8 TOXICOLOGY OF ORGANOPHOSPHATE NERVE AGENTS

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INTRODUCTION

The organophosphate (OP) anticholinesterases include the chemical warfare nerve agents, a variety of OP pesticides (Ballantyne and Marrs, 1992) and, less well-known, a natural compound, anatoxin-As (Dittmann and Wiegand, 2005). The active ingredients of OP pesticides are used as drugs in human medicine, e.g. malathion to treat head-lice and metrifonate/trichlorfon in tropical medicines. OPs are also used in veterinary medicine notably as ectoparasiticides (Beesley, 1994). The chemical warfare nerve agents have a much higher mammalian acute toxicity, particularly via the percutaneous and inhalation routes, than the OP pesticides. Additionally many chemical warfare agents are phosphonofluoridates and phosphonothioates, while many pesticides are = S type-phosphorothioates. Qualitatively, the anticholinesterase toxicology of the OP nerve agents and pesticides is similar and in general treatment strategies are alike so that much information on OP pesticides is relevant to nerve agent toxicology.

History

OP compounds were intensively investigated in Germany in the 1930s. The German conglomerate IG Farbenindustrie looked at a number of these compounds for use as insecticides and a programme of synthesis of a large number of compounds was undertaken. Tabun and sarin, OPs of little use as insecticides but of very high mammalian toxicity, were synthesized by Schrader in 1937 and a small pilot production plant was set up at Münster-Lager. Later, at Dühernfurt near Breslau in Prussian Silesia (now Bzerg Dolny and Wrocław in Poland), a production plant for these agents was established and both tabun and sarin were manufactured in quantity: in the case of tabun, 12 000 tonnes were produced. Soman, another nerve agent, was also synthesized in Germany during the war, but only manufactured in small quantities. Strangely, perhaps in view of the large stocks held by Germany, the nerve agents were not used in World War II (UK Ministry of Defence, 1972) and appear to have been dumped in the Baltic sea. The V agents similarly arose out of studies of putative insecticides and VX has been variously reported as being first synthesized at the Chemical Defence Experimental Establishment (CDEE) at Porton Down, UK (now Dstl, Porton Down) or by Ghosh at Imperial Chemical Industries in 1952 (SIPRI, 1971). Other nerve agents were developed subsequently and stocks have been held by a number of countries, including USA, the former USSR and successor states, the UK, France and Iraq. However, nerve agents have rarely been used in warfare; the only notable instance of use being by Iraq against that country's own Kurdish population (le Chêne, 1989). There have also been allegations of use of OP nerve agents during the Iran/Iraq war. The nerve agent sarin, in an impure form, was used in two terrorist attacks in Japan, respectively, in Matsumoto 1994 (Okudera, 2002) and Tokyo in 1995 (Nagao *et al.*,

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Table 1. Possible targets for nerve agents in warfare.[a] Adapted and reproduced from Chapter 34, 'Organophosphorus compounds as chemical warfare agents', by Maynard RL and Beswick F, in *Clinical and Experimental Toxicology of Organophosphates and Carbamates* (B Ballantyne and TC Marrs, eds), 1992, with the kind permission of the publishers, Elsevier, and the authors

	Type of agent			
Target	Sarin	Soman	VX	Delivery system
Rear areas				
Airports/airfields	—	L	L	Aircraft (bombs, cluster spray bombs, spray tanks, missiles)
Seaports	—	L	L	Aircraft (bombs, cluster spray bombs, spray tanks, missiles)
Railways, especially junctions	—	L	L	Aircraft (bombs, cluster)
Headquarters and communication centres	—	L	L	Aircraft (bombs, missiles)
Storage sites	—	L	L	Aircraft (bombs, cluster missiles)
Troop concentrations	—	L	L	Aircraft (bombs, spray tanks)
Forward areas				
Nuclear delivery weapons, other key weapons and systems	L	—	L	Multiple rocket launchers, aircraft (bombs, rockets)
Defence positions	L	—	L	Multiple rocket launchers, artillery, mortars, aircraft (bombs), rockets
Own flanks	L	—	L	Mines
Own defence front generally	L	—	L	Artillery, mortars, mines
To produce casualties, to harass and reduce combat efficiency	L	—	L	Multiple rocket launchers, artillery, mortars, aircraft (bombs, rockets)
To deny ground	—	L	L	Aircraft (spray, mines)
Harass civilian populations	L	—	—	Aircraft (bombs, rockets, sprays)

Note:
[a] L, likely use.

1997), as was, almost certainly, VX, but this agent was used for assassination of individuals (Nozaki *et al*., 1995).

Use

Possible roles of OP nerve agents in warfare are outlined in Table 1. Until the use of sarin in Matsumoto and Tokyo, the use of chemical weapons as terrorists' tools had been considered unlikely. Since then, and the emergence of al-Qaida, this has been reconsidered and Table 2 gives some of the roles whereby terrorists might make use of chemical weapons. Note that the synthesis of tabun is easier than the other G agents, so that tabun is more likely to be used in terrorist scenarios (see below). The reason is that tabun has no fluorine in its structure. Incorporation of the fluorine leaving group requires the use of hydrofluoric acid during the synthesis and this is, of course, corrosive to glass. Early bulk synthesis of nerve agents with fluorine leaving groups was carried out using special apparatus made or lined with pure silver. Such a process is inevitably costly, although the difficulties did not deter a Japanese terrorist group (the Aum Shinrikyo). It is of interest that the nerve agent likely to have been used by Iraq against the Kurds was tabun,

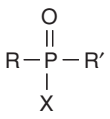
Table 2. Possible terrorist targets for nerve agents

Major target	Specific target
Air transport	Airport terminals/interiors of planes
Railways/subway systems	Stations, interiors of carriages
Road transport	Freeways/motorways, especially intersections and service stations
Public meeting places	Concert halls, major sporting events, political meetings, churches and synagogues
Financial centres	Headquarters of financial institutions, exchanges, e.g. trading floors
Energy supply	Power stations, gas terminals, oil terminals
Communications	Television and radio broadcasting stations, telephone exchanges

the first nerve agent to be synthesized on a large scale.

Structure and physical properties

The nerve agents comprise a group of OPs of high acute mammalian toxicity. They are derivatives of phosphoric or phosphonic acids (more often the latter) and contain two alkyl groups (R and R′) and a leaving group. The general formulae of the OP nerve agents is similar to the OP pesticides:



The nerve agents are traditionally divided into the G agents and V agents (Table 3). According to Watson *et al*. (2005), G stands for German and V for venom. In the case of the G agents the leaving group is often a fluorine atom and, exceptionally in GF (cyclosarin), one of the alkyl groups is replaced by a cyclohexyl group. Soman is distinguished by the fact that one of its alkyl groups is a bulky pinacolyl group, while tabun does not contain a fluorine atom and is a cyanidate. The G agents include GB (sarin, isopropyl methylphosphonofluoridate), GD (soman, pinacolyl methylphosphonofluoridate), GA (tabun, ethyl *N*,*N*-dimethylphosphoramidocyanidate) and GF (cyclosarin, cyclohexyl methylphosphonofluoridate). The V agents are phosphonothioates of the P–O type in which the leaving group is linked to phosphorus through a sulphur atom, except for VG which is a phosphorothioate. The V agents are exemplified by VX (*O*-ethyl *S*- (2-(diisopropylamino)ethyl) methylphosphonothioate).

All of the nerve agents are colourless liquids, although impure agents may be yellow to brown in colour. However, these compounds differ amongst themselves in physical properties (Table 4), for example, the V agents are much less volatile than the G agents, the latter being volatile liquids (tabun less volatile than sarin or soman). Soman may be thickened to increase persistence (Marrs and Maynard, 2001). VX is a non-volatile liquid with the result that the VX (unless aerosolized) is not an inhalation hazard, a fact that may significantly affect its role in warfare. Tabun is said to have a fruity odor, while the other agents are said to be odorless. Sarin may be mixed with tributylamine (sarin type I) or diisopropylcarbodiimide (sarin type II), to prevent spontaneous hydrolysis.

As is discussed below, more information on cyclosarin (GF, cyclohexyl methylphosphonofluoridate) has accumulated recently, because, during operations 'Desert Shield' and 'Desert Storm', it was discovered that cyclosarin was among the nerve agents that Iraq possessed. Thus, some limited information is now available on the physical properties of this nerve agent (gulflink, 2005). The material is a liquid at room temperature with a boiling point of 239°C and a freezing point of −30°C. The vapour pressure is 0.044 mmHg at 20°C and the volatility is 438 mgm^{-3} at 20°C.

TOXICOLOGY

Until recently, attention has almost entirely been given to the acute toxicity of nerve agents and, in the military context, this is still the most

Table 3. Formulae of nerve agents. Adapted and reproduced from Chapter 34, 'Organophosphorus compounds as chemical warfare agents', by Maynard RL and Beswick F, in *Clinical and Experimental Toxicology of Organophosphates* (B Ballantyne and TC Marrs, eds), 1992, with the kind permission of the publishers, Elsevier, and the authors

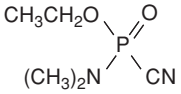
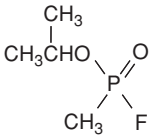
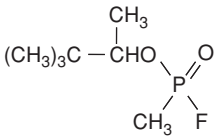
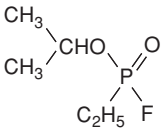
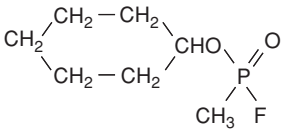
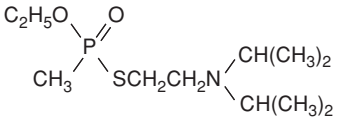
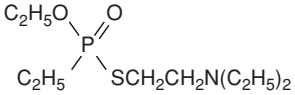
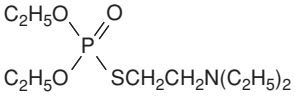
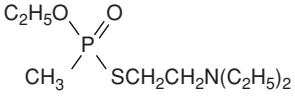
Abbreviation	Common name	Proper name
GA	Tabun	Ethyl *N*, *N*-dimethylphosphoramidocyanidate 
GB	Sarin	Isopropyl methylphosphonofluoridate 
GD	Soman	Pinacolyl methylphosphonofluoridate 
GE	—	Isopropyl ethylphosphonofluoridate 
GF	Cyclosarin	Cyclohexyl methylphosphonofluoridate 
VX	—	*O*-Ethyl-*S*-[2(diisopropylamino)ethyl]methylphosphonothioate 
VE	—	*O*-Ethyl-*S*-[2-(diethylamino)ethyl]ethylphosphonothioate 
VG	—	*O*,*O*-Diethyl-*S*-[2-(diethylamino)ethyl] phosphorothioate 
VM	—	*O*-Ethyl-*S*-[2-(diethylamino)ethyl] methylphosphonothioate 

Table 4. Physico-chemical properties of nerve agents. Adapted and reproduced from Chapter 34, 'Organophosphorus compounds as chemical warfare agents', by Maynard RL and Beswick F, in *Clinical and Experimental Toxicology of Organophosphates* (B Ballantyne and TC Marrs, eds), 1992, with the kind permission of the publishers, Elsevier, and the authors

Property	Tabun, GA	Sarin, GB	Soman, GD	VX	GF
Molecular weight, MW (Da)	162.3	140.1	182.18	267.36	180.14
Specific gravity at 25°C	1.073	1.0087	1.022	1.0083	1.133[a]
Boiling point °C	246	147	167	300	—
Melting point °C	−49	−56	−80	−20	−12

Vapour pressure (VP) and Volatility (Vol)[b]	°C	VP (mmHg)	Vol (mg m^{-3})	VP (mm Hg)	Vol (mg m^{-3})	VP (mm Hg)	Vol (mg m^{-3})	VP (mmHg)	Vol (mm m^{-3})	VP (mm Hg)	Vol (mg m^{-3})
	0	0.004	38	0.52	4 279	0.044	470.9	—	—	0.006	63
	10	0.013	119.5	1.07	8 494	0.11	1 135.5	—	—	0.017	173
	20	0.036	319.8	2.10	16 101	0.27	2 692.1	0.00044	5.85[c]	0.044	438[d]
	25	0.07	611.3	2.9	21 862	0.40	3 921.4	0.0007	10.07	0.068	659
	30	0.094	807.4	3.93	29 138	0.61	5 881.4	—	—	0.104	991
	40	0.23	1912.4	7.1	60 959	—	—	—	—	0.234	2159
	50	0.56	4512.0	12.3	83 548	2.60	23 516.0	—	—	0.501	4480

Notes:
[a] Temperature, 20°C.
[b] Volatility = concentration of saturated vapour at specified temperature. Volatility calculated from $PV = nRT$.

$$\text{Vol} = \frac{VP \times 101\,325 \times \text{MW}}{760 \times 8.3143 \times A} = \frac{\text{VP} \times \text{MW} \times 16.035}{A}$$

where A is the Absolute temperature.
[c] Some authorities quote values as low as 0.1–1.0 mg m^{-3}.
[d] Where a range is given, this may be because materials of different purity have been studied.

important consideration. It is also the case when considering casualties from terrorist use. Two other exposure patterns have also to be considered, (1) the long-term effects of low dose exposure, particularly in relation to manufacturing workers and individuals involved in clean-up of contaminated sites, and (2) the delayed and long-term effects after recovery from acute exposure. It has been pointed out (Reutter, 1999) that the toxicity of chemical weapons is unchanged but our perception of toxicity has since these compounds were developed in the 1940s and 1950s.

Other scenarios that need to be considered include contamination of food and public places and, in such situations, levels to which it is necessary to decontaminate to ensure public safety. A further consideration of importance is the toxicity of nerve agent degradation products (see review by Munro *et al*., 1999) and, particularly in the case of terrorist use, manufacturing contaminants.

Acute toxicity

The toxic actions of all of the nerve agents are very similar, although some differences have been observed. There are differences between the OPs with respect to their relative central and peripheral effects (Ligtenstein, 1984; Misulis *et al*., 1987) and, within the central nervous system (CNS), some differences between the OPs may exist. For example, in the case of soman, there is some indication that there are differences in the sensitivity of acetylcholinesterase within the nervous system (Sellström *et al*., 1985). Acute toxicity figures are available for the nerve agents tabun, sarin, soman and VX in many species (Table 5). The acute toxic dose in humans in not known with any exactitude as poisoning with nerve agents has only rarely been observed in man (Sidell, 1974; Maynard and Beswick, 1992). Where poisoning has been observed in man, detailed information on dose has usually not been available and this was the case with the sarin casualties in Japan, where the material was, in any case, impure. Therefore, lethal doses and likely clinical effects in man have to be inferred from experimental poisoning in animals and from OP pesticide poisoning. The cases that have been studied, together with low-dose volunteer studies with nerve agents, do not suggest any major differences between man and other animals in clinical response to nerve agents.

Acute toxicity figures for nerve agents other than tabun, sarin, soman and VX are scanty, with the exception of cyclosarin. Since the 1st Gulf War, a considerable amount of work has been carried out on the treatment of poisoning with cyclosarin and data have also become available on the acute toxicity of this compound. In studies by Anthony *et al*. (2004), the LCt50s in the rat for inhaled cyclosarin were of a similar order of magnitude to the LCt50s for sarin (Table 6). Although cyclosarin is a powerful acetylcholinesterase inhibitor, rapid ageing of the inhibited enzyme as seen with soman, does not occur, while spontaneous reactivation does (Worek *et al*., 1998) (see below). Qualitatively, the toxic effects of cyclosarin seem similar to other nerve agents (Young and Koplovitz, 1995).

It should be noted that the nerve agents have one or more chiral centres and the toxicity of the enantiomers may differ dramatically from one to another and the racemic mixtures (Spruit *et al*., 2000).

Mechanism of toxicological actions

The action of OP nerve agents on the nervous system results from their effects on enzymes, particularly esterases. The most notable of these esterases is acetylcholinesterase. The active site of acetylcholinesterase comprises a catalytic triad of serine, histidine and glutamic acid residues and other important features of the enzyme are a 'gorge' connecting the active site to the surface of the protein and a peripheral anionic site (Bourne *et al*., 1995,1999; Sussman *et al*., 1991; Thompson and Richardson, 2004), The OPs phosphylate[1] the serine hydroxyl group in the active site of the enzyme.

The binding to acetylcholinesterase of acetylcholine (the natural substrate of the enzyme) results in acetylation of the serine at the active site of the enzyme, with loss of the choline moiety. The reaction for acetyl choline can be envisaged as shown below, with E being the

[1] Phosphylation includes phophorylation and phosphonylation, the latter being more common with nerve agents.

Table 5. Comparative acute toxicity of nerve agents. Adapted and reproduced from Chapter 34, 'Organophosphorus compounds as chemical warfare agents', by Maynard RL and Beswick F, in *Clinical and Experimental Toxicology of Organophosphates* (B Ballantyne and TC Marrs, eds), 1992, with the kind permission of the publishers, Elsevier, and the authors

Species	Route[a]	Term	Unit (duration)	Tabun	Sarin	Soman	VX
Man	pc	LD_{50}	mg kg^{-1}	—	28 [b]	—	—
	pc	LCLO	µg kg^{-1}	—	—	—	86[c]
	pc	LDLO	mg kg^{-1}	23[b]	—	18[b]	—
	inhal	LDLO	mg m^{-3}	150[b]	—	70[b]	—
	inhal	LD_{50}	mg m^{-3}	—	70[b]	—	—
	inhal	ECLO	µg m^{-3}	—	90[d]	—	—
	iv	TDLO	µg kg^{-1}	14[b]	—	—	—
	iv	TDLO	µg kg^{-1}	—	—	—	1.5[e]
	oral	TDLO	µg kg^{-1}	—	2[f]	—	4[e]
	sc	LDLO	µg kg^{-1}	—	—	—	30[g]
	im	TDLO	µg kg^{-1}	—	—	—	3.2[g]
Rat	pc	LD_{50}	mg kg^{-1}	18[h]	—	—	—
	inhal	LC_{50}	mg m^{-3} (10 min)	304[h]	150[d]	—	—
	iv	LD_{50}	µg kg^{-1}	66[h]	39[i]	44.5[j]	—
	oral	LD_{50}	µg kg^{-1}	3700[h]	550[h]	—	12[m]
	sc	LD_{50}	µg kg^{-1}	193[j]	103[k]	75[l]	—
	im	LD_{50}	µg kg^{-1}	800[f]	108[n]	62[n]	—
	im	LD_{50}	µg kg^{-1}	130[G]	—	—	—
	ip	LD_{50}	µg kg^{-1}	—	218[i]	98[o]	—
Mouse	pc	LD_{50}	mg kg^{-1}	1[h]	1.08[h]	—	—
	inhal	LC_{50}	mg m^{-3} (30 min)	15[h]	5[p]	1[p]	—
	iv	LD_{50}	µg kg^{-1}	150[h]	113[q]	35[r]	—
	sc	LD_{50}	µg kg^{-1}	250[s]	60[p]	40[p]	22[s]
	sc	LD_{50}	µg kg^{-1}	—	319[q]	—	—
	sc	LD_{50}	µg kg^{-1}	—	172[E]	—	—
	im	LD_{50}	µg kg^{-1}	440[q]	222[q]	—	—
	ip	LD_{50}	µg kg^{-1}	—	420[u]	393[u]	50[c]
Dog	pc	LD_{50}	mg kg^{-1}	30[h]	—	—	—
	inhal	LC_{50}	mg m^{-3} (10 min)	400[h]	100[h]	—	—
	iv	LD_{50}	µg kg^{-1}	84[v]	19[v]	—	—
	oral	LD_{50}	µg kg^{-1}	200[p]	—	—	—
	sc	LD_{50}	µg kg^{-1}	284[l]	—	12[w]	—
	sc	LD_{50}	µg kg^{-1}	—	120[H]	14[H]	10[H]
Monkey	pc	LD_{50}	µg kg^{-1}	9300[h]	—	—	—
	inhal	LC_{50}	mg m^{-3} (10 min)	250[h]	100[h]	—	—
	sc	LD_{50}	µg kg^{-1}	—	—	13[x]	—
	im	LD_{50}	µg kg^{-1}	—	22.3[y]	9.5[z]	—
Cat	inhal	LC_{50}	mg m^{-3} (10 min)	250[h]	100[v]	—	—
	iv	LD_{50}	µg kg^{-1}	—	22[h]	—	—
Rabbit	pc	LD_5	µg kg^{-1}	2500[h]	925[h]	—	—
	inhal	LC_{50}	mg m^{-3} (10 min)	840[h]	120[h]	—	—
	iv	LD_{50}	µg kg^{-1}	63[h]	15[A]	—	—
	oral	LD_{50}	µg kg^{-1}	16300[h]	—	—	—
	sc	LD_{50}	µg kg^{-1}	375[B]	30[C]	20[w]	14[D]
	ip	LD_{50}	µg kg^{-1}	—	—	—	66[D]

(*cont.*)

Table 5. (*cont.*)

Species	Route[a]	Term	Unit	Tabun	Sarin	Soman	VX
Guinea Pig	pc	LD_{50}	mg kg^{-1}	35[h]	—	—	—
	inhal	LC_{50}	mg m^{-3} (2 min)	393[h]	—	—	—
	sc	LD_{50}	μg kg^{-1}	120[C]	—	—	—
	sc	LD_{50}	μg kg^{-1}	—	30[B]	24[C]	8.4[C]
Hamster	sc	LD_{50}	μg kg^{-1}	245[F]	95[B]	—	—
Farm Animal	pc	LD_{50}	μg kg^{-1}	1100[h]	—	—	—
	inhal	LC_{50}	mg m^{-3} (14 min)	400[h]	—	—	—
Chickens	sc	LD_{50}	μg kg^{-1}	—	—	50[w]	—
	ip	LD_{50}	μg kg^{-1}	—	—	71[o]	—
Frog	ip	LD_{50}	μg kg^{-1}	—	—	251[o]	—

Notes:
[a] pc, percutaneous; inhal, inhalation; iv, intravenous; sc, subcutaneous; im, intramuscular; ip, intraperitoneal. [b] Robinson (1967). [c] WHO (1970). [d] Rengstorff (1985). [e] Sidell (1974). [f] Grob and Harvey (1958). [g] National Academy of Sciences (1982). [h] Gates and Renshaw (1946). [i] Fleisher *et al*. (1963). [j] Pazdernik *et al*. (1983). [k] Brimblecombe *et al*. (1970). [l] Bosković *et al*. (1984). [m] Jovanovic (1982). [n] Schoene *et al*. (1985). [o] Chattopadhyay *et al*. (1986). [p] Lotts (1960). [q] Schoene and Oldiges (1973). [r] Brezenoff *et al*. (1984). [s] Maksimović *et al*. (1980). [t] Fredriksson (1957). [u] Clement (1984). [v] O'Leary *et al*. (1961). [w] Berry and Davies (1970). [x] Clement *et al*. (1981). [y] D'Mello and Duffy (1985). [z] Lipp (1972). [A] Wills (1961). [B] Coleman *et al*. (1968). [C] Gordon and Leadbeater (1977). [D] Leblic *et al*. (1984). [E] Inns *et al*. (1992). [F] Coleman *et al*. (1966). [G] Cabal *et al*. (2004). [H] Weger and Scinicz (1981).

enzyme, AX acetylcholine, EAX is a reversible Michaelis–Menten complex and A is acetate:

$$E + AX \xrightarrow{k_{+1}} EAX \xrightarrow{k_2} \begin{matrix} EA \\ +X \end{matrix} \xrightarrow{k_3} E + A$$

where k_{+1}, k_{-1}, k_2 and k_3 are rate constants. Inhibition with OPs takes place by a process analogous to the reaction of acetylcholine with the enzyme, by a reaction in which the leaving group of the OP is lost so that the esterase is phosphylated at the hydroxyl group of the serine residue instead of acetylated. In the above equation, AX would be the OP nerve agent, EAX a reversible Michaelis–Menten complex of the enzyme and nerve agent, EA the phosphylated enzyme, X the leaving group and A, with nerve agents, (typically) an alkoxy alkylphosphonate. Reactivation of nerve agent-inhibited acetylcholinesterase occurs by hydrolysis of the alkoxy alkylphophonyl or phosphoryl enzyme, resulting in dephosphylation and the rates of phosphylation are very variable, which partly accounts for differences in acute toxicity between the nerve agents.

The affinity with which a substrate such as acetylcholine or an inhibitor such as an OP binds to acetylcholinesterase is described by the dissociation constant for the complex EAX, K_D. This is equal to k_{-1}/k_{+1}. For inhibitors whose

Table 6. Acute toxicity of cyclosarin (GF)

Species	Sex	Route[c]	LD_{50} (μg kg^{-1}) or LCt_{50} (mg min m^{-3})
Rat	Male	inhal	371 (10 min)[b] 396 (1 h)[b] 585 (4 h)[b]
Rat	Female	inhal	253 (10 min)[b] 334 (1 h)[b] 533 (4 h)[b]
Rat	Male	im	80[c]
Mouse	Male	sc	243[d]
Guinea pig	Male	sc	44[e]
Monkey, rhesus	Male	im	46.6[f]

Notes:
[a] Inhal, inhalation; sc, subcutaneous; im, intramuscular.
[b] Anthony *et al.* (2004).
[c] Kassa and Cabal (1999).
[d] Clement (1992).
[e] Lundy *et al*. (1992).
[f] Koplovitz *et al*. (1992).

complexes with acetylcholinesterase reactivate slowly, such as OPs, k_3 can be ignored and the reaction with acetylcholinesterase can be described by a bimolecular rate constant, k_i as follows:

$$E + AX \xrightarrow[k_i]{} X + EA$$

Some useful relationships can then be derived, e.g. $k_i = k_2/K_D$ (Main and Iverson, 1966). In addition, $k_i = \ln 2/I_{50}$ (Aldridge, 1950) which allows easy estimation of k_i (The I_{50} is the concentration of inhibitor, which inhibits the enzyme by 50%). These constants have been measured for many OP chemical warfare agents and also pesticides (e.g. Gray and Dawson, 1987). The hydrolysis reaction for acetylated acetylcholinesterase is fast (Koelle, 1992), in the region of 100 μs (Lawler, 1961; O'Brien, 1976). The key to the powerful anticholinesterase effects of OPs is what happens after inhibition by these compounds. In the case of OPs, hydrolysis of the phosphylated serine residue is much slower[2] than the acetylated analogue.

Rates of phophorylation or phosphonylation (inhibition) are a function of the whole OP molecule [see Maxwell and Lenz (1992) for a discussion of the structure-activity effects that underlie cholinesterase inhibition]. Rates of reactivation (hydrolysis) are determined by the structure left attached to the active-site serine residue after loss of the leaving group. Reactivation by hydrolysis of the phosphylated enzyme, which is a nucleophilic displacement reaction, occurs at a clinically significant rate, with nearly all important OPs but always much slower than when the enzyme is acetylated by its natural substrate: thus the enzyme deacetylates in μs, but dephosphorylates in a matter of hours to days, In general, spontaneous reactivation is faster, the smaller the alkyl groups; thus dimethyl phosphoryl acetylcholinesterase reactivates faster than the diethyl analogue and diisopropyl phosphorfluoridate (DFP)-inhibited enzyme hydrolyses extremely slowly, in reality so slowly that the binding of the organophosphate to the enzyme has been described as irreversible (WHO, 1986) (for a tabulation of the $t_{1/2}$s for spontanous reactivation of various phosphylated acetylcholinesterases, see Wilson *et al*., 1992). While the active site of the enzyme is phosphylated it is of course unavailable for hydrolysis of acetylcholine. With some organophosphorylated and organophosphonylated acetylcholinesterases (but notably not most OP insecticides), hydrolysis is very slow. With soman, the situation is further complicated by an additional reaction known as ageing. This consists of monodealkylation of the dialkylphosphyl enzyme, creating a much more stable monoalkylphosphyl enzyme, the reactivation rate of which is negligible (see review by Curtil and Masson, 1993). On the basis of studies using acetylcholinesterase from *Torpedo californica*, Millard *et al*. (1999) suggested a number of non-covalent forces that might stabilize the aged enzyme, thereby preventing reactivation. Rates of ageing depend on the structure of the inhibited acetylcholinesterase produced with each nerve agent. Soman produces an inhibited acetylcholinesterase, where the active site serine is phosphylated with a pinacoloxy methylphosphonyl structure. This ages very rapidly by loss of the large pinacolyl group, with the result that reactivation of inhibited acetylcholinesterase does not occur to any clinically significant extent. Talbot *et al.* (1988) studied *in vitro* and *in vivo* ageing rates of soman-inhibited erythrocyte cholinesterase in several animal species. *In vitro*, $t_{1/2}$s were (mean ± sem) 8.0 ± 0.82 min for guinea pigs, 1.1 ± 0.08 min for marmosets, 1.4 ± 0.11 min for cynomolgus monkeys and 0.88 ± 0.03 min for squirrel monkeys. *In vivo* $t_{1/2}$s were 8.6 ± 0.94 min for the rat, 7.5 ± 1.7 min for the guinea pig and 0.99 ± 0.10 min for the marmoset. The ageing $t_{1/2}$ in human erythrocytes is known to be rapid (1.3 min) for soman-inhibited enzyme (Harris *et al*., 1978). Incidentally, this suggests that the guinea pig would not be a good model for humans in antidotal experiments in soman poisoning and that such experiments could only be undertaken realistically in primates. Because of the rapid rate of ageing of the soman-inhibited acetylcholinesterase, recovery of function depends on resynthesis of acetylcholinesterase (Gray,

[2] The term irreversible is often used for this reaction. This should not be taken to mean that reactivation of acetylcholinesterase by hydrolysis does not occur. The term is used because the OP is not recovered intact upon reactivation of the enzyme (Chambers, 1992).

1984). Substances which produce complexes with acetylcholinesterase which age virtually instantaneously have been synthesized, for example crotylsarin, *O*-(2-butenyl) methylphosphonofluoridate, which has been used as an experimental tool in the investigation of the action of oximes (van Helden *et al*., 1994). With most other OPs, including most other nerve agents, ageing occurs, by the same mechanism as soman (monodealkylation or, in the case of tabun, possibly P–N scission (Barak *et al*., 2000)), but more slowly and some spontaneous reactivation may take place. Where treatment is instituted late, or where there has been repeated exposure, significant amounts of enzyme may be in the aged state (for tabulations of ageing $t_{1/2}$s of various phosphylated acetylcholinesterases, see Wilson *et al*., 1992 and Maynard, 1999). The slow ageing process which occurs in regard to tabun and sarin has been taken by some to indicate that treatment with oxime acetylcholinesterase reactivators can be safely delayed. Nothing could be further from the truth. Ageing has little to do with the toxic effects of organophosphates: these are, of course, dependent on the accumulation of acetylcholine at essential sites and the patient will always benefit from treatment being instituted as soon as possible.

Tabun-inhibited enzyme (an *O*-ethyl *N*,*N*-dimethylamidophosphoro derivative) is slow to reactivate and Heilbronn (1963) found no detectable spontaneous reactivation with human acetylcholinesterase. The reasons for this are becoming more clear. Tabun binding of mouse acetylcholinesterase causes conformational changes in the enzyme that may stabilize the enzyme–inhibitor complex even without ageing of the complex (Ekstrom *et al*., 2006).

ACCUMULATION OF ACETYLCHOLINE

In normal circumstances, acetylcholine is hydrolyzed almost immediately by acetylcholinesterase close to receptors in the synaptic cleft at sites of action (see Bowman, 1993). The normal function of acetylcholinesterase is to hydrolyze acetylcholine in the synaptic cleft, parasympathetic effector organ or neuromuscular junction, in order to terminate transmission of a nerve impulse: failure of acetylcholinesterase activity results in accumulation of acetylcholine (Burgen and Hobbiger, 1951), which in turn causes enhancement and prolongation of cholinergic effects and also depolarization blockade. At the neuromuscular junction, where accumulation of acetylcholine initially causes fasciculation, continued accumulation produces flaccid type paralysis due to depolarization blockade.

CHOLINERGIC NEUROTRANSMISSION

Fully to undertand the effects of esterase inhibitors, such as OPs, it is necessary to discuss the cholinergic neurotransmission system. The essential features in this system are a synthetic enzyme, choline acetyltransferase (ChAT), the neurotransmitter itself (acetylcholine), a hydrolytic enzyme, acetylcholinesterase and specialized receptors, with which the acetylcholine interacts. Acetylcholine is one of a number of neurotransmitters in the nervous systems of mammals. It is synthesized from acetyl coenzyme A and choline by the enzyme ChAT in the perikaryon of cholinergic neurones and transported to nerve terminals, with ChAT existing in both soluble and membrane-bound forms. The ChAT gene also encodes another protein besides ChAT, the vesicular acetylcholine transporter. This transporter is responsible for transporting acetylcholine from the neuronal cytoplasm to the synaptic vesicles (Oda, 1999). At the nerve terminals, acetylcholine is released in response to an action potential, thereby triggering opening of voltage-gated calcium channels in the presynaptic terminal. The acetylcholine thus released crosses the synaptic cleft. Contact between the acetylcholine and specialized receptors (see below) at the proximal end of the post-ganglionic nerve fibre results in localized depolarization of the postsynaptic membrane and generation of a nerve impulse in the postganglionic nerve fibre. Transmission is similar at parasympathetic nerve endings except that the acetylcholine stimulates receptors in parasympathetic effector organs. At the neuromuscular junction, muscle fibre depolarization is initiated at the motor end plate following a similar sequence of events. In the cases of both autonomic ganglia and muscle, it is necessary for

the depolarization to exceed a threshold in order to produce postjunctional activity, e.g. initiate an action potential in a postsynaptic nerve cell or muscle fibre.

CHOLINERGIC RECEPTORS

Cholinergic receptors are divided into muscarinic and nicotinic on the basis of their sensitivity to pharmacological stimulation by muscarine and nicotine, respectively; muscarinic receptors are found in parasympathetic effector organs, and, prejunctionally at the neuromuscular junction, whereas nicotinic receptors are found at autonomic ganglia and the neuromuscular junction. More or less specific antagonists are available; thus, atropine antagonizes muscarinic agonists at muscarinic sites but has little effect at the neuromuscular junction, whereas the reverse is true of tubocurarine. The two types of receptor are fundamentally different – muscarinic receptors using a second messenger system, whereas nicotinic receptors are ligand-gated ion channels.

MUSCARINIC RECEPTORS

Five sub-types of muscarinic receptor, designated M_1 to M_5, have been cloned. Each receptor has a serpentine structure that spans the cell membrane seven times. The receptor is coupled via guanosine triphosphate-binding proteins (G-proteins) to the enzymes adenylate cyclase or phospholipase C. M_I receptors, activation of which leads to an increase in Ca^{2+} conductance of local ligand-gated Ca^{2+} channels, are found in neuronal tissue. These calcium channels are ligand-gated, unlike those in the presynaptic or prejunctional terminals which are voltage-gated. Influx of calcium ions via open channels is both electrically driven by the negative intracellular potential and concentration driven as the intracellular free Ca^{2+} concentration is about 100 nmol l^{-1} while the extracellular concentration is about 1.2×10^6 nmol l^{-1} (Ganong, 1991). Activation of M_2 receptors leads to a decrease in Ca^{2+} conductance. M_2 receptors may play a prejunctional role in modulating the activity at the neuromuscular junction. M_2 receptors are also found in the heart and mediate the depressor effects of parasympathetic activity via the vagus nerve. The distribution and some functions of the other sub-types have also been characterized (Eglen and Whiting, 1990; Jones, 1993). M_3 receptors are found in glandular tissue and M_4 receptors are found in the striatum while the M_5 subtype is present in the hippocampus and brainstem.

NICOTINIC RECEPTORS

Nicotinic receptors, found at all autonomic system ganglia and at the skeletal neuromuscular junction have been well characterized at a molecular level largely because the same type of receptor is found in the fish (*Torpedo californica)* electric organ. The structure of the nicotinic acetylcholine receptor has been reviewed (Kaminski and Ruff, 1999). These receptors are part of a 'superfamily' of ligand-gated ion channels with γ-aminobutyric acid A ($GABA_A$) receptors and glycine receptors (Ortells and Lunt, 1995). Each receptor comprises five sub-units arranged around a channel that passes through the cell membrane, but there are some differences between the receptors found at the neuromuscular junction and those in neuronal tissue, in structure and in inhibition characteristics (the former are inhibited by α-bungarotoxin). In neuronal tissue, the receptors are composed of α and β sub-units and a number of each type of sub-unit have been cloned (Boyd, 1997). The muscle receptor in adult innervated muscle contains 5 sub-units, 2 α, 1 β 1 δ and 1 ε; in denervated or embryonic muscle the ε is replaced by a γ sub-unit. Binding of acetylcholine leads to a widening of the channel and an increase in Na^+ conductance. Influx of Na^+ ions leads to depolarization of the cell membrane (see Lefkowitz *et al*., 1996).

Clinical Effects

The clinical effects of nerve agents are, to a large extent, those of acetylcholine accumulation and the effects of all of the nerve agents are similar. Those differences that have been observed are presumably due to a combination of different rates of inactivation and reactivation of the enzymes, together with different rates of ageing of the inhibited enzyme and differences in absorption, distribution, metabolism and

Table 7. Main effects of nerve agents at various sites in the body

Receptor	Target organ	Symptoms and signs
Central	Central nervous system	Giddiness, anxiety, restlessness, headache, tremor, confusion, failure to concentrate, convulsions, respiratory depression, respiratory arrest
Muscarinic	*Glands*	
	Nasal mucosa	Rhinorrhea
	Bronchial mucosa	Bronchorrhea
	Sweat	Sweating
	Lachrymal	Lachrymation
	Salivary	Salivation
	Smooth muscle	
	Iris	Miosis
	Ciliary muscle	Failure of accommodation
	Gut	Abdominal cramp, diarrhoea, involuntary defecation
	Bladder	Frequency, involuntary micturition
	Heart	Bradycardia
Nicotinic	Autonomic ganglia	Sympathetic effects, including pallor, tachycardia, hypertension
	Skeletal muscle	Weakness, fasciculation

excretion of the nerve agent. It is noteworthy that in the case of soman, kinetic differences have been recorded between different stereoisomers (Benschop *et al*., 1987).

The symptoms and signs of nerve agent poisoning may be divided into three groups, muscarinic, mediated by muscarinic receptors in parasympathetic effector organs, nicotinic, mediated by nicotinic receptors in autonomic ganglia and the neuromuscular junction, and effects in the central nervous system, which are mediated by receptors of both types. Acute effects of OP nerve agents are given in Table 7. The muscarinic symptoms and signs result from increased activity of the parasympathetic system and include bronchorrhoea, salivation, constriction of the pupil of the eye (miosis),[3] abdominal colic and bradycardia (Grob and Harvey, 1953). Nicotinic effects at autonomic ganglia can produce pallor, tachycardia and hypertension. The clinical effects in the cardiovascular system depend on whether muscarinic or nicotinic effects predominate; bradycardia or tachycardia may occur and, in some cases arrhythmias (see below). At the neuromuscular junction, nicotinic signs include muscle fasciculation and later paralysis. If the patient survives the acute cholinergic syndrome, the effects of nerve agents are largely reversible, although, as discussed above, with soman recovery may be very slow and in certain circumstances there may be long-term changes in the CNS (see below). Where death occurs, it is caused by respiratory paralysis, which may be central or due to the anticholinesterase action at the neuromuscular junction (Chang *et al*., 1990).

[3] Miosis is the term used to describe the constriction of the pupil. The term is often assumed to be derived from the same Greek root as meiosis, i.e. a diminution. This is in fact not the case. Miosis, and the earlier variant myosis, are derived from the Greek root myein (or muein): to close, blink (Shorter Oxford English Dictionary, Webster's Third New International Dictionary).

NON-ANTICHOLINESTERASE EFFECTS

Clinically, the most important effects of OP nerve agents are anticholinesterase actions. However, it should not be forgotten that OPs bind to a variety of enzymes, including esterases other than acetylcholinesterase, e.g. carboxylesterase, (long-chain fatty acid hydrolase), serine

peptidases, amidases and proteases and others (see Lockridge and Schopfer, 2005). Moreover, there is some evidence that some OP anticholinesterases can act directly on muscarinic and nicotinic receptors (Bakry *et al*., 1988; Silveira *et al*., 1990; Mobley, 1990; Eldafrawi *et al*., 1992). More complex direct effects at cholinergic receptors may be important: Rocha *et al*. (1999) showed that VX, at concentrations that had little effect on cholinesterase, interacted directly with presynaptic muscarinic receptors, causing a selective inhibition of the evoked release of GABA. This would increase excitatory neurotransmission and these workers hypothesized that this might account for the ability of VX to induce convulsions.

It has also been demonstrated that OPs, including nerve agents, can affect neurotransmission pathways other than cholinergic ones, for example γ-aminobutyric acid (GABAergic) receptors as well as glutamatergic, dopaminergic, somatostatinergic and noradrenergic systems (Lau *et al.*, 1988; Fletcher *et al*., 1989; Fosbraey *et al*., 1990; Naseem, 1990; Smallbridge *et al*., 1991; Chechabo *et al*., 1999; Tonduli *et al*., 1999; Rocha *et al*., 1999). It is likely that most if not all such perturbations are secondary to effects on cholinergic systems, but there is evidence that recruitment of non-cholinergic systems is responsible, at least in part, for seizure activity (Solberg and Belkin, 1997). Furthermore, the efficacy of some experimental treatments not directed at the cholinergic neurotransmission system suggests a role for other neurotransmission systems. Thus, Braitman and Sparenborg (1989) found that a potent antagonist at the *N*-methyl-D-aspartate (NMDA)-type glutamate receptor (MK-801), together with other antidotes, protected against seizures induced in guinea pigs by soman. Moreover, Filliat *et al*. (1999) found that learning deficits produced in rats by soman were reduced by antagonists at α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors and NMDA-type glutamate receptors, together with atropine. It has also been suggested that the protective action of caramiphen aginst OP poisoning may be, in part, due to the ability of this drug, which is also an anticholinergic agent, to modulate the NMDA receptor (Raveh *et al*., 1999)

Kovacic (2003) suggested that oxidative stress might play a part in the toxic manifestations of OPs.

It is possible that some of the CNS effects of nerve agents are secondary to changes in the blood brain barrier. There is little evidence that nerve agents will cause OP-induced delayed polyneuropathy at doses likely to be encountered and survived by man (see below).

EFFECTS ON SPECIFIC ORGANS

The eye

Unlike OP insecticides, the G-type nerve agents are most likely to be encountered as vapours and eye effects occur early. Nerve agents produce miosis (constriction of the pupil); this produces a feeling that the surroundings are dim, or that illumination has been reduced. The onset is rapid and may last for several days. Miosis is a very sensitive indicator of exposure to nerve agents and van Helden *et al*. (2004a) concluded, on the basis of studies in guinea pigs and marmosets with sarin, that miosis would occur during low-level exposure at levels that would not be detectable by the currently fielded alarm systems, assuming that humans are of similar sensitivity to these experimental species.

Spasm of the ciliary muscle may impair accommodation and is associated with severe headache. Long-lasting miosis, associated with eye pain, was a notable clinical sign in the Tokyo Subway (underground railway) terrorist attack with sarin and the same was true of the sarin attack at Matsumoto (Nohara and Segawa, 1996).

Dilatation of subconjunctival blood vessels occurs and the eye becomes bloodshot. After exposure to high concentrations of nerve agent, the eyes take on a glassy appearance: the appearance is sometimes compared to that of a glass marble. The lachrymal glands do not seem to be much affected by exposure to nerve agent vapour and tearing is not a reliable early sign of exposure.

The Respiratory Tract

The upper respiratory tract contains two components that are under cholinergic control. These

are the mucous glands and smooth muscle. The response to nerve agents by the former is an increase of secretions resulting in bronchorrhea and rhinorrhea (runny nose) and, in severe cases, foaming around the nose may occur. The effect on smooth muscle is to produce bronchospasm. Respiratory function may also be affected by the action of nerve agents on the neuromuscular junction of the (striated) muscles of respiration and by central effects on the respiratory centre.

Heart

The acute effect on the heart depends on the relative predominance of muscarinic or nicotinic effects. Bradycardia or tachycardia may occur, as well as arrhythmias, including atrioventricular block and various ventricular arrhythmias. An arrhythmia characteristic of poisoning with OP pesticides is Torsade de Pointes (Ludomirski *et al*., 1982), while soman and other OPs have been reported to produce histopathological changes in the myocardium in both experimental animals and humans (McDonough *et al*., 1989; Singer *et al*., 1987; Pimentel and Carrington da Costa, 1992; Koplovitz *et al*., 1992; Britt *et al*., 2000). A few patients in the Matsumoto nerve agent attack had arrhythmias (Okudera, 2002).

Nervous system and skeletal muscle

Systemically, nerve agents produce fasciculation and then blockade at the neuromuscular junction with weakness and paralysis. Paralysis of the muscles of respiration (see above) may interfere with respiration and is potentially life-threatening. Some of these effects may be mediated by direct actions at the receptor-ion channel complex. Soman and sarin were reported by Goldstein *et al*. (1987) to alter muscle spindle function.

Separate from the acute effects of anticholinesterases upon the neuromuscular junction (discussed previously), two further syndromes involving the neuromuscular system have been associated with OP poisoning. These are (1) the intermediate syndrome (IMS), and (2) organophosphate-induced delayed polyneuropathy (OPIDP).

INTERMEDIATE SYNDROME (IMS)

Senanayake and Karalliedde (1987) described a new form of neurotoxicity following intoxication by organophosphorus insecticides. As it occurred after the acute syndrome and before the onset of classical OPIDN, they called it the 'Intermediate Syndrome'. This phenomenon consists of marked weakness of the proximal skeletal musculature (including the muscles of respiration and neck muscles) and cranial nerve palsies. IMS comes on 1–4 days after acute poisoning; respiratory support is often necessary and, if it is provided, recovery occurs within 4–18 days. Although the IMS has not been described in cases of accidental nerve agent poisoning, it is likely that it would occur, at least in some cases. The pathogenesis of IMS is not known with certainty, although it appears to be a post-junctional non-depolarizing blockade. IMS is probably a consequence of cholinergic overactivity at the neuromuscular junction (NMJ), perhaps through down-regulation of post-junctional nicotinic receptors (i.e. a reduced density of functioning nicotinic receptors), with a possible contribution from prejunctional muscarinic receptors (Marrs *et al*., 2005). It has also been suggested that failure to hydrolyze acetylcholine may reduce the supply of choline, which is a substrate for ChAT, the enzyme that synthesizes acetylcholine. Furthermore, connection has been suggested between the IMS and OP-induced myopathy (Senanayake and Karalliedde, 1992). Myopathy associated with OPs was first observed many years ago and has been observed histologically in experimental animals with the nerve agents tabun, soman, and sarin (Preusser, 1967; Ariens *et al*., 1969; Gupta *et al*., 1987a,b; Bright *et al*., 1991; Hughes *et al*., 1991; Koplovitz *et al*., 1992; Britt *et al*., 2000). The changes characteristic of OP-induced myopathy seem to be initiated by calcium influx (Leonard and Salpeter, 1979) as a consequence of acetylcholine accumulation at the neuromuscular junction (Marrs *et al*., 1990; Inns *et al*., 1990). In general the distribution of the myopathy parallels the distribution of muscles affected by IMS, although that was not true of the study in rhesus macaques by Britt *et al*. (2000). If it is these histological changes that underlie IMS and, as they have been observed

with chemical warfare nerve agents, the development of the syndrome can be anticipated in the recovery phase of nerve agent poisoning in at least some cases. However, it should be noted that oximes are reported to protect against the myopathy, whereas there have been reports that, in insecticide poisoning, oximes have not always protected against IMS. Furthermore, the time-course of the myopathy observed in experimental animals is not similar to the time-course for the development of IMS. Karalliedde *et al*. (2006) concluded that IMS arose from down-regulation of the nicotinic acetylcholine receptor, caused by acetylcholine accumulation, and that myopathy and IMS are not causally related, but have a common origin in acetylcholine accumulation.

NERVE AGENT-INDUCED DELAYED POLYNEUROPATHY

Organophosphate-induced delayed polyneuropathy (OPIDP) is a symmetrical sensory-motor neuropathy, with both central and peripheral components, tending to be most severe in long axons and occurring 7–14 days after exposure to OPs. In severe cases, it is an extremely disabling condition, the central component being irreversible. Inhibition of neuropathy target esterase (NTE), an esterase of unknown function present at several sites, including neurones, where it is an integral membrane protein (Glynn, 2000), appears to be necessary for OPIDP to develop (see reviews by Somani and Husein, 2000 and Senanayake, 2001). This is followed by an ageing reaction similar to that described for soman with acetylcholinesterase above (Johnson, 1975). By contrast with IMS, it is unlikely that nerve agents possess the capability to cause OPIDP. In experimental studies, nerve agents do not usually bring about OPIDP (Anderson and Dunham, 1985; Crowell *et al*., 1989; Johnson *et al*., 1985; Goldstein *et al*., 1987; Parker *et al*., 1988; Henderson *et al*., 1992). Gordon *et al*. (1983) reported studies in hens (Ross white or Sussex) *in vivo,* protected with physostigmine, atropine sulphate and pralidoxime mesilate (P2S): OPIDP (as assessed clinically) with NTE inhibition was only seen at 30–60 $\times$ LD_{50} for sarin, but not at 38 $\times$ LD_{50} for soman or 82 $\times$ LD_{50} for tabun. Furthermore, in the case of soman, Johnson *et al*. (1985) showed that only a tiny proportion of inhibited NTE from hen brain and spinal cord underwent ageing. Crowell *et al*. (1989) studied the potential for sarin and soman to cause OPIDP. Sarin type I[4] was given by gavage at single doses of 61, 200 and 400 $\mu g\ kg^{-1}$, sarin type II at doses of 70, 140 and 280 $\mu g\ kg^{-1}$ and soman at doses of 3.5, 7.1 and 14.2 $\mu g\ kg^{-1}$ to groups of five Leghorn hens protected from acute cholinergic toxicity with atropine. There were appropriate vehicle controls and tri-*o*-cresyl phosphate was used as a positive control. NTE was measured in brain, spinal cord and lymphocytes. With sarin type I, the highest dose was lethal within 24 h and the treatment did not significantly depress NTE in any tissue. The highest dose of sarin II decreased lymphocyte NTE to 33% of the control value. In the brain and spinal cord, significant depression of NTE was not seen at any dose. Soman did not produce significant depression of NTE. In all cases tri-*o*-cresyl phosphate produced significant depression of NTE. Despite this, there have been reports that sarin may produce histopathological changes in rodents similar to those found in OPIDP (see Somani and Husain, 2001).

Studies *in vitro* also suggest that the development of OPIDP is unlikely to be a clinical problem in humans. Thus, Gordon *et al*. (1983) compared the inhibitory potency of the nerve agents, sarin, soman, tabun and VX, and some other OP compounds, including diisopropyl phosphorofluoridate (DFP), mipafox and related compounds, against NTE and AChE. The ratio I_{50} for inhibition of acetylcholinesterase/I_{50} for inhibition of NTE was 0.0056 for sarin, 0.0012 for soman, 0.0005 for tabun and 10^{-6} for VX. In the case of the neuropathic OPs, the ratio was 1.13 for DFP and 1.8 for mipafox. Moreover, structure–activity considerations lend no support to suggestions that nerve agents would be neuropathic (Aldridge *et al*., 1969).

Thus, the reason for the expectation that nerve agents would be non-neuropathic in man is that concentrations of nerve agent required to produce AChE inhibition are low, by comparison with concentrations required for inhibition of

[4] Types I and II refer to the stabilizers used.

NTE. Nevertheless, OPIDP was one hypothesis for Gulf War Syndrome (Institute of Medicine, 1996).

CENTRAL NERVOUS SYSTEM

OPIDP, which has a central component, was discussed above. The nerve agents produce a wide range of effects upon the central nervous system, ranging from anxiety and emotional lability at low doses, to convulsions and respiratory paralysis at higher ones. It is important to note that doses considerably below lethal doses can markedly degrade performance of tasks in behavioral studies (Brimblecomb, 1974; Wolthuis and Vanwersch, 1984; D'Mello and Duffy, 1985; D'Mello, 1993; DiGiovanni, 1999) and there is evidence that the decremental effects of exposure to single doses of nerve agents may be prolonged (McDonough *et al*., 1986). Clearly, this is of importance as it is likely that military performance of personnel would be impaired: affected servicemen might not only lose the motivation to fight but also lose the ability to defend themselves and be unable to carry out the complex tasks frequently required in the modern armed forces. Effects on skilled personnel, such as pilots and navigators, would be particularly disabling. In a terrorist attack, rescuers may be affected and their skills impaired.

Long-term and delayed effects

Previously, experimental work with nerve agents has been concentrated on the acute effects. However with the possibility of mass casualties from terrorist outrages, the possibility of persistent neurobehavioral and psychiatric effects, such have sometimes been observed with excessive exposure to OP pesticides, needs to be considered. Such effects have included headache, anxiety, agitation, insomnia, irritability, impaired memory and difficulty in concentrating (Annau, 1992; Jamal, 1995; Lader, 2001; Feldman, 1999).

The long-term effects of low dose exposure and the delayed effects of acute exposure are often conflated. The latter are easier to understand. A number of studies have been performed to investigate subtle changes in humans exposed to various sorts of OPs, and with differing patterns of exposure, and these have been reviewed (Ray, 1998a; Committee on Toxicity of Products in Food, Consumer Products and the Environment, 1999; Karalliedde *et al*., 2000; Romano *et al*., 2001). Many of the studies reviewed refer to OP insecticides. It is difficult to summarize this work as the patterns of exposure have been very varied. Of course, long-term low dose exposure and acute high dose exposure are two ends of a spectrum of different exposure scenarios and many intermediate patterns of exposure are possible. Studies have also been undertaken in experimental animals (including primates) and some of these are discussed below.

Delayed effects of high dose exposure

The anticholinesterase effects induced by nerve agents in the CNS are reversible and histopathological changes are usually exiguous in fatalities and in animals dying during acute toxicity studies. Thus Anzueto *et al*. (1986) found that, in baboons, given intravenous infusions of soman, only minimal CNS histopathological changes were observed. The absence of specific changes hinders diagnosis at autopsy; moreover, it implies that complete recovery after successful treatment is probable. However, the situation may be different after survival of near-lethal doses and where humans survive, it is probable that functional deficits would be observed. It is also likely that were humans to survive for an appreciable period before dying, histopathological changes would be seen in the CNS. In animals, after substantial doses of soman, the initial histopathological changes seen are edema, particularly astrocytic and perivascular hemorrhages. Neuronal degeneration and necrosis, sometimes diffuse, may be observed, together with more discrete infarcts, with necrosis of all cell types. Such changes may be detected particularly in the hippocampus and piriform cortex (McLeod, 1985). This picture, which does not seem to correlate with areas in which the blood-brain barrier is compromised, may proceed to an encephalopathy. Most commonly, this affects the cortex, hippocampus and thalamic nuclei, a distribution that suggests anoxia is a likely cause (McLeod, 1985; McDonough *et al*., 1986,1989). The hypothesis that hypoxia secondary to convulsions is the

cause, is supported by the fact that the nerve agent-induced effects have been correlated with seizure activity (Anzueto *et al*., 1986; Britt *et al*., 2000) and that anticonvulsant γ-aminobutyric acid ($GABA_A$) agonists, such as diazepam or midazolam, alleviate the effects (Martin *et al*., 1985; Anderson *et al*., 1997). Nevertheless, the attribution of nerve-agent-induced pathological changes to hypoxia and/or convulsions is not the view of all (Petras, 1981) and in an *in vitro* ultrastructural study using rat rat hippocampal slices, Lebeda *et al*. (1988) found morphological differences between the effects produced by hypoxia and soman. OP poisoning is indeed sometimes associated with long-term CNS changes, both in experimental animals and in humans (Holmes and Gaon, 1956; Korsak and Sato, 1977; Rosenstock *et al*., 1991) and this is likely to be the case both with pesticides and nerve agents (see reviews by Marrs and Maynard, 1994 and Ray, 1998a).

Human data on nerve agent exposure is scanty compared to that on OP pesticides. A notable study (on workers) involving nerve agents was carried out by Duffy *et al*. (1979). Behavioral signs and subtle EEG changes were noted after exposure to sarin, severe enough to cause symptoms and clinical signs. Burchfiel *et al*. (1976) described changes, which persisted for a year in the EEGs of rhesus monkeys after a single large dose or repeated small doses of sarin. The lower dose used was 1 μg, given by ten weekly injections (total dose 10 μg = 7% LD50). This result, which is somewhat surprising, must be interpreted cautiously as the group size was small (three per group) and similar but not identical electroencephalographic changes were seen after the organochlorine cyclodiene pesticide, dieldrin, namely a relative increase in ß activity. Subjects from volunteer programmes have been studied in the USA (National Academy of Sciences, 1982) and here there was no clear evidence of CNS effects or any effect on mortality. In a 1- and 2-year follow-up of survivors of the Matsumoto sarin exposure, epileptiform EEG abnormalities were found in four out of six severely poisoned subjects (Sekijima *et al*., 1997). In a study of 18 victims of the Tokyo subway sarin incident, 6–8 months after exposure, the event-related and visual evoked potentials were significantly prolonged compared to matched controls, although no subject had obvious clinical abnormality (Murata *et al*., 1997). A case-report from Japan noted specific interference with memory in a victim of the sarin exposure in Tokyo: this persisted to 6 months after exposure and extended to the period in a retrograde fashion to 70 days before exposure (Hatta *et al*., 1996). In a suspected case of VX poisoning, antegrade and retrograde amnesia was observed (Nozaki *et al*., 1995). In a study of members of a rescue team, who attended the Matsumoto sarin exposure, all symptoms of the affected personnel had resolved a year after exposure (Nakajima *et al*., 1997). In a cross-sectional study of rescue staff and policemen approximately 3 years after they were exposed to sarin on the Tokyo subway, effects were observed on memory (Nishiwaki *et al*., 2001). Yokoyama *et al*. (1998) reported vestibulocerebellar effects 6–8 months after exposure to sarin in the Tokyo incident. This comprised low-frequency sway, which was more severe in females. Of course, in the aftermath of a terrorist use of a nerve agent, psychological effects are to be expected in addition to any effect from damage to the CNS (DiGiovanni, 1999).

The implications for treatment are, at this time, uncertain; the most logical course would be to avoid convulsions and/or anoxia as much as possible during treatment of acute nerve agent poisoning.

Low dose exposure

A variety of effects in human and animals have been attributed to OP exposure but it is less clear whether low doses, particularly subconvulsive ones, can bring about long-term changes in CNS function. If that were the case, it is clearly desirable to know if there is a threshold dose below which effects are not observed. Effects observed after long-term low dose exposure to OPs are less easy to explain than delayed effects of high dose exposure, but it should be noted that OPs, including nerve agents, can react with targets other than cholinesterases (see above). Richards *et al*. (1999) found two novel OP-reactive sites in rat brain homogenates by inhibition of ^{3}H-DFP labeling using several OP insecticides or their oxons. One of the sites had a molecular mass

of 85 kDa and Richards *et al*. (2000) purified the 85 kDa site from porcine brain and characterized it as an acylpeptide hydrolase (see reviews by Ray, 1998a,b).

After reviewing the available data, both in humans and in experimental animals, Moore (1998) concluded that the available data indicated that exposure to nerve agents at doses producing no clinical signs or symptoms of acute toxicity did not produce chronic illness (see also Brown and Brix, 1998). Pearce *et al*. (1999) were unable to find significant persisting effects of single im doses of sarin producing 36.4 to 67.1% erythrocyte acetylcholinesterase inhibition in marmosets on EEG or the CANTAB automated test battery. There was a marginal increase of beta 2 EEG activity attributable to an affect in one subject: Haley (2000) speculated that this might be due to a polymorphism for the enzyme paraoxonase 1 (PON1) in one marmoset. van Helden *et al*. (2004b) have shown effects on the EEG from sarin vapor at concentration × time values (Cts) of 0.2 and 0.1 mg min m^{-3} (time of exposure was 5 h) in vehicle and pyridostigmine-treated marmosets; these effects were persistent. Furthermore, Bajgar *et al*. (2004) found persistent (4 weeks) effects on a few behavioural parameters in guinea pigs given single inhalation exposures to soman. The concentrations used were 1.2, 1.5 and 2.7 mg m^{-3} and exposure was for 1 h. Only butyrylcholinesterase was inhibited at the lowest concentration at 1 day, while red cell cholinesterase was 66% control at the mid-concentration and 28% control at the highest concentration, both at 1 day. Brain acetylcholinesterase measured at 4 weeks was only inhibited > 20% at the highest concentration and then only in the basal ganglia, not the other areas in which enzyme activity was estimated. Significant increases in neuroexcitability score were seen at all test concentrations, compared to controls at 4 weeks, although the differences were small in magnitude. Unsurprisingly, larger differences between test groups and controls were seen at 1 day. The same group (Kassa *et al*., 2004) reported a study in rats, in which exposure to sarin by inhalation was studied. Exposure was for 1 h at concentrations of 0.8 $\mu g\,l^{-1}$, which produced red cell acetylcholinesterase depression of < 20% compared to controls, 1.25 $\mu g\,l^{-1}$, which produced red cell acetylcholinesterase depressions of 20–30%, and 2.5 $\mu g\,l^{-1}$, which produced red cell acetylcholinesterase depression of 40–50%. Group sizes were 10. Exposures were on single occasions, except that exposure to the middle concentration was carried out repeatedly and additionally in a separate group of animals. A functional observational test battery (FOB) of behaviour was carried out at 3 months. Significant abnormality was seen after single exposure at the highest concentration in respect of a number of observations (gait disorder and score, mobility score, activity and stereotypy and at the mid-concentration only in respect of the last – more changes were seen after repeated exposure). Changes in spatial discrimination in the Y-maze were seen at all doses shortly after exposure at all test concentrations, but at the highest concentration this persisted for 3 weeks. This study could be interpreted as suggesting a single exposure threshold for long-term effects at 20–30% erythrocyte acetylcholinesterase depression in the rat.

Sleep disruption was seen in mice during a study on soman by Crouzier *et al*. (2004). The dose used was 50 $\mu g\,kg^{-1}$ bw sc.

Visual field defects persisting for 1 year, which had disappeared 17 months after exposure, were found in a single subject exposed to sarin in Matsumoto (Sekijima *et al*., 1997).

Reproductive toxicity, developmental toxicity and developmental neurotoxicity

On the basis of a study with VX (Goldman *et al*., 1988), there was little evidence that OP nerve agents presented a reproductive hazard at doses below those toxic to the parents. On the basis of studies with sarin (Denk, 1975; La Borde and Bates, 1986), there is little evidence that OP nerve agents have a potential for teratogenesis or fetoxicity at doses below those toxic to the dams. There is no direct evidence that exposure to nerve agents has produced developmental toxicity in humans; data on exposure of pregnant women to nerve agents are unsurprisingly extremely scanty. Four pregnant women were exposed to sarin in the Tokyo exposure at 9–36 weeks' gestation (Ohbu *et al*., 1997). All had normal offspring. Nevertheless, because of the profound effects of

OPs on neurotransmission, there is a strong theoretical basis for concern that effects which, in adults would be reversible, would in the embryo, fetus and neonate be irreversible. With pesticidal OPs, there are data in animals to suggest a potential for developmental neurotoxicity. Thus, studies have suggested the possibility that chlorpyrofos may have effects on developing organisms (Tang *et al*., 1999; Qiao *et al*., 2001) and a body of work is accumulating on OP pesticides in response to the development of a standardized developmental neurotoxicity test in the rat (US EPA, 1998; OECD, 2003; Fenner-Crisp *et al*., 2005). However, formal developmental neurotoxicity tests on OP nerve agents have not been done and the effects have to be inferred from knowledge on OP pesticides [see reviews by Kitos and Suntornwat (1992), Slotkin (2005) and Makris (2005)]. There is little doubt that, with the appropriate pattern of exposure to pregnant animals, including humans, nerve agents would be developmental neurotoxicants. Further infants and children also have developing nervous systems and these individuals may be at greater risk of long-term effects on the nervous system than adults (Guzelian *et al*., 1992). Nevertheless, with pesticides, it has been suggested that most exposures of pregnant women have not shown adverse effect on the offspring, presumably because exposure is of short duration and occurs only once (McElhatton, 1987; Minton and Murray, 1988) and it seems likely that that would be the case with nerve agents.

Delayed effects outside the CNS

The nerve agents are generally considered to be acute toxicants and their delayed effects have been relatively little investigated. Li *et al*. (1998) reported an increase in sister chromosome exchange (SCE) in the lymphocytes of victims of the Tokyo sarin disaster. Because of the probability that the victims were exposed to by-products of sarin synthesis, diisopropyl methylphosphonate, diethyl methylphosphonate and isopropyl ethyl methylphosphonate, these were also studied. The frequency of SCE was determined in human lymphocytes exposed to these by-products: all three compounds increased the frequency of SCE compared with controls. Sarin and soman did not produce unscheduled DNA synthesis in isolated rat hepatocytes but decreased repair synthesis was seen with sarin (Klein *et al*., 1987). Decreased excision repair capacity has also been seen with the insecticide diazinon, and so this may be a more general effect of OPs.

EFFECT OF ROUTE OF EXPOSURE

Vapour

Onset is rapid and the eyes and respiratory system are most affected. Low-level exposure causes tightness of the chest, rhinorrhea and salivation. Dimming of vision due to miosis, eye pain and headache then follow. On examination, the pupils are constricted and the conjunctivae hyperaemic. These effects may last several hours after cessation of exposure and the headache and visual problems several days. In severe cases, salivation and rhinorrhea are more marked, and wheezing and dyspnea are prominent. Other effects, such as abdominal pain, vomiting, involuntary defecation and micturition, weakness, fasciculation and convulsion, follow depending on the degree of systemic absorption. Death may occur from respiratory failure.

The attack with sarin on the Tokyo Subway (underground railway) has added considerably to our knowledge of the clinical effects of sarin vapor. The symptoms observed were largely as expected, namely cough, difficulty in breathing and tightness of the chest, bradycardia and eye pain (Masuda *et al*., 1995; Nozaki *et al.*, 1995; Ohbu *et al*., 1997) (see Chapter 13).

Skin contamination

Local effects at the site of contamination include sweating and local fasciculation. Fasciculation may spread to involve whole muscle groups, while the onset of systemic symptoms and signs is generally slower than after vapour exposure.

Ingestion

Ingestion of nerve agent may occur from contaminated food or water. Colicky pain occurs,

together with nausea, vomiting, diarrhoea and involuntary defecation, would be expected.

SUB-GROUPS PARTICULARLY LIABLE TO NERVE AGENTS

Most work on nerve agents has been directed at management of poisoning in military personnel, particularly soldiers; soldiers are generally physically fit and young. With the possibility of terrorist use of chemical weapons in public places, other groups need to be considered. Developmental neurotoxicity and nerve agents has already been discussed (see above) and in this respect (and possibly others) the fetus, infant and young child might represent a susceptible subgroup. It has already been noted that four pregnant women were exposed to sarin in the Tokyo disaster and that all had normal offspring. Nevertheless, it must be recognized that OP pesticides are embryo- and fetocidal and OP pesticide exposure of mothers has been associated with effects including cardiac defects in offspring (see review by Pelkonin *et al*., 2005). In a mass-casualty situation with a single high-dose exposure, it is likely that the outcome for the embryo/fetus would be largely determined by the outcome for the mother.

The elderly also represent a potentially susceptible sub-group to toxicants (Stevenson, 1990) including nerve agents; the reasons for this are numerous but may include impaired organ function, most notably, in acute intoxication, poor cardiovascular and respiratory status. Furthermore, decreased hepatic and renal function may impair metabolism and excretion of nerve agents (and also antidotes). Other groups, those with chronic conditions such as diabetes, may represent challenges particularly in management.

In healthy people, variation in activity of metabolizing enzymes, including that due to polymorphisms, may cause differences in susceptibility. One enzyme that has received a lot of attention is paraoxonase 1 (PON1). The Q-type allozyme (Gln_{192}) of PON1 hydrolyzes sarin and soman more effectively than type R (Arg_{192}) (Costa *et al*., 1999,2005). The clinical significance of the PON1 192 polymorphism remains to be established in respect of nerve agents (see reviews by Costa *et al*., 2002,2003,2005). A connection between this polymorphism and Gulf war syndrome has been suggested based on epidemiological studies (Haley *et al*., 1999) as it has with ill-health in UK sheep farmers and shepherds, using OP sheep dips (Cherry *et al*., 2005). It should be noted that the PON1 192 polymorphism (and the 55 polymorphism) have been associated with ill-health outcomes independent of OP exposure, including coronary heart disease (Shih *et al*., 2002); this may confound some of the studies mentioned above. There are several variants of butyrylcholinesterase, and individuals are known who completely lack plasma butyrylcholinesterase activity (Østergaard, 1992). It has been suggested that in nerve agent poisoning butyrylcholinesterase acts as a 'sink' for nerve agents. If that is the case it would be expected that those with low or absent butyrylcholinesterase activity would be more susceptible to nerve agents.

LABORATORY INVESTIGATIONS

Measurement of cholinesterase activity

The cornerstone of laboratory diagnosis of nerve agent poisoning is measurement of enzyme activity. Numerous methods are available for determination of both acetylcholinesterase and butyrylcholinesterase, most of which measure catalytic activity (see St Omer and Rottinghaus (1992) and Swaminathan and Widdop (2001). Of these, the Ellman method (Ellman *et al*., 1961) and its subsequent modifications are most widely used. Plasma butyrylcholinesterase activity correlates badly with brain acetylcholinesterase activity and is best thought of as a marker of poisoning rather than a prognostic indicator, although in certain circumstances it may have a role in cholinergic neurotransmission (See review by Casida and Quistad, 2004). Even activity of the preferred red cell acetylcholinesterase correlates poorly with central nervous acetylcholinesterase activity, seriously limiting the use of the former to assess the severity of poisoning (Jimmerson *et al.*, 1989; Karalliedde, 2002). The reasons for this are complex. Butyrylcholinesterase is a different

enzyme than acetylcholinesterase, with different substrate specificities and with different rates of inhibition, reactivation and ageing from acetylcholinesterase. Butyrylcholinesterase is predominantly synthesized in the liver and activity is affected by conditions such as liver disease and a large number of other factors, including the existence of inherited variants with different or even no enzymatic activity (Østergaard *et al*., 1992; Swaminathan and Widdop, 2001). These factors present difficulties with using butyrylcholinesterase in the diagnosis and management of nerve agent poisoning (see discussion by Brock and Brock, 1993). The activity of butyrylcholinesterase in an individual poisoned with an OP is, at any time, a function of inhibition, spontaneous reactivation and resynthesis, with the added possibility of ageing, all occurring concurrently. It should be noted that the rate constants for these processes are different for butyrylcholinesterase and acetylcholinesterase in any organism, as the two enzymes are not the same gene product. Acetylcholinesterase activity is the sum of the same processes, but there are two important additional considerations, i.e. (1) it is the same gene product as acetylcholinesterase in the nervous system (the enzyme of interest), and (2) resynthesis cannot occur in the erythrocyte and the $t_{1/2}$ for recovery here is consequently the same as the half-life of the red blood cell, while resynthesis can occur at other sites, and may not be insubstantial (Wehner *et al*., 1985). Additionally, the enzymes in the plasma and red blood cell may be more accessible to inhibitor than that in the nervous system, especially the central nervous system (for discussion, see Marrs, 2001 and Karalliedde, (2002). It is unlikely that a peak erythrocyte acetylcholinesterase depression of 30% or less would produce clinical signs or symptoms, and below 40% any signs would be expected to be very mild.

George *et al*. (2003) have studied the use of antisera to distinguish between native and inhibited acetylcholinesterase, providing a basis for measuring biomarkers of OP, including nerve agent, exposure.

Alkylphosphates

OPs, both pesticides and nerve agents, lose their leaving groups when they react with acetylcholinesterase. Hydrolysis of the reaction product produces an alkylphosphate or alkylphosphonate. Methods have been developed for measuring the VX–acetylcholinesterase hydrolysis product, *O*-ethyl methylphosphonic acid, and the sarin–acetylcholinesterase hydrolysis product, *O*-isopropoxy methylphosphonic acid (IMPA) (Noort *et al*., 1998) (for review, see Noort *et al*., 2002). IMPA and methylphosphonic acid were detected in patients from the Matsumoto sarin exposure (Nakajima *et al*., 1998). An alternative is to release isopropyl methylphosphonylserine or methylphosphonoserine from the active site of inhibited erythrocytic acetylcholinesterase and measure the sarin hydrolysis product and this was done on some victims of the Tokyo sarin exposure (Nagao *et al*., 1997). Another approach is to release the inhibitor bound to butyrylcholinesterase within the enzyme–inhibitor complex, using fluoride and measure the resulting phosphofluoridate using gas chromatography (van der Schans *et al*., 2004).

Black *et al*. (1999) found that sarin and soman bind to a tyrosine residue in human plasma and suggested that this could form the basis of a biomarker of exposure to these agents.

DIAGNOSIS POST-MORTEM

There may be signs of the acute cholinergic crisis preceding death, e.g. excess secretions in the form of foam around the nose. Furthermore, devices may have detected nerve agents in the atmosphere or on surfaces. Diagnosis of acute nerve agent poisoning at autopsy would be made difficult because of the paucity of histopathological changes that would be present in the nervous system. Cerebral edema may be present. One of the few histopathological changes to be expected *post-mortem* would be changes in skeletal muscle, sometimes called ‘segmental myopathy’. If biological fluids could be obtained, marked inhibition of acetyl- and butyrylcholinesterase activity would be present and, depending on the nerve agent involved, urinary alkylphosphates might be present if the patient has survived any length of time (see above). Histochemistry has been used to diagnose OP pesticide poisoning (Petty, 1958) and could doubtless be used to detect nerve agent poisoning, although it is, at most,

semiquantitative (Marrs and Bright, 1992). Methods may be available to measure the nerve agent itself in body fluids. Measurement of cholinesterase activity and demonstration of inhibition is not, of course, specific for particular types of OP, whereas alkylphosphates may be (see review by Ballantyne, 1992).

REFERENCE DOSES/HEALTH-BASED GUIDANCE VALUES FOR NERVE AGENTS

In the past, the notion of reference doses for nerve agents would have been thought a little preposterous. However, chronic reference doses/acceptable daily intakes have been established for chemical warfare agents, *inter alia* the nerve agents, soman, sarin, tabun and VX for exposure by the oral route. This was at the behest of the US Army, which asked the National Research Council to review the US Army's interim reference doses (Bakshi *et al*., 2000; Subcommittee on Chronic Reference Doses for Selected Chemical-Warfare Agents, 2000a–g). This was chiefly a result of the consideration of environmental contamination at various sites in the USA, but such reference doses could also be useful in deciding detection limits that would be needed where food was contaminated with nerve agents, either accidentally or deliberately. As many effects of nerve agents are not route- or pathway-specific, these reference doses could probably be applied to other routes of exposure. The data used to calculate these reference doses, in comparison with dossiers available for regulated substances, such as pesticides, are deficient. There was a lack of long-term studies, and with some nerve agents, multigeneration and developmental toxicity studies. Furthermore, there were problems with study design, in terms of numbers of animals and good laboratory practice. Allowance was made for these problems by the use of extra uncertainty factors.

CONCLUSIONS

The organophosphate nerve agents continue to pose major problems in chemical defence. Important measures in prevention of military casualties include adequate detection measures, physical protection, chemical prophylaxis and proper treatment. In the terrorist context, proper emergency planning is key. Treatment of poisoning by organophosphorus nerve agents is dealt with in other chapters in this volume.

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