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# 5

# Sarin

Sarin is a highly toxic nerve agent produced for chemical warfare. It was synthesized in 1937 in Germany in a quest for improved insecticides (Somani, 1992). Although its battlefield potential was soon recognized, Germany refrained during World War II from using its stockpiles. Sarin's first military use did not occur until the Iran–Iraq conflict in the 1980s (Brown and Brix, 1998).

Exposure to sarin can be fatal within minutes to hours. In vapor or liquid form, sarin can be inhaled or absorbed, respectively, across the skin, eyes, or mucous membranes (Stewart and Sullivan, 1992). Because of its extreme potency, sarin is lethal to 50 percent of exposed individuals at doses of 100 to 500 mg across the skin, or 50–100 mg/min/$m^3$ by inhalation (in an individual weighing about 70 kg) (Somani, 1992).

Sarin is a member of a class of chemicals known as organophosphorus esters (or organophosphates). There are about 200 distinct organophosphate insecticides marketed today in thousands of formulations (Klaassen et al., 1996). A few highly toxic members of this large class are chemical warfare agents, but most are insecticides (Table 5.1) (Lotti, 2000). The drug pyridostigmine bromide (PB) is pharmacologically similar to sarin and other organophosphates, but it is a member of a different chemical class, the carbamates (see Chapter 6). Both PB and sarin exert their effects by binding to and inactivating the enzyme acetylcholinesterase (AChE). The binding of sarin to AChE is irreversible, whereas the binding of PB is reversible.

Since AChE is responsible for the breakdown of the neurotransmitter acetylcholine (ACh), the inactivation of this enzyme results in a dramatic elevation of ACh levels at cholinergic synapses (Gunderson et al., 1992). The term "cho-

linergic synapses" refers to sites throughout the body where acetylcholine exerts its actions at the synapse, or junction, between nerve cells or between nerve cells and skeletal muscles. Widespread overstimulation of muscles and nerves induced by excessive levels of acetylcholine is primarily responsible for the acute cholinergic syndrome triggered by exposure to sarin and other organophosphate (OP) nerve agents.

## ACUTE CHOLINERGIC SYNDROME

In humans, exposure to high doses of sarin produces a well-characterized acute cholinergic syndrome featuring a variety of signs and symptoms affecting the peripheral and central nervous systems (Gunderson et al., 1992) (Table 5.2). The peripheral effects are categorized as either muscarinic or nicotinic, in reference to the type of receptor stimulated by acetylcholine. The muscarinic signs and symptoms usually appear first (Lotti, 2000), although the sequence of effects may vary according to the route of sarin's absorption (Stewart and Sullivan, 1992). If the dose of sarin is sufficiently high, death results after convulsions and respiratory failure (Lotti, 2000). Medical management of the acute cholinergic syndrome includes mechanical ventilation and the administration of several medications (anticholinergics, anticonvulsants, and drugs that break the chemical bond between sarin and AChE) (Sidell and Borak, 1992).

The acute health effects of sarin are exquisitely dependent on dose. Because the actual doses to humans under battlefield or terrorist circumstances cannot be measured or are difficult to reconstruct, they can be inferred on the basis of their acute clinical effects. A high level of sarin exposure of humans (after single or multiple exposures) is presumed to have occurred when the acute cholinergic syndrome is manifest. An intermediate-level exposure is presumed to have

**TABLE 5.1** Examples of Organophosphates

| |
|---|
| **Nerve Agents** |
| Sarin (GB) |
| Soman (GD) |
| Tabun (GA) |
| Cyclosarin (GF) |
| *o*-Ethyl-*S*-[2-(diisopropylamino)ethyl]methylphosphonothiolate (VX) |
| **Insecticides** |
| Parathion |
| Malathion |
| Dichlorvos |
| Diazinon |
| Chlorpyrifos |

**TABLE 5.2** Acute Cholinergic Syndrome

| Site of Action | Signs and Symptoms |
|---|---|
| **Muscarinic** | |
| Pupils | Miosis, marked, usually maximal (pinpoint), sometimes unequal |
| Ciliary body | Frontal headache, eye pain on focusing, blurring of vision |
| Nasal mucous membranes | Rhinorrhea, hyperemia |
| Bronchial tree | Chest tightness, prolonged wheezing, dyspnea, chest pain, increased bronchial secretion, cough, cyanosis, pulmonary edema |
| Gastrointestinal | Anorexia, nausea, vomiting, abdominal cramps, epigastric and substernal tightness with heartburn and eructation, diarrhea, tenesmus, involuntary defecation |
| Sweat glands | Increased sweating |
| Salivary glands | Increased salivation |
| Lacrimal glands | Increased lacrimation |
| Heart | Bradycardia |
| Bladder | Frequency, involuntary micturition |
| **Nicotinic** | |
| Striated muscle | Easy fatigue, mild weakness, muscular twitching, fasciculations, cramps, generalized weakness or flaccid paralysis (including muscles of respiration), with dyspnea and cyanosis |
| Sympathetic ganglia | Pallor, transitory elevation of blood pressure followed by hypotension |
| **Central nervous system** | |
| | *Immediate (acute) effects*: generalized weakness, depression of respiratory and circulatory centers with dyspnea, cyanosis, and hypotension; convulsions, loss of consciousness, and coma |
| | *Delayed (chronic) effects*: giddiness, tension, anxiety, jitteriness, restlessness, emotional lability, excessive dreaming, insomnia, nightmares, headaches, tremor, withdrawal and depression, bursts of slow waves of elevated voltage on electrogram, drowsiness, difficulty concentrating, slowness of recall, confusion, slurred speech, ataxia |

SOURCE: Gunderson et al., 1992.

occurred when the acute cholinergic effect is limited to miosis (contraction of the pupil), rhinorrhea (an extreme type of runny nose), and depressed cholinesterase levels in the blood. Finally, low-level exposure may have occurred even though there are no immediately detectable cholinergic signs and symptoms (Brown and Brix, 1998). The health effects of low levels of sarin exposure are of

most interest to Gulf War veterans because of their possible exposure from demolition of Iraqi munitions at Khamisiyah, Iraq (see discussion below).

## POSSIBLE U.S. TROOP EXPOSURE

In March 1991, during the cease-fire period, troops from the U.S. 37th and 307th Engineering Battalion destroyed enemy munitions throughout the occupied areas of southern Iraq (PAC, 1996). The large storage complex at Khamisiyah, Iraq, which contained more than 100 bunkers, was destroyed. Two sites within the complex—one of the bunkers and another site called the "pit"—contained stacks of 122-mm rockets loaded with sarin and cyclosarin (Committee on Veterans' Affairs, 1998). U.S. troops performing demolitions were unaware of the presence of nerve agents because their detectors, which were sensitive only to lethal or near-lethal levels of nerve agents (CDC, 1999), did not sound any alarms before demolition. It was not until October 1991 that inspectors from the United Nations Special Commission (UNSCOM) first confirmed the presence of a mixture of sarin and cyclosarin at Khamisiyah (Committee on Veterans' Affairs, 1998).

At the request of the Presidential Advisory Committee (PAC), the Central Intelligence Agency (CIA) and the Department of Defense (DoD) conducted exposure modeling to determine the extent of exposure of U.S. military personnel to the nerve agents. Since there was no air monitoring at the time of the Khamisiyah demolition, various models were employed to develop estimates of ground level concentrations of sarin and cyclosarin as a function of distance and direction from the detonation sites (PAC, 1996). The CIA–DoD report integrated four different components: (1) UNSCOM reporting and intelligence summaries of the amount, purity, and type of chemical warfare agents stored at Khamisiyah; (2) the results of experiments[1] performed later at Dugway Proving Ground to simulate the demolition at Khamisiyah and thus estimate the amount of sarin and cyclosarin released, the release rate, and the associated type of release (instantaneous, continuous, or fly-out); (3) a combination of dispersion models, which incorporated meteorological conditions at the time (including wind direction), to simulate the transport and diffusion of the plume in order to estimate agent concentrations downwind; and (4) unit location information to determine the position of troops in relation to the plume's path (CIA–DoD, 1997). The result of this modeling effort is a series of geographic maps of the Khamisiyah area that overlays known troop unit locations with the projected path of the sarin–cyclosarin plume. According to the model, the plume includes two levels of potential exposure, the first is "a first-noticeable-effects" level (approximately 1 mg/min/$m^3$), where the estimated exposure was high enough to

[1]These experiments, employing a substitute chemical (triethyl phosphate) to simulate chemical warfare agent, measured agent release concentrations after replicating the rockets in the pit, terrain, original warhead design, stacking of rockets, and other relevant information.

cause watery eyes, runny nose, tightness of chest, muscle twitching or other early signs of chemical warfare (CW) agent exposure; the second is a lower-exposure area where the estimated dosage was less than that needed to produce first noticeable effects (CIA–DoD, 1997). The CIA–DoD report estimated that approximately 10,000 U.S. troops had been located within a 25-km radius of Khamisiyah and thus might have been exposed over a period of hours to the lower exposure level (CIA–DoD, 1997). Uncertainties with the model led to DoD's doubling these figures to 20,000 U.S. troops with possible exposure within a 50-km radius; however, the dose levels remained unaltered.

The CIA–DoD findings were challenged in a U.S. Senate report (Committee on Veterans' Affairs, 1998). The Senate report took issue with the methodology, especially the reconstruction of the pit site, the nature of the demolition, and the number of exposed troops. At the request of the Senate Committee on Veterans' Affairs, the Air Force Technical Applications Center (AFTAC) prepared another exposure model. The AFTAC report summary—the only portion of the report made public—indicates that AFTAC used different models than those employed by CIA–DoD to simulate atmospheric chemistry (Committee on Veterans' Affairs, 1998). The report indicated additional geographic areas of low-level exposure not modeled by CIA–DoD. Neither the AFTAC nor the CIA–DoD report appears to have undergone independent peer review.

DoD is conducting a complete remodeling of the Khamisiyah demolition, which is projected to be completed by the end of 2000. This remodeling, unlike the initial effort, is expected to be peer reviewed. It incorporates improved intelligence information, improved transport and diffusion modeling, and improved knowledge of unit locations. The committee encourages DoD to complete its ongoing remodeling efforts and to publish results in the peer-reviewed literature to enable broad review and independent validation of its work.

Although exposure to sarin and cyclosarin was estimated by CIA–DoD modeling, there were no medical reports by the U.S. Army Medical Corps at the time of the release that were consistent with signs and symptoms of acute exposure to sarin (PAC, 1996). Further, a 1997 survey mailed by DoD to 20,000 troops who were within a 50-km radius of Khamisiyah found that more than 99 percent of respondents ($n$ = 7,400) reported no acute cholinergic effects (CIA–DoD, 1997). Nevertheless, low-level exposure, as noted earlier, could have occurred without producing acute cholinergic effects.

Two other storage sites in central Iraq sustained damage from air attacks during the Gulf War, but chemical agent releases were too far removed from U.S. troops for exposure to have occurred (PAC, 1996). At one site (Muhammadiyat), munitions with 2.9 metric tons of sarin–cyclosarin and 1.5 metric tons of mustard gas were damaged. At the other site (Al Muthanna), munitions containing 16.8 metric tons of sarin–cyclosarin were damaged (PAC, 1996). Atmospheric modeling by the CIA and DoD determined that the nearest U.S. personnel—located 400 km away—were outside the range of contamination (PAC, 1996).

In summary, exposure models indicate that sarin–cyclosarin release occurred in March 1991 as a result of U.S. demolition of a storage depot in

Khamisiyah, Iraq. The degree of exposure of U.S. troops located within the path of a sarin–cyclosarin plume, which is being remodeled in an upcoming DoD study, is at this point presumed to be low on the basis of previous exposure modeling and in the absence of medical personnel or veterans' reporting symptoms of an acute cholinergic syndrome.

The remainder of this chapter examines the scientific literature on the adverse health effects of sarin. It begins with a discussion of the toxicology of sarin and its effects on animals. It then summarizes the modest number of published toxicology studies on cyclosarin. The chapter next proceeds to its major focus, the health effects of sarin in humans. Most, if not all, toxicological and epidemiological studies focused on the health effects of sarin, as opposed to sarin in combination with other agents.

## SARIN TOXICOLOGY

Sarin (GB; *o*-isopropyl methylphosphonofluoridate) is an organophosphate ester with high potency as an anticholinesterase nerve agent. It is a clear, colorless liquid with a molecular weight of 140.11, a boiling point of 158°C, and a vapor pressure of 1.48–2.9 mm Hg at 25°C (making it highly volatile). Sarin presents a liquid and a vapor hazard. In the liquid state, sarin can rapidly penetrate skin (as well as clothing), and in the vapor state it can contact the eye directly or be inhaled into the lungs, whereupon it is rapidly absorbed (Spencer et al., 2000). Exposure of the eye to vapor, which produces pinpoint pupils (miosis) and blurring of vision, accounts for one of the earliest signs of sarin exposure (Gunderson et al., 1992; Stewart and Sullivan, 1992).

### Mechanisms of Acute Toxicity

*Inhibition of Acetylcholinesterase*

There is widespread agreement that the principal mechanism of toxicity after sarin exposure is by inhibition of acetylcholinesterase and consequent rise in ACh, leading to overstimulation at cholinergic synapses (Somani, 1992; Lotti, 2000; Spencer et al., 2000). These effects are dose related. The degree of inhibition of AChE in the mouse brain depends directly on the administered intravenous (i.v.) dose of sarin (Tripathi and Dewey, 1989). High doses of sarin (100 µg/kg) administered subcutaneously to rats produce a 32 percent increase in ACh levels (Flynn and Wecker, 1986).

Sarin inhibits AChE by phosphorylating a serine hydroxyl on the ester portion of the active site of this enzyme.[2] The phosphorylated enzyme is hydrolyzed very slowly, with a half-life of reactivation of hours to days (Gray, 1984). The

[2]During its normal function, AChE hydrolyzes acetylcholine to produce choline, acetic acid, and the reactivated enzyme. The reactivated enzyme is available to bind to another acetylcholine molecule. AChE has one of the fastest turnover rates known.

phosphorylated enzyme then can undergo a second process, called aging, by loss of an alkyl group (dealkylation). The half-life for "aging" is about 5 hours after sarin exposure (Sidell and Borak, 1992). Only during this period prior to aging can treatment with oxime therapy (e.g., pralidoxime chloride) successfully remove sarin from the enzyme and thus block the aging process. After aging has occurred, the phosphorylated enzyme (now negatively charged) is resistant to cleavage or hydrolysis and can be considered irreversibly inhibited. Recovery of AChE function occurs only with synthesis of new enzyme. Inhibition of AChE prevents the breakdown of acetylcholine, which accumulates in central and peripheral nerve synapses, leading to the acute cholinergic syndrome.

Sarin also may exert its effects through other cholinergic mechanisms (unrelated to inhibition of AChE). A new line of research suggests that sarin (in picomolar concentrations) may interact directly with muscarinic ACh receptors (Rocha et al., 1998; Chebabo et al., 1999). Researchers uncovered this new mechanism by studying sarin's ability to reduce evoked GABA (gamma-aminobutyric acid) release from hippocampal neurons. This effect of sarin is blocked by the muscarinic receptor antagonist atropine, but not by nicotinic receptor antagonists (Rocha et al., 1998). These findings suggest that sarin may interact with presynaptic muscarinic receptors, thereby reducing action potential-dependent release of GABA in the postsynaptic neuron (Chebabo et al., 1999). It is reasonable to consider that sarin acts as a muscarinic receptor antagonist inhibiting the evoked release of GABA. Reductions in the levels of GABA, which is an inhibitory neurotransmitter, may contribute to the convulsive properties of sarin.

### *Noncholinergic Mechanisms*

For decades, researchers observed puzzling relationships between the extent of neurobehavioral toxicity and the degree of inhibition of AChE. For example, only sarin-induced tremor has a slight correlation with AChE inhibition in rat striatum, whereas chewing, hind-limb abduction, and convulsions have no clear correlation (Hoskins et al., 1986). Some sarin-treated rats with 90 percent inhibition of AChE in the striatum of the brain had no convulsions or hind-limb abduction, while rats with less enzyme inhibition exhibited both. From these findings, researchers have concluded that noncholinergic mechanisms may also contribute to toxicity induced by sarin and other organophosphates. The difficulty has been in disentangling which effects are mediated directly by sarin and which are secondary to its inhibition of AChE.

Several studies suggest that sarin may alter the level of neurotransmitters other than ACh. In most of these studies, however, the neurotransmitter effects are seen in brain regions where there are cholinergic synapses. Significant increases in catecholamines, measured histochemically, were found in the substantia nigra pars compacta and locus coeruleus of the brain following intramus-

cular (i.m.) injection of sarin at one-third of the median lethal dose ($LD_{50}$)[3] (Dasheiff et al., 1977). Catecholamine levels in the nucleus accumbens decreased. All changes, except for the latter, returned to normal within 10 days. It is not clear whether these changes represented the direct action of sarin on enzymes related to noncholinergic neurotransmission or were secondary to the production of excessive ACh (Somani, 1992). Alternatively, stress could activate catecholamine neurons.

Levels of the neurotransmitter serotonin 5-hydroxytryptamine (5-HT) were decreased, and its major metabolite (5-hydroxyindoleacetic acid, or 5-HIAA) increased, in rat striatum after subconvulsive doses of sarin. Since this effect was also seen after administration of the OP nerve agents soman and tabun, it most likely is not agent specific, but rather is a likely consequence of an acute increase of acetylcholine in the striatum (Fernando et al., 1984).

Neuropathological damage in the hippocampus, dorsal thalamus, and piriform cortex was found in about 70 percent of rats within 24 hours of administering a single dose of sarin (95 µg/kg, i.m., or 1 $LD_{50}$ (Kadar et al., 1995). These animals had prolonged convulsions, whereas the other 30 percent with short convulsive episodes had minimal brain damage. The authors interpreted these results to mean that convulsions may have caused the severe hypoxic damage. The neuropathology in the most affected animals continued to increase for 3 months, involving brain regions previously unaffected. The study attributed the progressive, long-term neuropathology either to delayed neurotoxicity of sarin or to secondary retrograde degeneration. It did not directly investigate potential neurochemical mechanisms underlying the neuropathology.

## Toxicokinetics

This section discusses the absorption, distribution, metabolism, and elimination of sarin. In general, these events occur very rapidly after exposure, although there is some variability depending on the route of administration and the species studied (Somani, 1992). Most of the research reported here comes from animal studies, but where possible, human toxicokinetic studies are also reported.

### *Absorption and Metabolism*

Sarin in vapor or liquid form is absorbed rapidly to produce local and systemic effects. Local effects, such as those on the eyes (e.g., miosis) and nose, are the product of sarin vapors directly interacting with AChE at the nerve endings near body surfaces (Sidell and Borak, 1992). Systemic effects, including those within the central nervous system (CNS), occur as a result of absorption of sarin into the circulation from the skin, respiratory tract, or gastrointestinal tract (Lotti, 2000).

[3] $LD_{50}$ is the lethal dose to half or 50 percent of the test subjects.

The fate of sarin in the blood is a major determinant of how much sarin reaches the central nervous system and other sites of systemic toxicity. In the blood, sarin first interacts with several esterases (a class of enzymes). Some of the esterases, such as paraoxonase, hydrolyze sarin to inactive metabolites (Davies et al., 1996; Lotti, 2000). Two other blood esterases—AChE and butyrylcholinesterase (BuChE)—irreversibly bind to sarin. AChE found on the surface of red blood cells (RBCs), although chemically indistinguishable from AChE in the nervous system, has unknown physiological functions (Sidell and Borak, 1992). These esterases in the blood are often described as "false targets"—by binding irreversibly to sarin, AChE and BuChE sequester sarin in the blood, thereby preventing some or all from reaching the CNS (Spencer et al., 2000). However, esterases in the blood can be overwhelmed by high doses of sarin. The acute cholinergic syndrome occurs when RBC AChE is inhibited by 75–80 percent (Sidell and Borak, 1992).

### *Distribution and Elimination*

The tissue distribution of sarin and its metabolites has been studied in rodents. In one study a single sublethal dose (80 µg/kg) of radiolabeled sarin was administered intravenously, after which tissues were examined at distinct points in time for 24 hours (Little et al., 1986). Within 1 minute, sarin was distributed to the brain (and thus crossed the blood–brain barrier), lungs, heart, diaphragm, kidneys, liver, and plasma, with the greatest concentrations found in the last three tissues. Thereafter, the concentrations in all tissues declined. Within 15 minutes, sarin concentrations declined by 85 percent, followed by a second, more gradual decline. Relatedly, within the first minute, about half of the labeled sarin was associated with the major sarin metabolite isopropyl methylphosphonic acid (IMPA). A nonextractable label was present in constant amounts in all tissues, except plasma, throughout the time course of the experiment.

The kidneys are the major route of elimination of sarin or its metabolites. In the above study, Little and colleagues (1986) determined that kidneys contained the highest concentrations of sarin and its metabolites, whereas much lower concentrations of metabolite were detected in the liver. This suggests a minor role for the liver in detoxification of sarin. Shih and colleagues (1994) injected rats subcutaneously with a single dose of 75 µg/kg of sarin. They then measured excretion of the hydrolyzed metabolites, the alkylmethylphosphonic acids, which include IMPA and other methylphosphonic acids. Urinary elimination was found to be quite rapid; the terminal elimination half-life of sarin metabolites in urine was 3.7 ± 0.1 hours. Nearly all of the administered dose of sarin was retrieved from the urine in metabolite form after 2 days.

Distribution, metabolism, and elimination of sarin in humans appear to resemble findings in animals. Minami and colleagues (1997) detected the sarin metabolite IMPA in urine of humans after the terrorist attack on the Tokyo subway system (see later description). They found peak levels of IMPA or methylphosphonic acid in urine 10–18 hours after exposure but did not report meta-

bolic rates. The levels of IMPA in urine correlated with the degree of clinical symptoms. They also found evidence of distribution of sarin to the human brain in 4 of the 12 people who died after exposure. Solubilized sarin-bound AChE from formalin-fixed cerebellar tissue of victims of the Tokyo attack contained a derivative of the sarin hydrolysis product methylphosphonic acid (MPA) (Matsuda et al., 1998). The estimated amounts of MPA ranged from 0.32 to 1.13 nmol/g tissue. Although no IMPA was found, it was assumed that IMPA had hydrolyzed to MPA in the formalin solution over 2 years of storage.

*Biomarkers of Exposure*

Biomarkers of acute sarin exposure can be detected in blood or urine. In blood, the extent of inhibition of RBC AChE is considered the best marker of acute exposure. Sarin preferentially inhibits RBC AChE more than BuChE; however, after high-level sarin exposure, complete inhibition of both esterases occurs (Sidell and Borak, 1992). Since inhibition of blood cholinesterases is a common feature of organophosphates and other anticholinesterases, this biomarker is not specific to sarin exposure. Further, its utility as a biomarker is limited to a short time after exposure, with a return to original blood esterase levels by about 1–3 months (Grob, 1963). The recovery times for blood esterases are somewhat different. BuChE is replaced after about 50 days following de novo synthesis in the liver. RBC AChE recovery is contingent upon the turnover rate of red blood cells, which is about 1 percent per day. This esterase is synthesized with the RBC (Sidell and Borak, 1992). Sensitive methods for detecting urinary metabolites as biomarkers of sarin exposure were recently developed by Japanese researchers in the aftermath of the Tokyo terrorism incident (Minami et al., 1997, 1998).

Black and colleagues (1999) recently found a sensitive biomarker that can specifically identify sarin at low concentrations in human plasma. The researchers found a novel phosphonylation site, presumably from human serum albumin, at which sarin interacts with a tyrosine residue. In contrast, the biomarkers noted above are indices of sarin exposure but do not uniquely identify sarin as opposed to other CW agents. The advantage of this potentially new method is that it can directly implicate sarin at low concentrations.

## Animal Studies

This section summarizes the toxic effects of sarin in laboratory animals. Most animal studies of sarin did not examine low-level exposure, but instead focused on lethal, near-lethal, or maximum tolerated doses (MTDs).[4] These high doses produced the acute cholinergic syndrome and in many cases necessitated

[4]The MTD is the highest dose used during a long-term study that will not alter the life span of the animal and slightly suppresses body weight gain (i.e., 10 percent) in a 90-day subchronic study.

pharmacological intervention to prevent death. Although these studies enable researchers to deduce with some certainty what organ systems will not be affected by low levels of sarin (i.e., those systems that are not affected by large doses), they are not useful in distinguishing between primary damage caused by sarin and secondary damage caused by hypoxic events following convulsions.

### *Acute Toxicity*

In animals, sarin is acutely toxic and fatal in microgram quantities in a matter of minutes. There is some variability depending on the species and the route of administration. Table 5.3 outlines the doses and routes of administration that produce acute lethality (within 24 hours) in the animal species tested. The $LD_{50}$ in the rat and mouse are similar, with subcutaneous (s.c.), intramuscular, and intravenous doses requiring 150–180 µg/kg. Oral administration requires nearly 10 times more sarin. The hen, guinea pig, and cat are more sensitive than rats and mice, with lethal doses ranging from 16–40 µg/kg s.c. to 561 µg/kg oral.

The immediate cause of death from sarin poisoning is respiratory arrest (Rickett et al., 1986). In baboons, sarin administered to the upper airway in vapor form (30 µg/kg) causes apnea within 5 minutes (Anzueto et al., 1990). Since the dose was twice the $LD_{50}$, mechanical ventilation was needed to keep the animals alive. Their apnea was correlated with the absence of activity in the phrenic nerve (which projects to the diaphragm), suggesting a central effect of sarin on respiration. Respiration recovered spontaneously within 1–2 days, al-

**TABLE 5.3** Acute Lethality of Sarin Administered to Various Species

| Species, Strain | Route | $LD_{50}$ (µg/kg) | Reference |
|---|---|---|---|
| Rat | s.c. | 158–165 | Landauer and Romano, 1984; Singer et al., 1987; Somani, 1992 |
| Mouse, CD-1 | s.c. | 160–170 | Clement, 1991 |
| Mouse | i.m. | 179 | Somani, 1992 |
| Mouse | i.v. | 109 | Little et al., 1986; Tripathy and Dewey, 1989 |
| Mouse, Swiss albino | inhalation | 600 mg/min/$m^3$ | Husain et al., 1993 |
| Hen | oral | 561 | Bucci et al., 1993 |
| Hen | s.c. | 16.5–16.7[a] | Gordon et al., 1983 |
| Guinea pig | s.c. | 53 (divided doses) | Fonnum and Sterri, 1981; Somani, 1992 |
| Cat | s.c. | 30–35 | Goldstein et al., 1987 |

NOTE: i.m. = intramuscular; i.v. = intravenous; s.c. = subcutaneous.

[a]Converted from 0.119 µmol/kg in Ross white or Light Sussex hens.

though AChE activity was still significantly inhibited. In the cat, an infused dose of 0.56 $LD_{50}$ caused respiratory arrest, while neuromuscular blockade required a dose in excess of five times the $LD_{50}$ (Rickett et al., 1986). The diaphragm was still responsive to electrical stimulation at doses that inhibited respiratory nerve activity. The cells first affected were respiratory-related neurons in the medulla, and their inhibition preceded phrenic nerve inhibition. Therefore, the cause of death after sarin exposure is rapid inhibition of respiratory centers in the medulla followed by inhibition of phrenic nerve activity, which causes respiration to cease. The diaphragm muscle is paralyzed last.

*Neurotoxicity*

**Short- and long-term neurobehavioral toxicity.** Sarin's short-term behavioral effects are dose dependent. In several studies of rodents, behavior was assessed by flavor aversion, spontaneous motor activity, and motor coordination. Following subcutaneous administration of 61–115 µg/kg, sarin led to conditioned flavor aversion at doses greater than 70 µg/kg. Motor coordination, as measured by rotarod performance, was decreased at 98 µg/kg, but not at lower doses (Landauer and Romano, 1984). This study also found an increase in spontaneous locomotion at 61 µg/kg and a decrease at higher doses (measured only within 10 minutes of sarin administration). Nieminen and colleagues (1990) studied rats given intraperitoneal doses of 12.5 and 50 µg/kg, neither of which was sufficient to produce acute toxicity. By monitoring locomotor activity up to 72 hours, they found a decrease in rodent locomotion only with the highest dose until 6 hours of administration, after which time there was no difference from controls. In separate behavioral tests, they also found the highest dose of sarin to decrease certain behaviors (e.g., grooming) at 40–50 minutes after injection (Nieminen et al., 1990).

Short-term behavioral effects also have been examined in marmosets, a nonhuman primate. Doses at 33 to 55 percent of the $LD_{50}$ disrupted the performance of animals' food-reinforced visually guided reaching response. Performance returned to normal by 24 hours after sarin administration (D'Mello and Duffy, 1985). The only other studies of short-term behavioral consequences of low-dose exposures in nonhuman primates were carried out with soman, an organophosphate nerve agent that also inhibits AChE. Hartgraves and Murphy (1992) studied the effects of different dosing regimens—which did not produce signs of acute toxicity—on equilibrium performance, as measured on the primate equilibrium platform (PEP). This device requires the animal to manipulate a joystick in order to keep a rotating platform as level as possible. After administration, doses of soman, less than 2.0 µg/kg did not induce decrements in PEP performance, while doses greater than 2.75 µg/kg did induce decrements. Decrements were measured for 5 days after soman administration but later returned to normal. These findings, although not from sarin, are reported here because vestibular dysfunction has been reported as a long-term effect in humans after sarin exposure (see next section).

Long-term changes in the electroencephalogram (EEG) of rhesus monkeys occur after a single high dose of sarin (5 µg/kg, $n = 3$) or a series of 10 small doses (1 µg/kg per week, i.m., $n = 3$) (Burchfiel et al., 1976; Burchfiel and Duffy, 1982). The high dose was sufficient to produce an acute cholinergic syndrome, whereas each small dose produced few, if any, signs of acute poisoning. Animals given the large dose were pretreated with gallamine triethiodide and artificially respired to preclude the possibility of anoxic brain damage. At 24 hours after the single large dose or after the final small dose, there were significant increases in high-frequency beta activity (13–50 Hz) in the temporal lobe compared with the monkey's own pre-exposure EEGs. The increase in beta activity persisted for 1 year after sarin administration, although it did not appear to have any behavioral or psychological significance. Control animals ($n = 6$) did not exhibit any significant changes in EEG. The second component of this study, in which the same EEG change was found in humans after accidental occupational exposure to sarin, is reported later in this chapter.

A subsequent study in marmosets ($n = 17$) examined the long-term effects of a single low dose (3.0 µg/kg) of sarin on EEG and cognitive behavior (Pearce et al., 1999). In comparison with controls, which received saline injection, the sarin-dosed group experienced a 36–67 percent inhibition of RBC AChE within 3 hours. From then until 12–15 months later, no significant changes in EEG were detected, but the increase in the beta 2 amplitude (22–40 Hz) approached significance ($p = .07$). The dose did not produce a decrement in touchscreen-mediated discrimination tasks, which are indices of cognitive functioning. Pearce and colleagues attributed the discrepancy between their EEG findings and those of Burchfiel and Duffy (1982) to methodological differences. The more recent study did not use anesthesia or restraints immediately before monitoring animals' EEG.

**Delayed neurotoxicity.** Exposure to some, but not all, organophosphates produces a delayed neurotoxic syndrome known as organophosphate-induced delayed neuropathy (OPIDN) (Somani, 1992; Moore, 1998; Lotti, 2000). OPIDN is a progressive neuropathy that becomes manifest approximately 1–4 weeks after an acute exposure to some organophosphates; motor symptoms of ataxia and flaccid paralysis of the lower extremities are exhibited. Symptoms persist for up to a year and may be permanent in severe cases (De Bleecker et al., 1992). Research conducted in the 1970s determined that OPIDN results from the chemical interaction between certain organophosphates and an enzyme known as neuropathy target esterase (NTE), whose normal function in blood and other tissues is unknown. After the organophosphate covalently binds to NTE, the complex undergoes a further reaction known as aging through dealkylation of the bonded ester or amide. NTE activity in the brain typically must be decreased by 70 percent before eventual manifestation of symptoms. That different OPs produce different degrees of inhibition of NTE explains some of their variability in triggering delayed neurotoxicity. OPIDN is associated with histopathological evidence of axonal degeneration of peripheral nerves and spinal

cord. It is also associated with slightly reduced nerve conduction velocities. The specific pathophysiological steps giving rise to delayed manifestation of symptoms are not well understood (Somani, 1992; Lotti, 2000; Spencer et al., 2000; see Chapter 6 also).

In some animal models, massive doses of sarin can cause delayed neurotoxicity, which becomes manifest by ataxia and paralysis appearing days to weeks after a single high exposure or multiple lower exposures (Somani, 1992; Lotti, 2000; Spencer et al., 2000). The doses of most OPs capable of producing these neurotoxic effects in experimental animals are typically higher than the lethal dose. Therefore, to study delayed neurotoxicity, most species must be protected from death through pharmacological and other interventions collectively referred to as "protection."

This line of research in animals is an outgrowth of historical episodes (dating back to the 1880s) of human poisoning by organophosphates. The most dramatic episode occurred in the 1930s when 20,000–40,000 people developed a delayed neurotoxicity 10–14 days after drinking an illicit alcoholic beverage containing an organophosphate contaminant (TOCP, or tri-*o*-cresyl phosphate) (De Bleecker et al., 1992).

Table 5.4 summarizes findings from animal studies of OPIDN or other forms of delayed neurotoxicity after administration of sarin. The findings are based on abnormal behaviors exhibited by the study animal. The development of delayed neurotoxicity is dependent on the animal species (e.g., hen is the species of choice because of its sensitivity to sarin), dose, route of administration, number of doses, and protection used.

In several studies, sarin did not produce delayed neurotoxicity. The negative findings in hens were attributed by Crowell and colleagues (1989) to sarin's inability to significantly inhibit brain NTE at nonlethal doses. Sarin did produce delayed neurotoxicity in six studies. In four of them, the doses were either at the lethal level or at least 30 times higher than the lethal level (Davies et al., 1960; Davies and Holland, 1972; Willems et al., 1983), or about 30–60 times the $LD_{50}$ (Gordon et al., 1983). Animals displayed severe signs of acute cholinergic toxicity but were protected from death by administration of atropine and other agents. From these studies, most investigators concluded that sarin was unlikely to produce delayed neurotoxicity at sublethal doses.

In two more recent studies, however, sublethal doses were administered. Husain and colleagues (1993) administered sarin by inhalation (5 $mg/m^3$ for 20 minutes, daily for 10 days) to Swiss albino mice ($n = 6$). In this strain, the $LD_{50}$ of sarin was 600 $mg/min/m^3$ (Husain et al., 1993). By the fourteenth day after the beginning of the study, animals developed muscular weakness of the limbs and slight ataxia. Significant inhibition of NTE was found in the brain (59 percent), spinal cord (47 percent), and platelets (55 percent), and the spinal cord exhibited pathological evidence of focal axonal degeneration. Both biochemical and morphological changes were more severe in animals ($n = 6$) exposed to the positive OP control compound mipafox (2.5 mg/kg, s.c., daily for 10 days; Husain et al., 1993). None of the changes was detected in negative control ani-

mals ($n$ = 8) exposed to fresh air in an exposure chamber. At no time did sarin-exposed animals show signs of cholinergic toxicity, although AChE activity was inhibited by 27 percent (blood) and 19 percent (brain). A subsequent study in white leghorn hens (*Gallus domesticus*, $n$ = 5) given subcutaneous doses of sarin (50 µg/kg, daily for 10 days) found moderate ataxia on the fourteenth day (Husain et al., 1995). The dose is reported to be one-tenth of the $LD_{50}$ (Husain et al., 1995). NTE activity was inhibited in brain (53 percent), spinal cord (38 percent), and platelets (54 percent). Sarin caused moderate axonal degeneration and axonal swelling, while the effects of mipafox ($n$ = 6) were much more severe (Husain et al., 1995). Platelet acetylcholinesterase activity was inhibited by 72 percent, but no indication is provided on whether cholinergic symptoms were observed. In summary, the findings of the studies reviewed indicate evidence that sarin can cause OPIDN in some animal species, particularly at doses that produce otherwise lethal effects.

### *Genotoxicity*

In a comprehensive study of the genotoxicity of sarin, no mutagenesis, chromosomal damage, unscheduled DNA synthesis, or sister chromatid exchange was found. In vitro doses of sarin ranging from 0.2 to 200 µg/ml and in vivo exposures in rats at 360 µg/kg did not produce toxicity in any gene toxicity assays performed (Goldman et al., 1988). Klein and colleagues (1987) measured unscheduled DNA repair and synthesis in rat hepatocytes exposed to sarin. No increase in DNA synthesis was observed, but a decrease in repair synthesis was seen after administration of two different formulations of sarin ($3.0 \times 10^{-4}$–$2.4 \times 10^{-3}$ moles [M] sarin, with different stabilizers). This study did not control for the stabilizers, and variability between experiments casts doubt on these results.

### *Sub-Chronic Toxicity*

A standard subchronic (90-day) toxicology study of sarin was performed at the National Center for Toxicological Research (Bucci and Parker, 1992; Bucci et al., 1992). Rats were administered sarin in two formulations (type I with tributylamine stabilizer and type II stabilized with diisopropylcarbodiimide) at three different doses: a maximum tolerated dose, MTD/2, and MTD/4 (corresponding to 300, 150, and 75 µg/kg per day, given by gavage). Both formulations produced profound inhibition of acetylcholinesterase and some deaths. No neoplastic lesions were detected after sarin (type I), but nonneoplastic lesions (necrosis in the cerebrum, related to hypoxia) were detected and were thought to be the cause of death in 3 of 36 female rats (1 at 75 µg/kg, 2 at 300 µg/kg.). Sarin (type II) was associated with one neoplastic lesion, a lymphoma, in one male in the high-dose group ($n$ = 12). No studies have been conducted to catalog the effects of chronic exposure to sarin.

**TABLE 5.4** Delayed Neurotoxicity of Sarin

| Species | Dose (μg/kg) | Route of Administration | Frequency and/or Duration | Protection | Neurobehavioral Outcomes | Reference |
|---|---|---|---|---|---|---|
| Chicken | 25 (1 $LD_{50}$) | i.m. | 1×/day for 26–28 days | Atropine, P2S, PAD | 5/8 slight ataxia | Davies and Holland, 1972 |
| Hen | 500–2,500 | i.m. | 1×/day for 5 days (20% of total dose given) | Atropine, P2S | 9/28 ataxia at minimum dose of 1000 μg/kg[a] | Davies et al., 1960 |
| Hen | 252 | s.c. | 1× | Physostigmine, atropine, P2S | 0/4 ataxia | Gordon et al., 1983 |
| | 504–1,962 | s.c. | 1× | | 12/12 ataxia to paralysis | |
| Chicken | 70.2–281 | Gavage | 1× | Atropine | None | Bucci et al., 1993 |
| | 23–94 | Gavage | 1×/week for 3 weeks | Atropine | None | |
| Hen | 50 (1/10 $LD_{50}$) | s.c. | 1×/day for 10 days | None | Moderate ataxia[b] | Husain et al., 1995 |
| Hen | 600 | i.m. | 1×/day for 2 days | Atropine, Physo-stigmine, P2S | 0/4 DN | Willems et al., 1983 |
| | 900 | | 1×/day for 3 days | | 1/3 DN | |
| | 1,500 | | 1×/day for 5 days | | 8/9 DN | |
| | 900 | | 1×/day for 1 day | | 3/4 DN | |
| | 1,200 | | 1×/day for 1 day | | 4/4 DN | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Rat | 75–300 | Gavage | 5×/week for 13 weeks | NA | None | Bucci and Parker, 1992; Bucci et al., 1992 |
| Mouse | 5 mg/m$^3$ | Inhalation | 20 min for 10 days | None | Slight ataxia[c] | Husain et al., 1993 |
| Cat | 1,000 | s.c. | 1× | Physostigmine and atropine | None | Goldstein et al., 1987 |
| | 3.5 | s.c. | 1×/day for 10 days | None | None[d] | |
| | 7 | s.c. | 1×/day for 5 days | None | None[d] | |

NOTE: DN = delayed neuropathy; i.m. = intramuscular; i.p. = intraperitoneal; i.v. = intravenous; s.c. = subcutaneous; NA = not available; PAD = dodecyl iodide salt of P2S; 2-PAM = pralidoxime chloride; P2S = pralidoxime mesylate, 2-hydroxyiminomethyl-*N*-methylpyridinium methyl methanesulfonate.

[a]No hens were ataxic at 500 µg/kg. Figures not provided for doses higher than 1,000 µg/kg.
[b]Study does not report how many of five dosed animals developed moderate ataxia.
[c]Study does not report how many of six dosed animals developed slight ataxia.
[d]No behavioral signs of neurotoxicity, but sarin decreased conduction velocity of muscle spindle afferents and altered the frequency response of primary and secondary nerve endings.

*Reproductive or Developmental Toxicity*

Sarin appears to produce no reproductive effects in rats, rabbits, or dogs. Pregnant female rats were administered sarin (100, 240, and 380 µg/kg per day) by gavage on gestational day (gd) 6–15 and were sacrificed on gd 20. There was no evidence of developmental toxicity related to any dose or formulation of sarin, even at doses that produced maternal toxicity and 28 percent mortality in the high dose group (LaBorde et al., 1996).

Pregnant female rabbits (New Zealand White) were studied in a similar fashion, receiving sarin on gd 6-19 and sacrificed on gd 29. None of the groups had any evidence of developmental toxicity at doses that produced maternal toxicity and 25 percent mortality in the high-dose group (LaBorde et al., 1996). Male dogs exposed to sarin vapor concentration of 10 mg/min/$m^3$ for 6 months successfully mated and produced normal litters (Jacobson et al., 1959).

## CYCLOSARIN TOXICOLOGY

Cyclosarin (cyclohexyl methylphosphonofluoridate) also belongs to the organophosphate group of nerve agents. Like other OPs, cyclosarin exerts its toxic effects by inhibition of AChE. This section reports on the limited number of toxicological studies of cyclosarin, whereas a later section reports on a study of military volunteers exposed to anticholinesterase nerve agents, including sarin and cyclosarin.

Cyclosarin produces maximal inhibition of AChE in less than 1 minute, with inhibition rate constants of 7.4 and $3.8 \times 10^8$ $M^{-1}$ $min^{-1}$ for AChE and BuChE, respectively (Worek et al., 1998). The aging half-life of the cyclosarin–esterase complex is 8.7 hours for AChE and 2.2 hours for BuChE (Worek et al., 1998).

The $LD_{50}$ for cyclosarin in mice is estimated at 243 µg/kg by subcutaneous administration (Clement, 1992). In comparison, sarin's $LD_{50}$ in this same study was somewhat lower (170 µg/kg). In protection studies, a $3LD_{50}$ dose was used, and pralidoxime chloride (2-PAM) was found to be ineffective against cyclosarin, but the antidotes toxogonin and HI-6 are effective at higher doses than were necessary to protect against sarin.[5] The author correlated the rapid recovery of HI-6-treated mice with a 67 percent reactivation of AChE 30 minutes after cyclosarin administration.

The $LD_{50}$ for an intramuscular dose of cyclosarin in rhesus monkeys was 46.6 µg/kg (Koplovitz et al., 1992). Animals dosed with 30–75.4 µg/kg became unconscious within 2 minutes of administration. Those that survived were able to sit in their cages by 5–12 hours, and clinical signs disappeared by 12–24 hours. The primary pathological findings in most of the animals that died soon after exposure were neuronal degeneration or necrosis of the brain and spinal cord and spinal cord hemorrhage. The most affected brain regions were the

[5]HI-6 = 1-[[[4-(aminocarbonyl)pyridinio]methoxy]methyl]-2-[(hydroxyimino)methyl]-pyridinium dichloride monohydrate.

frontal and entorhinal cortex, amygdala and caudate nuclei, hippocampus, and thalamus—regions frequently affected by organophosphate poisoning. Cardiomyopathy and skeletal muscle lesions were the primary nonneural lesions.

This study also compared the efficacy of pretreatment with pyridostigmine and treatment with atropine and either 2-PAM or HI-6 given immediately after cyclosarin administration. All animals survived lethal doses of cyclosarin regardless of the oxime they received, and all were clinically normal 24 hours after dosing. Minimal nervous system lesions were observed in these animals. Cardiomyopathy and skeletal muscle lesions were apparent in about a third of protected animals.

In a subsequent study using an identical protection paradigm in rhesus monkeys, Young and Koplovitz (1995) examined biochemical and hematological parameters. They found elevated creatine kinase, lactate dehydrogenase, aspartate and alanine transaminases, and potassium ion in both oxime treatment groups 2 days after cyclosarin poisoning. The elevated biochemical markers are indications of striated muscle damage. The blood values returned to normal at 7 days. The RBC count, hemoglobin, hematocrit, and serum protein and albumin were significantly decreased at 7 days.

## SUMMARY OF TOXICOLOGY

Sarin is toxic to animals in a dose-dependent manner. Animals exposed to high doses display the same acute cholinergic syndrome as displayed by humans. The main mechanism of toxicity is through inhibition of AChE. Sarin is readily and rapidly absorbed into the circulation where it is hydrolyzed or bound to blood esterases. Sarin that is not inactivated in the blood quickly distributes to the brain and other tissues where it inhibits AChE. Massive acute doses of sarin, through the inhibition of NTE, can induce delayed neurotoxicity in some, but not all, animal species. Lower doses over longer periods may also exert this effect, but more research is needed to substantiate these findings. Long-term alterations in the EEG of nonhuman primates were found after sarin administration at high doses, as well as at doses that did not produce acute signs of toxicity. The clinical significance of the EEG changes is unclear. There is no evidence of genotoxicity or reproductive or developmental toxicity. The toxicology of cyclosarin appears to be similar to that of sarin, but few studies have been reported. There are no studies of the long-term or delayed effects of toxic interactions between sarin–cyclosarin and pyridostigmine.

## HUMAN STUDIES

This section reviews studies of sarin's acute and long-term health effects on humans. Four human populations have been studied following exposure to sarin: military volunteers who were exposed several decades ago to nonlethal doses of sarin and other chemical warfare agents (NRC, 1982, 1985); industrial workers

with documented acute exposure to sarin (Duffy et al., 1979); and victims of the sarin terrorist attacks in Matsumoto City in 1994 and Tokyo in 1995 (Morita et al., 1995; Okumura et al., 1996). Other studies on military volunteers have been summarized (Marrs et al., 1996) but have not been published; thus, the latter studies were not considered by the committee in reaching its conclusions.

Given the extreme dose dependence of sarin's *acute* health effects—which are literally a matter of life and death—a key question is, Do nonlethal doses of sarin have *long-term* health effects and, if so, are they too dose dependent? The possibility of low-level sarin exposure of U.S. troops during the Gulf War has generated much interest in whether sarin has *long-term* effects after a relatively short exposure at levels that are insufficient to produce an acute cholinergic syndrome.

A major limitation of most human studies of either long- or short-term health effects is the inability to document actual exposure levels. Most studies of sarin were undertaken in the aftermath of occupational accidents or terrorist attacks. In such cases, the exposure levels were inferred from clinical effects. As explained earlier, high-level exposure is inferred from the acute cholinergic syndrome (see Table 5.2) with outcomes including miosis, rhinorrhea, apnea, convulsions, and possibly death. High-level exposure requires hospitalization or emergency treatment. Intermediate-level exposure is inferred from minimal or threshold cholinergic effects such as miosis or rhinorrhea and limited decline in cholinesterase activity measured in the blood (<20 percent). Low-level exposure can be inferred from proximity to a documented exposure with no clinically detectable cholinergic signs or symptoms or detectable change in blood cholinesterase activity (Brown and Brix, 1998).[6]

As seen in the following review, there have been relatively few human studies of sarin's long-term health effects. There are more human studies of OP insecticides, whose mechanism of action is similar to sarin. Although the committee was charged with evaluating the literature on sarin, it also examined relevant studies on OP insecticides to contribute to its understanding of sarin. The committee's evaluation of the long-term effects of OP insecticides is contained in Appendix E. The studies described there do find a higher prevalence of neurological and/or psychiatric symptoms, as measured through either self-reports or standardized questionnaires, at all levels of acute OP exposure. With intermediate- or high-level acute exposures, higher symptom reporting is supported by poorer performance on standardized neuropsychological tests several years later. With low-level acute exposures (insufficient to produce cholinergic signs and symptoms), the higher symptom reporting is not consistently supported by poor performance on standardized neuropsychological tests.

[6]The U.S. federal guidelines for general population exposure to military OP nerve agents are based on values for minimum clinical cholinergic effects (i.e., the intermediate level) reduced by a hundredfold safety factor (Brown and Brix, 1998).

## Studies of Military Volunteers

### *U.S. Military Studies*

Between 1958 and 1975, the U.S. Army studied servicemen exposed voluntarily to an array of chemical warfare agents (NRC, 1982, 1985). During the program, the Army investigated only acute short-term effects. Approximately 6,720 soldiers (between the ages of 20 and 25 years) were exposed at Edgewood Arsenal, Maryland, to one or more of 254 chemicals in five classes. About 1,406 of the soldiers were exposed to 15 anticholinesterases. Of this group, 246 were tested with sarin under different conditions (e.g., i.v. or inhalation of sarin vapor), but the committee was unable to determine the actual doses to most of the soldiers by either route. However, for approximately 10 percent of this group, i.v. doses were reported to range from 3.0 to 4.0 μg/kg, alone or in combination with other agents (NRC, 1982); twenty-one soldiers were exposed to cyclosarin.[7] The servicemen were above average in physical and mental ability.

Five years after the program ended, the Department of the Army requested that the National Research Council's (NRC's) Board on Toxicology and Environmental Health Hazards examine the possible long-term health effects in servicemen tested in the research program. In a series of reports, the NRC designed and conducted a follow-up survey and examined soldiers' hospitalization and mortality. Two comparison groups of soldiers in the testing program were used as controls (i.e., those who received no test chemicals[8] and those who received chemicals other than the one under scrutiny). The NRC results and conclusions were based primarily on anticholinesterases as a class, rather than on sarin or cyclosarin.

The NRC questionnaire contained 27 outcome variables relating to health, social adjustment, and reproductive experience of the participants. Mailed survey questionnaires were returned by 64 percent of the overall population of soldiers tested. No long-term health consequences were reported by those responding to the questionnaire, including those exposed to anticholinesterases. Nonrespondents reported having had no health problems to report, when contacted later about their reasons for not returning the questionnaire. Nevertheless, the NRC cautioned that the study had low statistical power and that the exposed group was a highly selected, healthier subset than those who were unexposed. Thus, despite no major identifiable long-term effects, the NRC concluded that "the limited information available from the follow-up on these soldiers does not permit definitive conclusions regarding the nature and extent of possible long-term problems resulting from chemical exposure at Edgewood" (NRC, 1985).

The NRC also reviewed Army data tapes for hospitalizations of volunteers while still in the service (1958–1983) and reviewed Veterans Administration

[7]The committee was unable to find information regarding the dose of cyclosarin that was administered to the soldier volunteers.

[8]These were soldier volunteers in the same testing program who were used in tests of equipment or of "innocuous" substances such as caffeine or alcohol.

(VA) hospitalizations occurring after Army discharge (1963–1981). Hospitalizations of exposed volunteers were not elevated in relation to both comparison groups. Conclusions in the hospitalization study were for all anticholinesterases considered as a group. There was no evidence of increased mortality rates among participants in the entire program, as well as in the subgroups of anticholinesterases. Among soldiers ($n$ = 149) exposed to sarin (alone or in combination with other agents) the number of deaths was lower than that expected for U.S. males, based on age-specific death rates for each calendar year of follow-up. The NRC noted that the lower death rate was expected because of the "healthy-soldier effect" (see Chapter 3). It concluded that there was no evidence of a long-term effect on mortality among servicemen exposed to chemical warfare agents.

The Institute of Medicine's Medical Follow-Up Agency is currently conducting a follow-up study on the cohort of soldiers experimentally exposed to sarin and other anticholinesterase chemical warfare agents at Edgewood to further examine possible long-term health effects attributable to that exposure.

### *U.K. Military Study*

One of the clinical syndromes occurring after high exposure to certain OP pesticides[9] is referred to as a delayed intermediate syndrome (Senanayake and Karalliedde, 1987; Brown and Brix, 1998). It is a life-threatening paralysis of respiratory, neck, and limb muscles. It appears after recovery from the acute cholinergic syndrome, but before the expected time of onset of delayed neuropathy. The symptoms are reversible and disappear within about 2 weeks. Although the mechanisms are unknown, this condition probably results from damage to the neuromuscular junction or the muscle. There has been scant study of the intermediate syndrome after sarin exposure. In one uncontrolled study of male U.K. military volunteers ($n$ = 8) exposed to sarin vapors at 15 mg/min/m$^3$, soldiers quickly displayed some signs of the acute cholinergic syndrome (e.g., miosis and depressed RBC AChE levels) (Baker and Sedgwick, 1996). Although soldiers did not experience muscular weakness, they developed a subclinical change detected by single-fiber electromyography of the forearm muscle, an increased "jitter" at 3 hours postexposure. Jitter refers to a variation in the time of onset of a second action potential within a motor unit after an initial discharge. Jitter is one indication of potential failure of transmission at the neuromuscular junction. The change in jitter in the soldiers was apparent at about 1 year, but disappeared by the second follow-up at about 2 years postexposure. The findings were interpreted by the authors as possibly a subtle indicator of the onset of the intermediate syndrome, but the intermediate syndrome itself did not become manifest.

[9]The OP insecticides were fenthion, monocrotophos, dimethoate, and methamidophos.

### Accidental Exposure of Industrial Workers

One of the first studies to raise questions about possible long-term CNS effects of OPs was an uncontrolled study of industrial workers exposed in the 1950s and 1960s (Metcalf and Holmes, 1969). This case series identified long-term alterations in workers' EEG and cognition. It provided the impetus for studies in rhesus monkeys (Burchfiel et al., 1976, described earlier) and the first controlled study of long-term CNS effects in workers accidentally exposed to sarin (Duffy et al., 1979; Burchfiel and Duffy, 1982). Researchers studied a population of 77 workers with previously documented accidental exposure at a manufacturing plant and compared them to unexposed controls from the same plant ($n$ = 38) on EEG activity. None had been exposed within a year of the study. Exposed workers had one or more exposure incidents within the previous 6 years. At the time of exposure, they had clinical signs and depressed erythrocyte cholinesterase activity (by at least 25 percent). The EEG investigation consisted of spectral analysis of tape-recorded EEGs, visual inspection of routine clinical EEGs, and visual inspection of all-night sleep EEGs. Univariate and multivariate analysis of the EEG power spectra showed significant increase in high-frequency, beta activity (15–30 Hz) in temporal, central, and occipital regions in workers exposed to sarin compared to the control group ($p < .001$). There was a discrepancy between increased amounts of slow-wave activity in the delta and theta frequency bands (0–8 Hz) seen on visual inspection of EEG and the absence of such a finding by spectral analysis for the group exposed to sarin. Analysis of all-night sleep recordings showed a significant increase in the amount of REM (rapid eye movement) sleep only in the workers exposed to sarin. The clinical significance of these changes was not clear. Exposed workers also reported increased dreaming, instances of irritability, disturbed memory, and difficulty in maintaining alertness and attention (Burchfiel and Duffy, 1982), although methodological details of the symptom reporting were not provided. The increase in EEG beta activity in both monkeys (see earlier discussion) and humans years after acute exposure to sarin lends credence to a chronic CNS effect of sarin.

### Matsumoto, Japan, Terrorist Attack

In the late evening of June 27, 1994, Japanese terrorists spread sarin vapor, using a heater and fan mounted on a truck, in a residential neighborhood near the center of Matsumoto, Japan (Nakajima et al., 1997). About 600 people (residents and rescue teams) developed acute symptoms of sarin exposure (i.e., the acute cholinergic syndrome); 58 were admitted to hospitals, 253 sought medical assistance, and 7 people died. Sarin was later detected in air and water samples by gas chromatograph-mass spectrometry (GC-MC) (Nakajima et al., 1998). Several case reports, case series, and a population-based epidemiologic study emerged from this attack on a civilian population. The population-based study, the first of its kind on sarin exposure, identified symptoms persisting up to 3

years after exposure. In all of the studies reported here, doses are inferred on the basis of clinical effects. No dose reconstruction appears to have been performed.

A case series at one of the nearby hospitals reported that 17 of 18 patients admitted soon after the attack had an average reduction of plasma cholinesterase activity of 94 percent (Suzuki et al., 1997). In a larger case series, medical records were collected for 264 people who sought treatment, and health examinations were performed on 155 residents 3 weeks postexposure (Morita et al., 1995). This case series found that severely symptomatic patients examined at 3 weeks continued to exhibit decreased activity of plasma cholinesterase and RBC AChE; reduced serum triglyceride, serum potassium, and chloride; and elevated serum creatinine kinase, leukocytes, and ketones in urine. Blood cholinesterase levels returned to normal within 3 months. Most patients recovered by 6 months. Yet two of the nine severely poisoned patients displayed epileptiform abnormalities (although details of these abnormalities and their timing were not given) (Morita et al., 1995).

In a later follow-up examination by the same research team, four of six severely poisoned patients were reported to display visual field defects, hypoxia, low-grade fever, and what were described as "epileptic electroencephalographic changes" up to 2 years postexposure (Sekijima et al., 1997). At 7 months postexposure, one patient also developed sensory polyneuropathy and reduced sensory nerve conduction velocity. The minimal clinical information reported on this single case is not consistent with classic OPIDN, which manifests primarily as a motor deficit, or a mixed motor–sensory deficit, but never as an isolated sensory deficit (Lotti, 2000). With the exception of this poorly documented case of delayed sensory neuropathy, there appear to be no other cases of delayed neurotoxicity resembling OPIDN among the numerous cases of documented accidental or experimental exposure to sarin. Nevertheless, on the basis of animal studies (see earlier), researchers assert that OPIDN is possible in individuals who are rescued from otherwise lethal doses of sarin or in those exposed to lower levels for prolonged periods (Brown and Brix, 1998; Spencer et al., 2000).

The Matsumoto incident also triggered the first population-based study of the long-term effects of a single exposure to sarin. Nakajima and colleagues (1998, 1999) surveyed all residents ($n$ = 2,052) living within a defined geographic area surrounding the sarin release site (1,050 meters from north to south; 850 meters from east to west).[10] They mailed questionnaires at various times until 3 years after the incident. At the outset of the study (3 weeks postexposure), about 27 percent of the cohort ($n$ = 471) was classified as "victims" based on their reports of either receiving a diagnosis or reporting symptoms of acute cholinergic syndrome. They were compared with so-called "nonvictim" controls ($n$ = 669) who lived in the same geographic area as the victims but did not report having acute cholinergic symptoms or diagnosis.

[10]It was estimated, from police reports, that 12 liters of sarin may have been released (Nakajima et al., 1998); however, the exact amount of sarin and its purity are unknown.

At 1 year, 54 of 318 victims (17 percent) still reported being symptomatic. More than 80 percent of victims lived closest to the site of sarin release. There were no age or gender differences between those whose acute symptoms either persisted or resolved. The most common symptoms were asthenopia[11] (38/54), fatigue (35/54), blurred vision (30/54), shoulder stiffness (19/54), and asthenia[12] (18/54). At 3 years, 27.5 percent of 167 victims reported being symptomatic, compared with 5.4 percent of controls. The odds ratios were highest for fatigue, headache, and visual disturbances (asthenopia, blurred vision, and narrowing of visual field) (Table 5.5). The limitations of the study were low response rate at 3 years (41.8 percent) and possible recall bias (Nakajima et al., 1999). It must also be pointed out that the controls were not necessarily unexposed; they likely were a mixed population of unexposed and low-level exposed individuals.

The Matsumoto experience shows that direct exposure to sarin, particularly at intermediate to high levels, is associated with the acute cholinergic syndrome. In the majority of sarin victims in Matsumoto, clinical signs and symptoms of acute sarin poisoning disappeared within a matter of days or weeks if victims survived the acute effects of respiratory failure and convulsions. Follow-up population-based studies of sarin victims in Matsumoto show that significant chronic symptoms from sarin exposure persist and include visual disturbance (asthenopia, blurred vision), fatigue or asthenia, and headache. These chronic symptoms appear to be dose dependent, given the geographic exposure data and documented clinical and laboratory findings. These follow-up studies, however, lack a well-defined control population.

### Tokyo, Japan, Terrorist Attack

On the morning of March 20, 1995, terrorists simultaneously released diluted sarin vapor into three convergent lines of the Tokyo subway system (Yokoyama et al., 1998c). About 5,000 people sought medical evaluation, 1,000 of whom were symptomatic and 12 of whom died (Woodall, 1997). The hospital in closest proximity to the attacks, St. Luke's International Hospital, treated the largest group of patients ($n$ = 641) (Okumura et al., 1996; Ohbu et al., 1997). Medical staff assessed most of these patients (83 percent) as having an intermediate level of exposure based on miosis (the most common symptom), blurred vision, and headache. Seventeen percent of the patients were presumed to have had high-level exposure. This patient group, which was admitted to the hospital, had more severe cholinergic signs and symptoms including marked miosis, weakness, difficulty breathing, fasciculations, convulsions, and >20 percent depression of cholinesterase activity in the blood. Most of these patients were given standard treatment for acute sarin intoxication (atropine, pralidoxime chloride, and diazepam). Five patients were critically ill with cardiac arrest, res-

[11]Weakness or fatigue of the visual organs, accompanied by pain in the eyes.
[12]General weakness, or loss of strength or energy.

**TABLE 5.5** Relationship Between Sarin Exposure and Symptoms 3 Years After the Matsumoto Incident

| Symptoms | Victims ($n$ = 167),[a] $n$ (%) | Controls ($n$ = 669),[b] $n$ (%) | Odds Ratio (95% CI) |
|---|---|---|---|
| Current symptoms | | | |
| No | 121 (72.5) | 633 (94.6) | 6.68 (4.15–10.78) |
| Yes | 46 (27.5)[c] | 36 (5.4) | |
| Fatigue | 25 (15.0)[c] | 22 (3.3) | 5.18 (2.84–9.44) |
| Asthenia | 14 (8.4)[c] | 11 (1.6) | 5.47 (2.44–12.29) |
| Shoulder stiffness | 15 (9.0)[d] | 25 (3.7) | 2.54 (1.31–4.94) |
| Bad dreams | 5 (3.0) | 7 (1.0) | 2.92 (0.92–9.32) |
| Insomnia | 9 (5.4)[e] | 15 (2.2) | 2.48 (1.07-5.78) |
| Blurred vision | 18 (10.8)[c] | 13 (1.9) | 6.10 (2.92–12.72) |
| Narrowing of visual field | 6 (3.6)[e] | 7 (1.0) | 3.52 (1.17–10.63) |
| Asthenopia | 40 (24.0)[c] | 21 (3.1) | 9.72 (5.54–17.04) |
| Difficulty in smoking | 0 (0) | 3 (0.4) | — |
| Husky voice | 2 (1.2) | 7 (1.0) | 1.15 (0.24–5.57) |
| Slight fever | 4 (2.4)[e] | 2 (0.3) | 8.18 (1.49–45.07) |
| Palpitation | 5 (3.0)[e] | 5 (0.7) | 4.10 (1.17–14.33) |
| Headache | 14 (8.4)[d] | 7 (1.0) | 8.65 (3.43–21.81) |

NOTE: Values given in absolute number of patients reporting symptoms (percentages). CI = confidence interval.

[a]Victims are those who lived in the geographic area of the incident and had one or more symptoms immediately after.
[b]Controls lived in the geographic area of the incident but did not have one or more symptoms immediately after.
[c]Significant differences noted between victims and controls at: $p < .001$.
[d]Significant differences noted between victims and controls at: $p < .01$.
[e]Significant differences noted between victims and controls at: $p < .05$.

SOURCE: Adapted from Nakajima et al., 1999.

piratory arrest, or convulsions, two of whom died. Patients in the highly exposed group improved by the time of discharge except for symptoms related to sarin's effects on the eyes—ocular pain, blurred vision, and visual darkness (Okumura et al., 1996; Ohbu et al., 1997). All but five patients were discharged from the hospital by the fifth day.

More than 20 percent of the hospital staff who treated victims developed acute cholinergic symptoms from secondary exposure (Nozaki et al., 1995; Ohbu et al., 1997). Although hospital staff quickly suspected sarin intoxication in patients, they did not take appropriate precautionary measures because they were first erroneously notified by the fire department that acetonitrile was the agent. Only hours later were they notified that sarin had been implicated by GC-MS (Okumura et al., 1998a,b). An organophosphorus anticholinesterase pre-

sumed to be sarin was later confirmed in serum samples from the victims (Polhuijs et al., 1997).

Questionnaires were distributed at 1, 3, and 6 months after the incident to 610 patients seen at St. Luke's International Hospital. Almost 60 percent of 475 respondents (290 patients) still reported symptoms related to the exposure, such as fear of subways, sleep disturbance, flashbacks, nightmares, and mood changes—symptoms that the authors interpreted as indicative of posttraumatic stress disorder (PTSD; Ohbu et al., 1997).

Six to eight months later, 18 symptom-free survivors with previous intermediate- and high-level exposure to sarin were tested for persistent CNS effects (Murata et al., 1997; Yokoyama et al., 1998a,b,c). At the time of their past admission to the hospital, their plasma cholinesterase had been depressed by about 25 percent of normal. Murata and colleagues (1997) first reported on their responses to sensory evoked potentials, a noninvasive method of detecting functional activity elicited by stimulation of specific nerve pathways, however any functional changes by EEG do not indicate their pathological basis. The event-related potential (ERP) (P300) and the visual-evoked potential (VEP) (P100) displayed slight yet significant prolongation in sarin-exposed subjects, compared with 18 sex- and age-matched control subjects (healthy volunteers).[13] There was no relationship in the sarin-exposed group between neurophysiological findings and scores for PTSD, which were significantly elevated compared to controls (Yokoyama et al., 1998c) Short-latency brain stem auditory evoked potentials and electrocardiography were not different between cases and controls. Findings were interpreted by the authors as suggestive of long-term neurotoxic effects of high-level exposure to sarin in those individuals who no longer reported symptoms.

The same sarin-exposed individuals underwent neurobehavioral testing and vestibulocerebellar testing (Yokoyama et al., 1998a,b). For neurobehavioral testing, cases and controls filled out a PTSD checklist and underwent nine tests: digit symbol (psychomotor performance); picture completion (visual perception); digit span (attention and memory); finger tapping (psychomotor performance); reaction time (psychomotor performance); continuous performance test (sustained visual attention); paired-associate learning (learning and memory); General Health Questionnaire (psychiatric symptoms); and the Profile of Mood States. The score on the digit symbol test for sarin-exposed cases was significantly lower than for controls. The scores on the General Health Questionnaire, fatigue (Profile of Mood States), and PTSD checklist were significantly higher for the sarin group. Their scores on the digit symbol test remained significantly decreased even after controlling for the effect of PTSD. It is important to control for PTSD because studies of military trainees under mock defensive chemical

[13]In the ERP test, subjects' EEG was measured in response to a random sequence of tones. In the visual-evoked potential, their EEG was measured after stimulation with a checkerboard pattern, which reversed at a rate of two times per second. P300 and P100 refer to the peak electrical potential recorded by the EEG.

warfare conditions revealed that 10–20 percent reported (in the absence of actual exposure to chemical weapons) moderate to severe psychological symptoms, including anxiety, claustrophobia, and panic (Fullerton and Ursano, 1990).

For vestibulocerebellar testing, Yokoyama and colleagues (1998a) used computerized posturography on sarin cases and controls. Computerized posturography is a standard means of assessing vestibular function by placing subjects in the middle of a platform and measuring how their movements displaced the platform (via pressure transducers connected from the platform to a computer). The study found significant impairment only in female cases ($n$ = 9) who performed more poorly (with their eyes open) in their ability to maintain postural sway and their center of gravity when they moved at low frequencies (0–1 Hz) in the anterior–posterior direction. Female patients also performed more poorly in the area of sway (i.e., the area on the platform over which the test subject moves to maintain balance). None of the postural sway tests were abnormal in male cases ($n$ = 9). The authors viewed their findings as suggestive of a gender difference in a "delayed" effect of acute sarin poisoning on the vestibulocerebellar system. Their characterization of this effect as "delayed" is questionable, since there is no evidence of this postural testing having been performed at an earlier point after sarin exposure. Thus, the effect may be chronic, rather than delayed.

The Tokyo sarin experience confirms that acute exposure to sarin leads to the acute cholinergic syndrome. Sarin exposure at high levels can be fatal if cardiopulmonary compromise or convulsions ensue. Visual disturbances are frequent sequelae of the acute exposure, particularly in individuals with high-level exposure. Neurophysiological testing of a small group of asymptomatic sarin-exposed individuals does show chronic changes in visual and event-related evoked potentials and vestibulocerebellar function months after the acute syndrome has subsided. These neurophysiological data are suggestive of subtle, persistent CNS effects from sarin. Except for digit symbol test abnormalities, significant cognitive deficits were not detected.

### Gulf War Veterans

As explained earlier in this chapter, CIA–DoD modeling determined that U.S. troops located within 25 km of the Khamisiyah weapons site demolition in March 1991 may have been exposed to low or intermediate levels of sarin (CIA–DoD, 1997). U.S. troops did not report acute cholinergic symptoms at the time, but the possibility of low-level, asymptomatic exposures cannot be discounted. In a series of studies on members of a naval battalion ($n$ = 249) called to active duty for the Gulf War, Haley and Kurt (1997) found that veterans who believed themselves to have been exposed to chemical weapons[14] were more

[14]Based on self-reports about their perceptions of CW exposure, rather than any evidence of symptomatology. Their geographical and temporal location in relation to the Khamisiyah demolition site was not reported. The questionnaire was sent to participants in 1994, before DoD reported that chemical weapons exposure could have occurred.

likely to be classified as having one of six new proposed syndromes (Haley et al., 1997; see also Chapter 2). Specifically, this syndrome—labeled by the investigators as "confusion–ataxia" or "syndrome 2"—features problems with thinking, disorientation, balance disturbances, vertigo, and impotence. This was the only syndrome of the six to have been associated with self-reported chemical weapons exposure (see Chapter 6).

A follow-up study of vestibular function was performed on a subset of those veterans ($n$ = 23) who had the highest factor scores on three of the syndromes identified in 1997 by Haley and Kurt (Roland et al., 2000). The study was designed to probe the nature of veterans' vestibular symptoms, rather than to examine the relationship between vestibular performance and exposure in the Gulf War. Of the 23 veterans in this study, 13 exhibited syndrome 2, whereas the others exhibited syndromes 1 (impaired cognition) and 3 (arthromyoneuropathy) (see Chapter 2). Based on a new questionnaire, veterans with syndrome 2 reported dizzy spells with greater frequency and longer duration than veterans with the other two syndromes. Veterans with syndrome 3, but not syndrome 2, performed significantly differently from controls on dynamic platform posturography (a test similar to that used by Japanese researchers to identify impairment in sarin-exposed females; see Yokoyama et al., 1998a). Veterans with other syndromes also had performance decrements on some of the measures of vestibular function. The study concluded that there was both subjective and objective evidence of injury to the vestibular system in this group of Gulf War veterans with newly defined syndromes. Haley and Kurt (1997) hypothesized that these newfound chronic syndromes represent variants of OPIDN caused by exposure to various combinations of organophosphates (pesticides and nerve agents) and carbamate pesticides that inhibit cholinesterases and NTE (see Chapters 2 and 6).

## Genetic Susceptibility to Sarin Toxicity

One of the mechanisms of sarin inactivation is by hydrolysis with the enzyme paraoxonase (PON1), an esterase found in liver and serum. The human PON1 gene has polymorphisms at positions 192 (*Arg/Gln*) and 55 (*Leu/Met*) (Furlong et al., 1993). The former accounts for three genotypes (QQ, RR, and QR) relating to the catalytic properties of two forms of an enzyme (types R and Q allozymes), which hydrolyze certain organophosphates at different rates. The R allozyme ($Arg_{192}$) hydrolyzes the organophosphate paraoxon at a high rate; however, it has a low activity toward OP nerve agents such as sarin and soman (Davies et al., 1996). Lower activity means that more sarin would be bioavailable to exert its anticholinesterase effects. The Q allozyme, on the other hand, has high activity toward organophosphate nerve agents (and low activity toward paraoxon). Thus, individuals with the Q allozyme (QQ or QR) are expected to have greater hydrolysis of sarin than individuals homozygous for the R allele (RR). Since hydrolytic activity with the same genotype can vary about tenfold, it is also important to determine the level of allozyme expression—in addition to

the genotype—in order to characterize an individual's PON1 status (Richter and Furlong, 1999). In Caucasian populations, the frequency of the R allele is about 0.3, but the frequency is 0.66 in the Japanese population (Yamasaki et al., 1997). This would make individuals in the Japanese population more sensitive to the toxicity of sarin, a fact that may have contributed to their morbidity and mortality after the terrorist attacks.

A recent study investigated PON1 genotype and serum enzyme activity in a group of 25 ill Gulf War veterans and 20 controls (Haley et al., 1999). Ill veterans were more likely than controls to possess the R allele (QR heterozygotes or R homozygotes) and to exhibit lower enzyme activity. This study raises the possibility that the R genotype (low sarin-hydrolyzing activity) may represent a risk factor for illness in Gulf War veterans. However, because of the very small size of the study, such findings necessitate further confirmation in a larger population (Furlong, 2000) (also see Chapter 6).

## CONCLUSIONS

The committee reached the following conclusions after reviewing the literature on sarin. The committee was unable to formulate any conclusions about cyclosarin because of the paucity of toxicological and human studies.

*The committee concludes that there is sufficient evidence of a causal relationship between exposure to sarin and a dose-dependent acute cholinergic syndrome that is evident seconds to hours subsequent to sarin exposure and resolves in days to months.*

The acute cholinergic syndrome has been recognized for decades and has been documented in human studies summarized in this chapter. This syndrome, as well as cholinergic signs and symptoms, is evident seconds to hours after exposure (see Table 5.2) and usually resolves in days to months. The syndrome and the cholinergic signs and symptoms are produced by sarin's irreversible inhibition of the enzyme acetylcholinesterase. Inactivation of the enzyme that normally breaks down the neurotransmitter acetylcholine leads to the accumulation of acetylcholine at cholinergic synapses. Excess quantities of acetylcholine result in widespread overstimulation of muscles and nerves. At high doses, convulsions and death can occur.

*The committee concludes that there is limited/suggestive evidence of an association between exposure to sarin at doses sufficient to cause acute cholinergic signs and symptoms and subsequent long-term health effects.*

Many health effects are reported in the literature to persist after sarin exposure: fatigue, headache, visual disturbances (asthenopia, blurred vision, and narrowing of the visual field), asthenia, shoulder stiffness, and symptoms of posttraumatic stress disorder; and abnormal test results, of unknown clinical

significance, on the digit symbol test of psychomotor performance, EEG records of sleep, event-related potential, visual evoked potential, and computerized posturography.

These conclusions are based on retrospective studies of three different exposed populations in which the acute cholinergic signs and symptoms were documented as an acute effect of exposure. The findings from those studies are based on comparisons with control populations. One population consisted of industrial workers accidentally exposed to sarin in the United States; the other two populations were civilians exposed during terrorism episodes in Japan. The health effects listed above were documented at least 6 months after sarin exposure, and some persisted up to a maximum of 3 years, depending on the study. Whether the health effects noted above persist beyond the 3 years has not been studied.

*The committee concludes that there is inadequate/insufficient evidence to determine whether an association does or does not exist between exposure to sarin at low doses insufficient to cause acute cholinergic signs and symptoms and subsequent long-term adverse health effects.*

On the basis of positive findings in a study of nonhuman primates and in studies of humans exposed to organophosphate insecticides (see Appendix E), it is reasonable to hypothesize the occurrence of long-term adverse health effects from exposure to low levels of sarin. Studies of low-level exposure of workers find that organophosphate insecticides are consistently associated with higher prevalence of neurological and/or psychiatric symptom reporting (see Appendix E). However, there are no well-controlled human studies expressly of sarin's long-term health effects at doses that do not produce acute signs and symptoms.

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