respiratory System

The main cause of death from nerve agents is respiratory failure, primarily failure of central driving mechanisms (Rickett, 1981; Johnson, 1985); Johnson, Anzueto, et al., 1988), but influenced by weakening of respiratory muscles and airway obstruction from bronchospasm and secretions. Mild respiratory symptoms—bronchospasm, rhinorrhea, and increased secretions—are an early sign of exposure. Even at a CT of 5 mg-min/m^3 of sarin can produce symptoms (Marrs et al., 1996), although effects at this level clear rapidly.

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[27]These findings are not typical of NTE-delayed neurotoxicity pathological findings, which also involve the cord.

Rats exposed to fairly high doses of sarin showed increased cellular proliferation in the lungs with interstitial thickening by day 4; by day 16, there was sign of bronchiolar damage, loss of alveolar spaces, and consolidation. Vigorous standard therapy prevented these changes (Pant et al., 1993).

Johnson, Anzueto, et al. (1988) compared respiratory and cardiopulmonary responses of anesthetized baboons to agent vapors delivered through a respiratory support apparatus in the upper airway using doses of soman or sarin in amounts in the range of estimated LD_{50}, from 0.5 to 2.0—all very severe exposures. The animals were followed for 28 days. Pulmonary artery pressure rose; hypoxia only arose after apnea occurred but continued after respirations resumed. Cardiac output fell, and arrhythmias were noted. Seizures preceded hypoxia. There was evidence of direct pulmonary damage from sarin and more from soman, with persisting neutrophil increase and decrease in alveolar macrophages extending four days beyond exposure (Anzueto et al., 1990). It was suggested that such effects might predispose to pulmonary infection. Soman has been shown to have a direct effect on bronchial smooth muscles, promoting constriction (Fonnum et al., 1984).

Cardiovascular and Circulatory System

Nerve agents and organophosphate pesticides induce myocardial injury, with subendocardial hemorrhage and myonecrosis (McLeod, 1985, Saidkarimov et al., 1985; Singer, Jaax, and McLeod, 1987). Unlike skeletal muscle injury, which is reversible, cardiac injury progresses to fibrosis (McLeod, 1985). This effect is only obvious in serious intoxications (Koplovitz et al., 1992) and resembles the cardiac injury sometimes seen from massive sympathetic outflow in central nervous system catastrophes. Prevention of seizures in animals can prevent the cardiac lesions in some situations (McDonough et al., 1989) but is not always effective (McDonough et al., 1995; Filbert et al., 1993).

Dangerous cardiac arrhythmias (e.g., ventricular arrhythmias (Johnson, Anzueto, et al., 1988)) are a frequent complication of nerve agent and pesticide poisoning. Hypotension and tachycardia with decreased cardiac output are common following large exposures (Johnson, 1985; Johnson, Anzueto, et al., 1988). The tachycardia probably reflects adrenergic effects (Marrs et al., 1996).

Some serious accident patients showed signs of ischemia but may have had premorbid atherosclerosis. Soman, in animal studies, induces coronary vasospasm with decreased coronary blood flow (McKenzie and Ballamy, 1993).

Musculoskeletal System

There is clinical and pathological evidence that nerve agents and other cholinesterase inhibitors can produce myopathy. Muscle pain and weakness

were common in occupational cases and serum creatine kinase levels were elevated (indicative of muscle injury) in many Japanese patients. Rhabdomyolysis with myoglobinuric renal failure has not been a feature of this apparently reversible injury. Early responses to substantial agent exposures are extensive twitching followed by flaccid paralysis (Marrs et al., 1996).

A subject of great attention has been the myopathy associated with motor end-plate degeneration, seen with nerve agents, organophosphate pesticides, and carbamate anticholinesterases. These effects are produced well below lethal levels. Two hours after agent administration, there is eosinophilia and swelling of sarcoplasm with loss of muscle striation. Later (within one day), there is leukocyte and histiocyte infiltration (Ariens et al., 1969). Activity and stimulation of motor end plates appears to initiate and exacerbate the problem. Oxime treatments that restore cholinesterase function are protective. The predominant evidence is that this myopathy is reversible. After tabun administration, recovery was seen in seven days (Gupta et al., 1987a).

Other Effects

Skin. The skin shows little reaction to the passage of agents. Increased sweating is seen, and V agents have produced erythema and itching (Sim, 1962). The stratum corneum may trap the agent and later serve as a reservoir for release.

Eye. The eye is extremely sensitive to nerve agents and can suffer effects at doses lower than other organs or systems. Agents coming into contact with the eye produce miosis, and induce vasodilation of conjunctival, scleral, and ciliary blood vessels, with ciliary muscle spasm producing pain. In some cases, intraocular pressure is lowered. There can be retinal effects with lowering of voltage in electroretinograms and decrease in dark adaptation with impairment of night vision, although the latter effect appears to be a central phenomenon (Rubin and Goldberg, 1957a; Rubin and Goldberg, 1957b; Rubin, Krop, and Goldberg, 1957). Eye changes from dermal exposures occur late in the intoxication, and in the case of this exposure route, pupillary dilatation is more likely than miosis. Japanese clinicians have reported a variety of other changes, including blurred disc margins and altered visual fields (Kato and Hamanaka, 1996; Nohara and Segawa, 1996).

Gastrointestinal Tract. Agents are rapidly absorbed from the gastrointestinal tract and produce early local symptoms (Karakchiev, 1973; Grob and Harvey, 1958; Sim et al., 1964), but gastrointestinal effects arise from all exposure routes, with cramping, increased peristalsis, vomiting, and diarrhea, even from low-level exposures. There is not an abundant literature about pathological changes in the bowel, and chronic diarrhea was not a long-term effect reported in occupational studies. Liver injury is reported from some organophosphate

pesticide poisonings (parathion) but has not been a clinical or pathological factor in nerve agent toxicity (Hayes, 1982).

Hematological. Hematological effects resulting from agent exposures have been identified, but they do not seem to be clinically important. It is not surprising that some hypocoagulation abnormalities have been seen (Kaulla et al., 1961), since thrombin is a serine-esterase. *In vitro* inhibition of thrombin by sarin has been demonstrated (Thompson, 1969).

Johnson (1985) mentions bone marrow depression in severely poisoned baboons but did not provide details. Sastry and Sadavongvivad (1979) mentioned ACh receptors on stem cells. Nerve agents and organophosphate pesticides may impair immune function, but they may also increase resistance to infection (Mierzejewski, 1970a, 1970b). Clement (1985) found indications of high-dose soman-induced immune suppression in mice, although soman-induced hypothermia could not be excluded as a factor.

Mutagenesis. There is little evidence that sarin, soman, and VX are mutagens (there is no information on cyclosarin), and they do not injure DNA (Goldman et al., 1987). Tabun at high doses is a weak mutagen (Wilson, Nicaise, and De Bisschop, 1994). The presence of a number of sarin production by-products (diethylmethylphosphonate [DEMP], diisopropylmethylphosphonate [DIMP], and ethylisopropylmethylphosphonate [EIMP]) and metabolites (such as ethylmethylphosphoic acid [EMPA]) in the urine of Tokyo sarin victims lead to the conduct of sister chromatid exchange studies using peripheral lymphocytes in nine exposed persons and in controls with positive findings in the victims.

German scientists have stressed the alkylating properties of organophosphate agents (Lohs, 1975), but apart from some pesticides, that does not appear to be a feature of military agents. With the exception of tabun's weak effect at large doses, such agents do not appear to be mutagenic (Goldman and Dacre, 1989; Wilson, Nicaise, and De Bisschop, 1994). A decrease in DNA repair systems *in vitro* has been seen with sarin but not soman (Klein et al., 1987).

Genitourinary. Although there is often increased urinary frequency from bladder-stimulation effects, renal disorders are not a clinical problem encountered with nerve agent exposure (Sidell, Takafuji, and Franz, 1997; Marrs et al., 1996). The kidneys receive considerable blood flow and eliminate agent metabolites, but renal problems related to agents have not come to light (Sidell, Takafuji, and Franz, 1997; Marrs et al., 1996). There are examples of renal failure associated with organophosphate pesticide intoxication (Abend et al., 1994).

Development. There is no evidence of developmental toxicity from sarin (Laborde, 1996). Resumption of weight gain in subacutely exposed young rats given sarin or tabun is an indication of tolerance (Dulaney et al., 1985).

Endocrine. Endocrine systems normally demonstrate considerable periodic activity—circadian variations in corticosteroids and growth hormone, or monthly menstrual cycles. DFP-treated animals did not show altered circadian patterns, although prolactin levels were elevated (Kant et al., 1991). Clement (1985) conducted extensive studies in rodents using several levels of soman subcutaneously. There were increased levels of corticosterone, thyroxine, and T3, which returned to normal in 22 hours. ACTH and testosterone levels were decreased, but only with high-level exposures. Repeated low exposures to soman in rats at 30 µg/kg/day did not produce signs of intoxication or changes in hormone levels. Higher doses (40 µg/kg) produced toxicity and increased levels of glucose and corticosteroids (Peoples et al., 1988). Severe soman intoxication impaired ACTH and prolactin responses (Kant et al., 1991). No detailed endocrinologic studies of humans exposed to low doses of nerve agents are available. It is known that some carbamate anticholinesterases can affect growth hormones (Cappa et al., 1993).

SHORT-TERM EXPOSURES AND LONGER-TERM EFFECTS

This section reviews the literature related to longer-term effects from short-term exposure. The reader should keep in mind that what is possible is described, but whether exposure took place in the Gulf or not is beyond the scope of this report.

Assumptions

Severe and moderately severe poisonings from nerve agents, which clearly can produce long-term effects, would have been recognized during the Gulf War. Nerve agent exposures, if they occurred, were therefore at lower levels capable of producing only mild intoxications, or none.[28] No assumptions were made that only single acute exposures could occur or that repeated or sustained lower level exposures did not, since such matters seemed still to be under investigation.

Information

As is often the case, there is an abundance of data on nerve agents, but studies that directly address the specific issues of concern here are not to be found. Most human data on the effects of nerve agents come from events for which the actual levels of exposure are unknown (accidents and attacks). Most information is short term (weeks at best). Studies of persons who may have been exposed but were not sick are rare. There is more follow-up information about

[28]This level of effects has been the main focus of the review.

patients who were hospitalized than patients who were not. The expectation that recovery from all but the most serious poisonings was always to be expected may have introduced a bias to decrease recognition of long-term effects. The situation with respect to animal studies is similar, with more interest in higher-dose exposures and limited follow-up. There is often more interest in demonstrating an effect, such as receptor downregulation, than there is in following the resolution of the effect—i.e., when do receptor levels return to normal? In thinking about longer-term effects, it should be kept in mind that, for rodents whose lives are short, an effect lasting several weeks could be considered a long-term effect in terms of life span.

Questions

Some specific questions about the effects of nerve agents seem relevant to the analyses of the Gulf War:

- Is it possible to have nerve agent effects from lower-level exposures to nerve agent without obvious typical signs and symptoms?
- Is it possible to have long-term effects from mild exposures?
- Is it possible to have delayed effects from unrecognized exposures?

The prevailing view of many experts (Sidell and Hurst, 1997; Marrs et al., 1996; Elson, 1996) and review panels (DSB, 1994; IOM 1996; PAC, 1996a, 1996b) is that long-term effects from exposure to lower levels of nerve agent are not to be expected, especially when no signs and symptoms are recognized. There is substantial evidence to support these views, ranging from the negative findings of long-term effects in volunteers exposed to nerve and other anticholinesterase agents (NAS, 1982; NAS, 1985), through largely negative findings in follow-ups of Japanese casualties (Nakajima and Sato, 1997). There is no evidence that nerve agents are carcinogens, and only tabun has been shown to be a weak mutagen in some assays. Likewise, there has been no expectation of long-term problems from exposures to organophosphate pesticides without overt toxicity (Sidell and Hurst, 1997; Hayes, 1982).

The lack of reports of overt toxicity resembling nerve agent exposure has caused many to dismiss a role for nerve agents in the varied illnesses of veterans of the Gulf War. Sidell (1997) is of the view that long-term effects in sarin workers would not have escaped attention because of the alertness of supervisors and plant physicians to workers whose behavior was unusual.

This review has found information that—from cases of unrecognized exposures, long-term effects from mild exposures, and perhaps delayed effects—makes the above reasonable points of view less certain. The variability of response to nerve agent by gender, time of day, and interactions with drugs and chemicals

has already been discussed. The clinical material above discussed a variety of circumstance where exposure might not be recognized at differing levels of exposure. With that in mind, the following subsections discuss the specific questions raised here.

Is It Possible to Have Nerve Agent Effects from Exposures That Are Unrecognized? Based on the published literature, the possibility cannot be ruled out, for the following reasons:

It is well documented that occupationally exposed persons were found who reported no acute signs and symptoms and who had very low cholinesterase levels (Gaon and Werne, 1955; Holmes, 1959; Freeman et al., 1956). Such persons were not subject to any special follow-up but in some cases were observed to be asymptomatic during the period when their cholinesterase levels were returning to normal.

Some of these individuals could have become tolerant of the agent as a result of their exposures. Similar situations are documented in organophosphate pesticide workers, in whom tolerance without preceding symptoms was found (Hayes, 1982). It is not certain that tolerance is without ill effects, but the situation is little studied. A clinical effect in tolerant persons and animals is an increased sensitivity to atropinelike anticholinergic drugs.

Although tolerance has been demonstrated in humans, the receptor downregulation of cholinergic receptors has only been demonstrated in experimental animals (Costa et al., 1982). However, there seems no reason to doubt that such downregulation occurs in humans. Tolerance induced by other non–nerve agent anticholinesterases (e.g., chlorpyrifos flea collars) would be expected to produce diminished clinical responses to low-level nerve agents.

Substantial mental changes (impaired thinking, memory, and calculating ability and anxiety) were documented in volunteers exposed to VX at levels that were asymptomatic for most (Bowers et al., 1964). No study of their recovery period was found; they were included in the negative findings of the NRC follow-up study (NAS, 1985).

There are several animal studies in which animals given doses of sarin, soman, or DFP were "sign free"—lacking overt signs of cholinesterase effects—showed definite behavioral and performance test decrements (Sirkka, 1990, intraperitoneal; Wolthuis, Groen, et al., 1995, intramuscular, acute; Buccafusco, 1997, repeated small doses). Only Buccafusco studied the process of recovery, documenting learning impairments and decreases in brain nicotinic receptors several weeks after exposure (a long-term effect for a rat).

There is comparable mental-effect information from a prospective study of organophosphate pesticide exposure in sheep farmers, which found that farm-

ers exposed to pesticides who reported no clinical symptoms showed decrements in performance on psychological tests some months later comparable to that of farmers who reported symptoms (Stephens et al., 1996). Korsak and Sato (1977) also reported deficits in varied neurobehavioral tests in organophosphate pesticide workers who had never reported having overt symptoms. The latter investigators noted the similarity to nerve agent effects.

Misinterpretation of nerve agent signs and symptoms of exposure as being due to other common illnesses is well documented in occupational settings (Gaon and Werne, 1955; Craig and Freeman, 1953; Holmes, 1959).

Nonhuman primates given doses of sarin that produced no signs or symptoms (1 µg/kg/day for ten days) showed EEG changes not present in controls a year after exposure (Sim, 1971; Burchfiel, 1976) This study has been controversial. The effect may not be clinically important but shows a long-term alteration of brain function.

A surprising report is that of Husain, Vijayaraghavan, et al., 1993, in which mice exposed daily for ten days to inhaled sarin (5 mg/m^3 for 20 minutes) developed typical delayed NTE type neuropathy by day 14. The notable matter is they showed no anti-AChE symptoms at any time.

In summary, there is evidence of asymptomatic or unrecognized nerve agent (and organophosphate pesticide) exposure producing effects of various durations in humans and animals. Alterations of mental function are the best-documented effect.

Is It Possible to Have Longer-Term (Months to Years) Effects from Mild, Lower-Level Exposures? The answer is yes. It does not seem to be common for a single mild exposure to produce long-term effects. The possibility of observer bias causing some cases to be overlooked was discussed earlier.

There are documented case reports of workers with mild exposures (one or two exposures) who had problems of fatigue, poor memory and concentration, and irritability 4 to 10 months after exposure (Gaon and Werne, 1955; Brody and Gammill, 1954).

Workers who had not been exposed to sarin within the previous year, (compared to nonexposed controls) with reported single or multiple mild exposures were found to report poorer health, to have poorer performance on psychological tests, and to have "soft" neurological findings of poor coordination. EEGs were nonspecific but differed from controls. Some of these workers may also have worked with organophosphate pesticides (Metcalf and Holmes, 1969).

In another study of workers a year after their last exposure to sarin, compared to nonexposed controls, Duffy and Burchfiel (1980) found nonspecific EEG changes in the workers that differed from controls.

The single exposures in Japan have shown little in terms of long-term effects. Some moderately severe cases have shown subtle changes on special tests of neurological function six months after exposure. A survey of Matsumoto residents, some of whom were moderately poisoned and others mildly so, showed some nonspecific "asthenia" and poor health at six months, with fewer such symptoms reported at one year (Nakajima, Ohta, et al., 1998). The many mild cases of exposure in Japan have not been reported in the available follow-up studies.

In summary, there is some evidence that mild exposures can have long-term effects. The evidence that a single mild exposure can produce long-term effects is slight, and there is more evidence that repeated mild exposures can result in effects a year or more after exposure.

Is It Possible to Have Delayed Effects from Low Doses of Nerve Agents? There is no compelling evidence that documents latent effects of nerve agents appearing long after unrecognized exposure or resolution of initial signs and symptoms. The subjects of previously noted follow-up studies were not observed during the year before study, and there is no indication that the findings noted had developed just at the time of study. See Table 5.14.

No human cases reported from nerve agent exposures clearly resemble NTE-delayed neurotoxicity, and the main weight of evidence is that nerve agents have little potential for producing delayed toxicity, which typically appears two to three weeks following an acute exposure. At very high doses, soman and sarin can produce the disorder in animals from acute exposures (Gordon et al., 1983), while sarin has produced delayed neurotoxicity in mice from repeated exposures to sarin that did not produce signs of inhibited AChE effects (Husain, Vijayaraghavan, et al., 1993). The whole matter of NTE-delayed neurotoxicity is difficult, since the natural function of the inhibited enzyme and the mechanism by which inhibition causes injury are not known. The effect can be produced in animals with repeated doses of other organophosphate chemicals at intervals of six weeks (Lotti, 1991), and there is a potential for complex interactions of multiple chemicals. Typical delayed neurotoxicity would be readily recognized. Reports of atypical presentations with more central nervous system manifestations in organophosphate poisoning with leptophos (Abou-Donia, 1981), or chlorpyrifos (Steenland et al., 1994), or late dementia in TOCP cases (Hayes, 1982) make it uncertain that the full clinical spectrum of NTE-based toxicity is understood.

Likewise, the mechanisms of long-term effects of nerve agents are not well studied. No studies emerged of animals exposed to low doses of nerve agents with later neurophysiological and biochemical studies. Mechanisms other than inhibition of AChE are required. The long-term effects of nerve agents on receptor abundance and function have not been studied.

The detailed mechanism by which IEGs affect adaptations in the brain merits attention. An interesting concept is that of Kaufer et al. (1998), as demonstrated in animals, that a variety of cholinergic stimuli (stress, PB, organophosphate pesticides, or nerve agents) are all capable of inducing the expression of an IEG c-fos, a regulatory gene that directs the expression of the regulatory protein FOS, known to influence the expression of genes involved in cholinergic brain mechanisms. The duration and end-effects of such induction are not known. The concept does provide an interesting hypothesis of how several environmental factors of interest might converge on a common biochemical pathway in the brain capable of producing longer-term changes in the brain.

In summary, there is no evidence of nerve agents producing delayed effects similar to those associated with NTE, but there is reason not to ignore the possibility totally, given the incomplete understanding of NTE delayed neuropathy and indications that there may be atypical syndromes from this mechanism.

WHAT TO LOOK FOR IN THE GULF CONTEXT

The eyes are highly sensitive to the effects of nerve agents (NAS, 1997). Clinical and epidemiological reviews of the Gulf War should note that vasodilation of conjunctival vessels from nerve agents can resemble conjunctivitis, but pain on focusing, dim vision, impaired night vision, and miosis all point strongly to anticholinesterase effects. Impaired night vision would likely have been reported by affected persons in a combat environment.

Upper and lower respiratory symptoms of rhinorrhea, tight chest, cough, or wheezing are not very specific and have historically been confused with other causes of these symptoms. The same is the case for gastrointestinal symptoms of nausea, abdominal cramps, and diarrhea. Reports of a distaste for cigarettes is common in low-dose nerve agent exposure and, if reported, suggest exposure. Mental changes, disturbed sleep, irritability anxiety, and muscle weakness are commonly found in mild nerve agent exposures but also might be overlooked or misdiagnosed.

However, if several of the different physiological symptoms noted above are present, suspicion should be high that they were produced by nerve agents (Holmes, 1959).

Cholinesterase levels are not a very effective biomarker, other than indicating that an exposure has occurred. Early experience with nerve agents showed poor correlation between enzyme levels and clinical findings, some cases showing no inhibition for a day or more (Craig and Freeman, 1953). Serum levels rise within days after the end of exposure; red-cell levels stay depressed until the affected cells are replaced (about 90 days). Even if frozen serum or red cells from the Gulf War period were available, lowered levels of enzyme would

not distinguish between nerve agents and other AChE inhibitors (e.g., chlorpyrifos or PB).

Should Gulf War material thought to have been exposed to nerve agents, such as mask filters or protective clothing, become available, there are now sensitive chemical techniques that might detect degradation products of the agents if they existed (Department of the Army, 1996).

The Armed Forces Institute of Pathology (AFIP) might be consulted on the possibility of obtaining evidence of nerve agent exposures from tissue material in their holdings from the Gulf War and the period immediately after.

Normal autopsy studies are directed at finding a cause of death. It might be useful to explore with AFIP whether material might be examined for indications of anticholinesterase activity, such as examining myoneural junctions for typical pathology.[29] Coronary blood flow is decreased in animals exposed to nerve agents (McKenzie and Ballamy, 1993). Review of material from heart attacks during the Gulf War might also be considered, looking for atypical findings.

AFIP's views could also be sought on the practicality of detecting degradation products of nerve agents in tissues, likely to be found from lower-dose exposures. There is some existing methodology for such measures (Sidell, Takafuji, and Franz, 1997, p. 296), but the applicability to formalin-fixed tissues is unknown to the reviewer. Japanese pathologists using large volumes of formalin-fixed tissues (the cerebellum) from fatal sarin cases have demonstrated sarin degradation products in formalin-fixed tissues (Matsuda et al., 1998), but such methods may or may not be applicable to lower levels of exposure. This is not a study suitable for random screening but might be considered, say, if there were material from a fatal accident after proximate exposure to the plume from a known release.

Any reviews of accidents during and immediately after the Gulf War should be aware of the historical information of high accident prevalence in workers following mild exposures to nerve agents (Gaon and Werne, 1955).

Epidemiologic studies of illnesses in veterans of the Gulf War might keep in mind the unproven possibility that the effects of several cholinergic stimuli (stress, organophosphate pesticides, PB, and nerve agents) might converge to produce longer-term changes in brain chemistry, resulting in a greater effect than any one of the factors alone. Looking at aggregate exposures of individuals and units over time might show correlations with later clinical problems that might not be evident in single-factor analysis. An example would be the previ-

[29]This would not, however, distinguish between nerve agents and other anticholinesterase effects.

ously mentioned increased clinic visits to a collective farm clinic on days when organophosphate pesticides were sprayed in the region (Richter, 1986).

There is a possibility that some persons in the theater may have become tolerant to anticholinesterases, including nerve agents. Such persons might come to attention because of adverse reactions to anticholinergic medications with atropine-like effects.

Although c-fos and FOS using PCR and immune methods could probably be detected in AFIP material, their clinical significance is not understood well enough to make such studies worthwhile at this time.

SUMMARY, ANALYSIS, AND COMMENT

Nerve agents inspire respect and fear. They are highly toxic and have steep dose-response curves, tabun being the least toxic and VX the most. They produce lethality in doses measured in micrograms per kilogram.

Their main effects are produced by irreversible inhibition of the enzyme AChE, producing signs and symptoms primarily resulting from excess ACh, overstimulating parts of the nervous system.

They also inhibit a variety of other enzymes in the body, but the biological consequences of this are poorly understood. The same situation applies to their direct interactions with neural receptors and cell membranes.

The nerve agents tabun and sarin are quite volatile, representing a considerable respiratory threat but with low persistence. Their volatility makes them less of a threat from dermal exposure. Soman and cyclosarin are somewhat less volatile and more persistent, and both pose significant dermal and respiratory threats. VX is not very volatile and is very persistent. It represents a serious dermal threat but has dangerous respiratory toxicity delivered as an aerosol in very fine droplets. All are subject to hydrolysis and degradation in the environment, lasting hours to a few days.

Severe intoxications with these agents produce signs and symptoms of seizures, respiratory distress, unconsciousness, and circulatory collapse (impossible to overlook during the Gulf War), which can produce brain and cardiac injuries that are sometimes long lasting. Moderately severe cases from Japan have usually recovered, but at six months, some cases showed subtle neurological changes on special tests despite generally seeming normal.

Delayed neuropathy from inhibition of NTE by nerve agents has never been reported in humans. Only sarin and soman have produced this neuropathy in animals, but exposure to extraordinarily high levels or prolonged exposure is required to produce the effect.

The effects of lower-level exposures to nerve agents have been less extensively studied in animals than those of higher doses. Most human cases of nerve agent exposures have been in the domain of mild or no symptoms. Since the most exposures arose from accidents or attacks, the exact exposure levels of these cases are unknown.

The eye is consistently the organ most sensitive to the effects of nerve agents applied by vapor or aerosol—with constriction of pupils (miosis), pain and difficulty focusing, dim vision, and dilated conjunctival vessels. NAS (1997) estimates these levels causing these effects to be as shown in Table 5.15.

Respiratory symptoms, headache, confusion, anxiety, dizziness, incoordination, nausea, gastrointestinal distress, and weakness all can arise in milder cases, at somewhat higher levels. The upper boundary of exposure that produces mild symptoms (patient can walk and talk but is definitely symptomatic) is not rigorously defined but is probably close to the ICT_{50}, as estimated in NAS (1997); see Table 5.16.

The onset of mild symptoms from lower-level respiratory exposures can be delayed for up to one hour, while it may take longer before symptoms from dermal exposures arise. The historical experience of mild recognized exposures is that symptoms last for hours to a few days before recovery, with 10 to 20 percent reporting effects to two to three weeks or beyond. Long-term effects are not expected.

The precise nature and amount of exposures, if any, of U.S. personnel to nerve agents during and after the Gulf War remains uncertain, as does the role of such exposures in producing illnesses in Gulf War veterans. The conventional view is that, historically, most low-level nerve agent exposures that were recognized produced interesting symptoms, which were cleared in a matter of days or weeks. Effects on memory, thinking, attention, and emotions were known but were considered to fade rapidly. For the majority of exposures, the number and duration of symptoms corresponded to the severity of the initial event. Neither the U.S. production experience nor the experiences in Japan (over 6,000 cases) produced a sustained set of complaints resembling the illnesses in the veterans of the Gulf War.

The low persistence of sarin makes it less likely that there have been effects from prolonged low-level exposures and from long-distance transportation from remote locations. Sarin absorbed in dust particles might persist longer or travel further. Cyclosarin might be more persistent, and less is known about this agent, although it has not been found to produce delayed neuropathy. There seem to be no reports of the dim vision or impaired night vision that would have been expected from low-level exposures, and these would have cre-

Table 5.15

Estimates of Threshold Levels for Eye Effects of Selected Nerve Agents

Nerve Agent	mg-min/m^3
Tabun	0.5[a]
Sarin	0.5[a]
Soman	0.2
Cyclosarin	0.2
VX	0.09

SOURCE: NAS (1997).

[a]Possibly higher.

Table 5.16

Estimates of Incapacitating Levels of Selected Nerve Agents

Nerve Agent	mg-min/m^3
Tabun	≤50
Sarin	≤25
Soman	≤25
Cyclosarin	≤25
VX	10

SOURCE: NAS (1997).

ated concern during a war. The exposure to nerve agents of U.S. experimental subjects does not seem to have produced notable long-term effects.

Humans retain about 85 percent of inhaled sarin, but some is trapped in the mucous of the respiratory tract and, when absorbed, encounters a number of nonspecific binding chemicals and enzymes in blood and tissue capable of hydrolyzing and degrading it. For very low-level exposures remote from Khamisiyah, these protective systems may have provided a "no effect" level of exposure. For example, the 0.01296 mg-min/m^3 exposure to sarin at Khamisiyah found in the CIA model can also be expressed as 0.01296 µg. During a 1-minute exposure for a human breathing 10 l of air per minute, 89 percent (0.115 µg) of the total inhaled sarin (0.12986 µg) would be absorbed. As a point of comparison, Somani (1992) estimates that a guinea pig can metabolize (degrade and detoxify) sarin at a rate of 0.013 µg/kg/min. Although the rate at which humans metabolize sarin has not been determined, if it is similar to the rate in guinea pigs, a 70 kg human should be able to metabolize 0.91 µg of sarin per minute. This calculated degradation rate is much higher than the calculated exposure from Khamisiyah. Studies of low-dose inhalation exposures

with absorbed doses of 0.08 to 9 µg/kg (Oberst et al., 1959; Freeman et al., 1952) indicate considerable individual variation in response and suggest that humans are less able to detoxify sarin than guinea pigs. Intraarterial administration of 3 to 4 µg/kg to humans produced no symptoms (Grob and Harvey, 1953).

The very low estimates of exposure arising from the Khamisiyah release make sarin-cyclosarin–induced delayed neurotoxicity essentially impossible. The amount of sarin required to produce this effect may be less than originally thought by researchers, but rather substantial amounts are required nonetheless.

On the other hand, it is not possible to eliminate nerve agents categorically from playing a role in some cases of illnesses of Gulf War veterans because of other information about the effects of nerve agents and related organophosphate pesticides.[30] The reasons include the following:

1. From occupational experience, it is known that persons have been discovered who had quite low cholinesterase levels, indicating exposure, who had not experienced any acute signs and symptoms, even many years later. Such persons were not studied after their cholinesterase levels returned to normal.[31]
2. Although there is no clear evidence of long-term effects arising from a single acute exposure producing mild effects, there are several reports of sarin workers with one or two mild exposures experiencing problems of fatigue, impaired memory and concentration, and irritability four to ten months after exposure.[32]
3. The population of workers who were in the plants where sarin was made but who did not report symptoms was never included in any long-term follow-up study. Any health problems they may have encountered could have been unrecognized as related to agent exposure. (Follow-up studies of field workers involved with organophosphate pesticides who had not reported clinical symptoms showed a variety of deficits on psychological testing long after exposure was possible; Korsak and Sato, 1977).
4. Follow-up studies of workers with mild responses to sarin exposure (some multiple) a year after the last sarin exposure, showed more health complaints, deficits on psychological testing, neurological findings of poor

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[30]This falls in the category of agents that "could" do certain things; whether they "did" is quite another matter.

[31]There is also a lack of information about persons with similar findings from organophosphate pesticides.

[32]There may have been problems recognizing long-term effects, since the prevailing view at the time was that they did not occur.

coordination, and nonspecific EEG changes than the controls. Another study with controls found nonspecific EEG changes in workers who had not been exposed to sarin in the previous year.

5. It is possible to have substantial mental effects from acute nerve agent exposure (VX) (impaired thinking, memory, calculating ability, and anxiety) at levels where other clinical signs and symptoms were absent (Bowers et al., 1964).

6. Similar data from animal studies with sarin (acute), soman (acute), and DFP (repeated doses) showed impaired test performances and altered behavior at levels not associated with other signs of toxicity. Recovery times for acute exposures were not reported, but the DFP study found impaired learning several weeks after stopping the agent.

7. The above studies make the point that some mild exposures with effects may be unrecognized and that, at least for some repeated low-level exposures, there can be long-term effects. Comparable data from organophosphate exposures of sheep farmers found decrements in the performance on psychological tests in asymptomatic individuals who were exposed that was comparable to that of those with symptoms, weeks to months after exposure (Stephens et al., 1996).

8. The controversial study that showed EEG changes lasting a year in nonhuman primates given 1 μg/kg of sarin for ten days, which did not produce any evident illness, may not be "clinically" important but does show a long-term change in brain function from doses that were without clinical signs (Duffy and Burchfiel, 1980).

9. The experience with occupational exposures is that it is possible for signs and symptoms of mild exposures to be misinterpreted as being due to other common health problems, such as upper respiratory infections or allergies, because of the nonspecific nature of the symptoms. The possibility that such misinterpretation could have occurred during the Gulf War should not be dismissed.

10. Based on self-reported exposures (Haley, Horn, et al., 1997), there is a possibility that some persons with sustained use of unauthorized flea collars containing chlorpyrifos (or other anticholinesterase exposures) might have developed tolerance to anticholinesterases and would not be expected to show typical signs and symptoms when exposed to nerve agents—providing yet another opportunity for unrecognized exposures.

11. Recovery from the tolerant state has been little studied, and the long-term effects (if any) are unknown. In animals with repeated exposures to DFP, there are suggestions of decreased abundance of nicotinic receptors being

associated with impaired performance on memory tests, for some weeks after stopping the agent (Buccafusco et al., 1997).

12. The effects of PB in modifying the clinical effects of low-level exposures have not been much studied. There is a report that humans pretreated with pyridostigmine and then exposed to 5 CT (mg-min/m^3) of sarin had less-severe miosis and a shorter period of visual symptoms (Gall, 1981). There are reports that pretreatment with PB (and other carbamates) lessens the effects of respiratory exposures to sarin at doses of about 50 CT in experimental animals (without other treatment) (Vijayaraghavan et al., 1992). This raises the possibility that PB pretreatment might modify some low-level exposures to a point where they would not be recognized. Since PB does not normally penetrate the blood-brain barrier, it would not be expected to protect the central nervous system from the effects of milder exposures. There is an animal study at somewhat higher levels using labeled sarin in pretreated and control animals that did not find increased label in the brains of the pretreated animals.

13. There are indications that there can be atypical syndromes, with more indication of central nervous system effects in NTE neurotoxicity (although not from nerve agents). The mechanism of toxicity is not well understood. Nerve agents alone seem unlikely to produce NTE effects, but nothing is known about their effects when combined with other organophosphate chemicals. Some organophosphate chemicals have shown development of toxicity when administered at six-week intervals. Cyclosarin has not been shown to produce delayed neurotoxicity in acute animal models, but it is a potent inhibitor of NTE. No studies examine combinations of sarin and cyclosarin in NTE toxicity models, and there are no animal studies of repeated exposures similar to those of Husain with sarin (Husain, Vijayaraghavan, et al., 1993)

This limited understanding and information about NTE effects creates uncertainty about whether it can be ignored. The other data create uncertainty that long-term effects can only arise after recognized exposures and that long-term effects from mild exposures cannot occur.

The effects of agents on nonneural tissues (lymphocytes, bone marrow) are poorly understood. There is animal research evidence that sign-free doses of soman permit viruses to enter the brain that would not normally enter. Modification of responses to infection by nerve agents cannot be excluded, although human clinical experience has not noted such effects.

There is little information about interactions of nerve agents with other chemicals at lower levels of exposure. There is some evidence that combined exposure of mustards with nerve agents can increase the toxicity of both. A person

taking an atropinelike compound (e.g., an antihistamine drug) might have decreased response to nerve agent—similar to the protective effect seen in animals pretreated with atropine (SIPRI, 1976).

The experience of workers recovered from mild exposures enough to resume work being prone to industrial and motor vehicle accidents deserves mention. The duration of the effect was not well defined, and the observation was not studied in detail. The effect should be kept in mind in analyses of accidents during the war (Writer, DeFraites, and Brundage, 1996) and in accidents of returned veterans (Kang and Bullman, 1996). Of course, many other factors are involved in increased accidents during periods of high operational activity and on return from overseas.

In 1998, a new Russian (Soviet-developed) nerve agent, Novichok—said to be a binary agent and highly toxic—was mentioned in the press (Englund, 1992a, 1992b; Adams, 1996; Tucker, 1996; Uhal 1997; "Russia Dodges . . . ," 1997). A Russian scientist-émigré was said to have indicated that some U.S. detectors might not recognize the agent and that it might have been available to Iraq (Smart, 1997). There are no peer-reviewed or scientific journal references to this agent, although there are some press reports. A Russian scientist, Dr. Vil Mirzaynov, has described a Soviet secret program of nerve agent developments. In an interview posted on the Internet, Dr. Mirzaynov said he was certain that the Soviets and Russia had not sent Novichok to Iraq.

It has been known for a long time that there are anticholinesterase chemicals that are 10 to 100 times more potent than current agents reviewed (SIPRI, 1971). The existence Novichok is scientifically undocumented, and its use has not been mentioned in any of the postwar revelations about Iraqi chemicals. The matter is mentioned here for completeness but does not appear relevant to a scientific review of chemical agents associated with the Gulf War.

RECOMMENDATIONS

The descriptions of the findings in mild nerve agent exposures should be kept in mind in any review of medical records using some of the distinguishing features mentioned in the clinical discussion, as well as the overall pattern and sequence of symptoms. Epidemiology reviews of medical experience in the theater should keep in mind the reports of increased outpatient visits for eye, headache, and respiratory complaints noted in a farm clinic on days when organophosphate pesticides were sprayed nearby.

It may be technically possible to document nerve agent and anticholinesterase exposure using material AFIP possesses. Whether it is practical or desirable to do so is a matter for discussion with the institute and relevant specialists.

Doing a study on a screening basis would be unadvisable, but such studies might be warranted if there is a probability of prior exposure to detectable amounts.

In the ongoing discussions with Japanese clinicians about their follow-up studies, it would be valuable to develop a clearer picture of the long-term outcomes of the many mild cases who did not require treatment.

It is not known what records exist for the occupationally exposed U.S. production workers or what health data on them exists. There would be interest in long-term follow-up of such workers, most of whom would be rather elderly now. Such a study would be difficult and expensive, especially in providing suitable controls and obtaining credible data about other occupational exposures.

The problem of illnesses in veterans of the Gulf War has inspired a large amount of research related to nerve agents, stress, PB, and pesticides. It was not within the scope of this report to survey such activity or to report on work in progress or in unreviewed drafts. Thus, any suggestions about research offered here may be redundant.

Further evaluations of NTE-based delayed effects from nerve agents should examine the effects of sarin-and-cyclosarin combinations. It would be helpful to use animal models of repeated subclinical exposure to replicate the effects Husain reported and to determine the threshold level of exposure that produces the effect. If the effects are consistently observed, it would be important to attempt their replication in some other species such as nonhuman primates, to better judge the hazard to humans from the effects of repeated subclinical exposures. Effects on higher-level brain performance might be examined as well. It is not certain that important combined effects of nerve agents and organophosphate chemicals could not occur. Those working in the field might be asked about experimental designs to look at combined effects that might relate to pesticides to which troops were exposed during the war, if any.

The role of receptor downregulation from sustained subclinical exposures is worth additional research. Some effects of this downregulation may be pathological. The hypothesis that ACh excess and receptor downregulation may produce long-term effects on the brain (Buccafusco et al., 1997; Kaufer et al., 1998) also merits further evaluation, with particular attention to the recovery process and duration of effects. Further documentation of effects from subclinical exposures to military agents and interactions with anticholinesterase pesticides and pretreatments appears to be important. The use of cholinergic drugs acting directly on receptors (e.g., nicotine) will no doubt be pursued, especially by the VA.

The consequences of the Gulf War have brought to light the possibility that humans subject to extreme stress may have alterations in the permeability of their blood-brain barrier, which permits the entry into the brain of molecules normally excluded, as is suspected with PB (Sharabi et al., 1991; Sharma et al., 1991; Friedman et al., 1996). There is also evidence in animals that nerve agents at lower doses may permit the entry of viruses normally excluded by the barrier (Grauer et al., 1996). These phenomena need much more research and documentation in humans.

Regional activity within the brain increases cerebral blood flow to the active region. This in turn may alter the regional distribution to the brain of lipophylic agents and toxins such as nerve agents. The state of cerebral activity at the time of exposure may influence the response to nerve agents and other toxins. This variable might be examined in modeling the effects of agents at lower levels of exposure.

The observation that a variety of cholinergic stimuli (stress, PB, organophosphate pesticides, and nerve agents) can induce the expression of a regulatory gene, c-fos, that is involved in adaptation of the brain to external stimuli appears to be important, but more investigation is needed to understand the duration of the effect and just what end-results occur biochemically and functionally.

Some thought should be given to study in animals of the aggregate effects of stress, PB, organophosphate pesticides, and low-level nerve agent. Longer-term observations, performance effects, and biochemical studies are indicated for these analog exposures, to simulate possible exposures of the Gulf War.

The possibility of a convergent mechanism with a common pathway for several exposures might be kept in mind in epidemiology studies that might develop an aggregate index of exposures of different kinds with which to compare later health effects.

It may well be that nerve agents had nothing to do with illnesses of veterans of the Gulf War, but enough is known—and unknown—about their effects (especially in combination with other factors) not to ignore them.