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REVIEW ARTICLE

Toxicology of organophosphorus compounds in view of an increasing terrorist threat

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Abstract The implementation of the Chemical Weapon Convention (CWC), prohibiting the development, production, storage and use of chemical weapons by 192 nations and the ban of highly toxic OP pesticides, especially class I pesticides according to the WHO classification, by many countries constitutes a great success of the international community. However, the increased interest of terrorist groups in toxic chemicals and chemical warfare agents presents new challenges to our societies. Almost seven decades of research on organophosphorus compound (OP) toxicology was mainly focused on a small number of OP nerve agents despite the fact that a huge number of OP analogues, many of these agents having comparable toxicity to classical nerve agents, were synthesized and published. Only limited physicochemical, toxicological and medical information on nerve agent analogues is available in the open literature. This implies potential gaps of our capabilities to detect, to decontaminate and to treat patients if nerve agent analogues are disseminated and may result in inadequate effectiveness of newly developed countermeasures. In summary, our societies may face new, up to now disregarded, threats by toxic OP which calls for increased awareness and appropriate preparedness of military and civilian CBRN defense, a broader approach for new physical and medical countermeasures and an integrated system of effective detection, decontamination, physical protection and treatment.

Keywords Organophosphorus compounds · Analogues and derivatives · Toxicology · Defense · Terrorism

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Introduction

We are now looking back on more than 160 years of research on highly toxic organophosphorus compounds (OP), a large group of organic phosphorus esters with the basic structure, proposed by Gerhard Schrader in 1937 (Holmstedt 1963), as shown in Fig. 1. Important milestones were the synthesis of tetraethyl pyrophosphate (TEPP) in the mid of the nineteenth century and of a close tabun analogue, *N*,*N*′-diethyl tabun, at the beginning of the twentieth century (De Clermont 1854, 1855; Michaelis 1903). Interest in OP as toxic compounds increased immediately before and during World War II. The synthesis of alkyl phosphorofluoridates by Lange and von Krueger and the extensive work by Schrader and colleagues may be considered as the starting point for the development of important OP pesticides but, unfortunately, also of highly toxic OP compounds which were further developed as chemical warfare nerve agents (Lange and von Krueger 1932; Schrader 1951). Further research on OP pesticides led to the discovery of phosphorylated thiocholine derivatives and finally to the development of even more toxic V-agents (Ghosh and Newman 1955; Baldit 1958; Tammelin 1958). The history of OP pesticides and nerve agents was extensively reviewed by various authors and shall not be addressed in more detail (Holmstedt 1963; Kabachnik et al. 1970; Maynard and Beswick 1992; Black 2016).

The intense research on OP started immediately after the end of World War II, and numerous compounds were further developed for use as pesticides (Eto 1974). The lower environmental stability, compared to DDT, and high insecticidal toxicity were major factors for the success of OP pesticides, especially in developing countries (Casida and Durkin 2013). The beneficial effects of widespread use of OP pesticides, among other factors the increase in crop

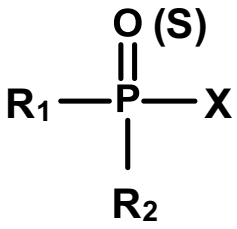


Fig. 1 Generic structure of organophosphorus pesticides and nerve agents according to Schrader with residues R_1 and R_2 and leaving group X

yields and the lowered incidence of vector-borne diseases, are accompanied by a rising insect resistance and a high number of accidental and intentional human poisonings (Eddleston et al. 2002).

The high incidence of suicidal OP pesticide poisoning with more than 250,000 fatal cases per year worldwide (Gunnell et al. 2007) initiated efforts to ban highly toxic OP pesticides. In consequence class I pesticides according to the WHO classification (International Programme on Chemical Safety 2010), e.g., parathion and mevinphos, are banned in most countries (Bertolote et al. 2006). In fact, recent studies provide evidence that this approach may lead to a decrease of OP pesticide-induced human poisonings (Chang et al. 2012; Knipe et al. 2014). However, these regulatory activities have only a limited effect on the ongoing challenge to implement effective strategies for the treatment of human OP pesticide poisoning (Eddleston et al. 2009, 2012). Moreover, the worldwide consumption of OP pesticides is still in the range of multiple kilotons per year (FAO Statistics Division 2013).

The rather accidental discovery of the highly toxic OP tabun by Gerhard Schrader in 1937 initiated extensive and long-lasting programs to identify even more toxic OP and to develop promising candidates as chemical warfare agents (Holmstedt 1963; Maynard and Beswick 1992). This led to large stockpiles of chemical warfare agents, e.g., sarin and VX in the USA, soman and Russian VX (VR) in the former Soviet Union (Szinicz 2005; Maxwell et al. 1997) and at a smaller scale in a number of other countries, notably Iraq and Syria (MacIlwain 1993; Pita and Domingo 2014).

It is noteworthy to mention that, although being fielded, the nerve agents tabun and sarin were not used by Germany during World War II, but repeated homicidal uses of tabun, sarin and VX occurred in later years. During the 1980s nerve agents were deployed against military forces during the Iran–Iraq war and against the civilian population by Iraqi forces (Black et al. 1994; Weimaster et al. 1995; Kadivar and Adams 1991). The deleterious terrorist attacks with sarin in Matsumoto 1994 and Tokyo 1995 as well as assassinations with VX in 1995 in Japan demonstrated the capability of non-state actors to produce and to disseminate nerve agents (Morita et al. 1995; Nozaki et al. 1995a, b). In 2013 attacks with sarin were conducted in Syria including the Ghouta incident in August 2013 with several thousand poisoned and up to 1500 killed humans (Pita and Domingo 2014).

The existence of large chemical warfare agent stockpiles and the fact that a huge number of exposed and intoxicated victims resulted from the repeated homicidal use of these agents convinced the international community to ban chemical warfare agents. The Chemical Weapon Convention (CWC), prohibiting the development, production, storage and use of chemical weapons, entered into force in April 1997 (United Nations Treaty Collection 1997). The CWC is administered by the Organisation for the Prohibition of Chemical Weapons (OPCW), which verifies the observance of the state parties to the convention. At present, more than 97 % of our world countries are members of this treaty which must be considered as an enormous success in the attempt to prevent future use of chemical warfare agents.

This very positive development decreases the likelihood of using chemical warfare agents on a large scale by states and by military forces. However, the use of nerve agents by a terrorist group in Japan and recent incidents in the Middle East give evidence for an increasing interest of non-state actors to get hold of chemical warfare agents and to use such toxic chemicals against the civilian population (Pita and Anadon 2015; European Parliamentary Research Service 2015; Hummel 2016; Hoette 2012). In fact, media reports indicate that the terrorist group Islamic State (ISIS) may have used chemical warfare nerve and blistering agents (Veterans Today 2015; CBC News 2015).

In consequence, we have to face the ongoing risk of accidental or intentional poisoning by OP pesticides and the potentially increasing threat of terrorist use of OP pesticides and nerve agents. This unpleasant situation calls for a closer look on toxicological and medical aspects of OP exposure in an environment with known and potentially new threats.

Toxicology of organophosphorus compounds

The toxicology of organophosphorus compounds was investigated extensively during the last decades by multiple research groups, and the mechanisms of acute toxicity are well defined (cf. Saunders 1957; Holmstedt 1963; O'Brien 1960; Eto 1974; Koelle 1992; Marrs 2007; Watson et al. 2015; Rice 2016). OP toxicology after subacute and chronic exposure as well as the long-term effects of OP poisoning is not within the scope of this paper, and the reader is referred to comprehensive reviews by experts in

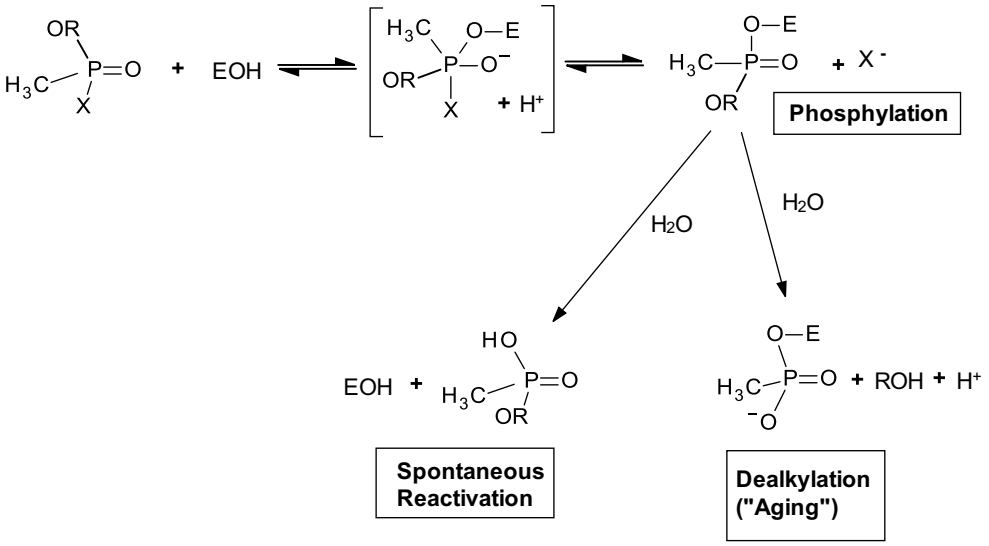


Fig. 2 Scheme of reactions between an OP and AChE. Incubation of a phosphonate with AChE (EOH) results in a Michaelis-type intermediate and finally in phosphylated AChE (phosphylation). The phosphyl-AChE complex may undergo two post-inhibitory reactions, the spontaneous cleavage of the phosphyl moiety (spontaneous reactivation) and of an alkyl residue leading to irreversible inhibition of AChE (dealkylation, "aging")

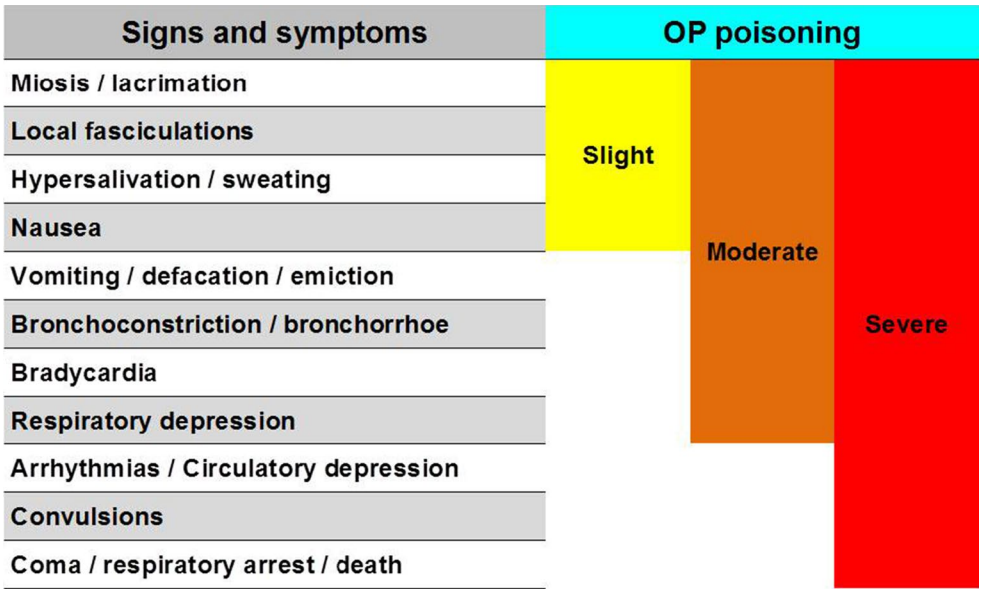


Fig. 3 Typical signs of OP poisoning in relation to the severity of intoxication

the field (cf. Krieger 2010; Scott 2007; Eyer 1995; Lotti and Moretto 2005; Abou-Donia 1981; Lohs 1975).

Basic aspects

The main mechanism of toxicologically relevant action of OP pesticides and nerve agents is the covalent binding to the active site serine OH-group at the base of a deep gorge of the pivotal enzyme acetylcholinesterase (AChE; EC 3.1.1.7; Fig. 2). Phosphylation of AChE, which denotes both phosphorylation and phosphonylation, leads to inhibition of its physiological action to hydrolyze the neurotransmitter acetylcholine (Aldridge and Reiner 1972).

Impaired hydrolysis of acetylcholine results in an overstimulation of muscarinic and nicotinic receptors at nerve–nerve and nerve–organ synapsis of the cholinergic system (Holmstedt 1959). This encompasses the central and vegetative nervous system and neuromuscular junctions. In consequence, OP poisoning may result in a broad spectrum of clinical signs (Fig. 3). The onset, sequence and severity of

clinical signs is strongly dependent on the intrinsic toxicity and dose of the OP and the route of exposure (Sidell 2007; Okumura et al. 1996; Peter et al. 2014). Signs of poisoning may develop within minutes after inhalation exposure to OP vapor but may take hours after percutaneous contamination by OP vapor or liquid (Sidell 1974; Okumura et al. 1996; Hamilton et al. 2004; Prinz 1969; Thiermann et al. 2007). Typically, miosis is an early sign after vapor exposure, local sweating and fasciculations after percutaneous exposure and gastrointestinal symptoms after oral intake of an OP (Lee 2003; Rengstorff 1994; Mumford et al. 2008; Goel and Aggarwal 2007).

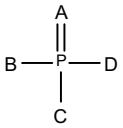
The phosphyl-AChE complex may undergo two post-inhibitory reactions, spontaneous reactivation and dealkylation ("aging"; Fig. 2). These reactions may have a major impact on the course of intoxication and the effectiveness of a specific class of therapeutic drugs, i.e., AChE reactivators (Jandorf et al. 1955). Aging proceeds extremely rapid with crotylsarin- ($t½ < 15$ s) and soman-inhibited human AChE ($t½ \sim 1$–2 min), slower with AChE inhibited by sarin and dimethoxy-OP pesticides ($t½ \sim 3$ h) and takes up to 40 h in case of AChE inhibition by diethoxy-OP pesticides and the nerve agent VX (Shafferman et al. 1996; Worek et al. 2004; Busker et al. 1991). Spontaneous reactivation, a process which can reverse the toxic OP effects, is negligible with G-type nerve agents (tabun, sarin, soman, cyclosarin), rapid with dimethoxy-OP pesticides ($t½ < 1$ h) but rather slow with diethoxy-OP pesticides and the nerve agent VX ($t½ > 30$ h) (Aurbek et al. 2006; Worek et al. 2004).

Besides AChE, being the main target of OP pesticides and nerve agents, other serine esterases, notably butyrylcholinesterase (BChE; EC 3.1.1.8) and carboxylesterase (CaE; EC 3.1.1.1), are inhibited by OP (Moralev and Rozengart 2007; Masson et al. 2009; Maxwell 1992). Inhibition of these enzymes by OP does not result in additional acute toxic effects, but BChE and CaE can serve as endogenous bioscavengers by binding and thus detoxifying a limited amount of incorporated OP (Maxwell et al. 1987; Lenz et al. 2007).

Structural aspects

The extensive and long-lasting work on OP by university and government research laboratories and private companies resulted in the identification of a huge number of compounds (Holmstedt 1963; O'Brien 1960; Eto 1974; Kabachnik et al. 1970; Moralev and Rozengart 2007; Timperley 2015). OP pesticides and nerve agents are based on the generic structure, proposed by Gerhard Schrader in 1937 (Holmstedt 1963; Fig. 1). Combination of different residues (R_1 and R_2) and leaving groups (X) enables synthesis of an unforeseeable number of derivatives. OP can be divided into different subclasses including phosphates, phosphonates, thiophosphates, phosphoramidates and phosphinates (Table 1; Ballantyne and Marrs 1992), having largely different physicochemical and toxicological properties.

Table 1 Classification of OP according to structural properties

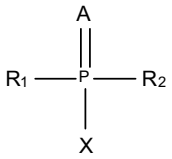


	A	B	C	D
Phosphate	O	O-R_1	O-R_2	O-R_3
Phosphonate	O	O-R_1	R_2	O-R_3
Phosphorofluoridate	O	O-R_1	O-R_2	F
Phosphonofluoridate	O	O-R_1	R_2	F
Thiophosphate	S	O-R_1	O-R_2	O-R_3
Thiophosphonate	S	O-R_1	R_2	O-R_3
Phosphorothioate	O	O-R_1	S-R_2	O-R_3
Phosphorodithioate	O	S-R_1	S-R_2	O-R_3
Phosphorotrithioate	O	S-R_1	S-R_2	S-R_3
Phosphonothioate	O	O-R_1	R_2	S-R_3
Phosphoramidate	O	O-R_1	O-R_2	N-R_3
Phosphoramidate	O	O-R_1	CN	N-R_3
Phosphorthioamidate	O	O-R_1	S-R_2	N-R_3
Thiophosphoramidate	S	O-R_1	O-R_2	N-R_3
Phosphinate	O	R_1	R_2	O-R_3

Table 2 shows selected OP pesticides and refers them to the WHO hazard classes, ranging from extremely (class Ia) to slightly hazardous (class III; International Programme on Chemical Safety 2010). An important factor for the human toxicity of OP pesticides is the presence of a P = S versus P = O bond. Thiophosphates have to be metabolized to the respective oxon, e.g., parathion to paraoxon or malathion to malaoxon, to become effective AChE inhibitors (Casida 1956; Eto 1974). This cytochrome P450-mediated reaction underlies large inter-individual variations and may have an essential impact on the individual susceptibility toward OP pesticides bearing a P = S bond (Mutch and Williams 2006; Foxenberg et al. 2007).

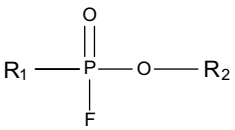
The development of highly toxic OP as chemical warfare agents (nerve agents) was focused on a rather small number of agents (Marrs 2007). Stockpiled and in part used nerve agents are the phosphonates sarin, soman and cyclosarin, the phosphoramidate tabun (designated as G-agents) and the phosphonothioates VX and VR. However, numerous nerve agent analogues were under investigation and their structures and in part details on the synthesis are available in the public domain, as can be derived from assigned Chemical Abstract Service registry numbers. By modification of the *O*-alkyl group a large number of alkylmethylphosphonofluoridates,

Table 2 Important OP pesticides, structural properties and classification according to the WHO recommended classification of pesticides by hazard (International Programme on Chemical Safety 2010)



OP	A	R_1	R_2	X	WHO classification
Parathion (CAS-No. 56-38-2)	S	*O*-Ethyl	*O*-Ethyl	*p*-Nitrophenyl	Ia
Diazinon (CAS-No. 333-41-5)	S	*O*-Ethyl	*O*-Ethyl	*O*-(2-Isopropyl-4-methyl-pyrimidin-6-yl)	II
Chlorpyrifos (CAS-No. 2291-88-2)	S	*O*-Ethyl	*O*-Ethyl	*O*-(3,5,6-Trichloro-2-pyridinyl)	II
Malathion (CAS-No. 121-75-5)	S	*O*-Methyl	*O*-Methyl	*S*-(1,2-Diethoxycarbonyl)ethyl	III
Dimethoate (CAS-No. 60-51-5)	S	*O*-Methyl	*O*-Methyl	*S*-(2-(Methylamino)-2-oxoethyl)	II
Fenamiphos (CAS-No. 22224-92-6)	O	*O*-Ethyl	NH-Isopropyl	*O*-(3-Methyl-4-methylthiophenyl)	Ib
Profenofos (CAS-No. 41198-08-7)	O	*O*-Ethyl	*S*-*n*-Propyl	*O*-(4-Bromo-2-chlorophenyl)	II
Methamidophos (CAS-No. 10265-92-6)	O	*O*-Ethyl	*S*-Methyl	NH_2	Ib

Table 3 Structural properties of selected alkylmethylfluorophosphonates (sarin analogues)

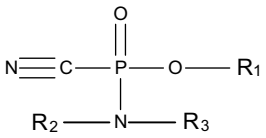


OP	R_1	R_2
Sarin (GB; CAS-No. 107-44-8)	Methyl	Isopropyl
Soman (GD; CAS-No. 96-94-0)	Methyl	Pinacolyl
Cyclosarin (GF; CAS-No. 329-99-7)	Methyl	Cyclohexyl
Methylsarin (CAS-No. 353-88-8)	Methyl	Methyl
Ethylsarin (CAS-No. 673-97-2)	Methyl	Ethyl
n-Propylsarin (CAS-No. 763-14-4)	Methyl	*n*-Propyl
n-Butylsarin (CAS-No. 352-63-6)	Methyl	*n*-Butyl
iso-Butylsarin (CAS-No. 2053-81-8)	Methyl	*i*-Butyl
n-Pentylsarin (CAS-No. 13454-59-6)	Methyl	*n*-Pentyl
iso-Pentylsarin (CAS-No. 22107-46-6)	Methyl	2-Methylbutane
sec-Pentylsarin (CAS-No. 468711-90-2)	Methyl	3-Methylbutane
neo-Pentylsarin (CAS-No. 372-62-3)	Methyl	2,2-Dimethylpropane
Crotylsarin (CAS-No. 138780-00-4)	Methyl	But-2-ene

i.e., sarin analogues, can be generated. A selection of agents is presented in Table 3. Likewise, variations of the *O*-alkyl groups and amide residues allow the synthesis of a large number of tabun analogues, Table 4 gives a selection of structures. The fundamental work of Tammelin and Ghosh in the early 1950s gave insight into the structure and function of organophosphorylthiocholines (Tammelin 1958; Ghosh and Newman 1955) and led to the presentation of amiton as a highly potent OP pesticide and VX as one of the most toxic nerve agents (Baldit 1958; Sidell 1997). Various VX analogues, e.g., VM and VE, were investigated and selected as chemical warfare agents (Russian VX; VR). Again, modification of the residues R_1–R_4 allows the synthesis of a huge number of V-agents (Table 5).

The acute human toxicity of OP pesticides and nerve agents can be estimated from the in vitro inhibitory potency toward human and animal AChE, the in vivo toxicity in different animal species and from human studies (Krieger 2010; Maynard and Beswick 1992; Gaines 1969; Rider et al. 1969). In general, the evaluation of OP toxicity is based on data from animal studies. However, one has to be aware of the difficulty to translate these data to humans due to considerable species differences in susceptibility toward OP (International Programme on Chemical Safety

Table 4 Structural properties of selected phosphoramidates (tabun analogues)



OP	R_1	R_2	R_3
Tabun (GA; CAS-No. 77-81-6)	Ethyl	Methyl	Methyl
N,*N*-Diethyltabun (CAS-No. 63815-60-1)	Ethyl	Ethyl	Ethyl
N,*N*-*n*-Propyltabun (CAS-No. 870124-33-7)	Ethyl	*n*-Propyl	*n*-Propyl
N,*N*-*i*-Propyltabun (CAS-No. 870124-37-1)	Ethyl	*i*-Propyl	*i*-Propyl
O-Methyltabun (CAS-No. 63815-56-5)	Methyl	Methyl	Methyl
O-*n*-Propyltabun (CAS-No. 162085-86-1)	*n*-Propyl	Methyl	Methyl

2010; Maynard and Beswick 1992). For obvious reasons, human studies with intentional exposure to OP are limited. In a few trials that were mostly performed decades ago, only sublethal OP doses were used (Sidell 2007; Rider et al. 1969; Hayes 1971). Moreover, relevant data of many highly toxic nerve agent analogues (cf. Tables 3, 4, 5) are not available in the open literature.

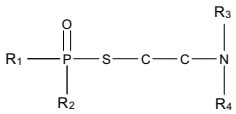
The determination of the inhibitory potency of OP toward human AChE in vitro is one option to get an initial insight into the potential toxicity of these compounds. Figure 4 shows the second-order inhibition rate constants relative to sarin of a broad range of pesticides, nerve agents and nerve agent analogues. These data demonstrate the wide range of inhibitory potencies as well as the exceptional high potency of a large number of sarin and VX analogues. It has to be emphasized that these data provide only initial, basic information of the potential to interact with human AChE, being the main target of OP toxicity, but do not necessarily reflect the in vivo toxicity. Comparison of the in vitro inhibitory potency and the estimated human LD_{50} and LCT_{50} values of relevant OP nerve agents show an in part substantial difference (Fig. 4, inset) which indicates that other factors are important for the in vivo toxicity of OP. These will be addressed in the following section.

Toxicokinetic and toxicodynamic aspects

The toxicity of OP nerve agents and pesticides is determined by their inhibitory potency toward AChE, the physicochemical properties, the chemical and biological stability and by additives. OP pesticides are marketed and used as formulations containing different organic solvents and emulsifiers, and there is evidence that these co-formulants may increase the toxicity of pesticides (Eddleston et al. 2012), while no information is available on the potential role of stabilizers used in weaponized OP nerve agents (Dacre 1984).

Physicochemical properties, notable volatility, are determining factors for the preferential OP exposure route. Table 6 shows the huge differences in volatility and hydrolytic stability of classical nerve agents, major factors for the largely different human toxicity estimates after vapor and liquid percutaneous exposure (cf. Table 7). The low volatile and persistent nerve agent VX is extremely toxic after skin exposure, while the more volatile G-agents are believed to

Table 5 Structural properties of selected organophosphorylthiocholines (VX analogues)



OP	R_1	R_2	R_3	R_4
Dimethyl-amiton (CAS-No. 3147-20-4)	*O*-ethyl	*O*-ethyl	Methyl	Methyl
Amiton (VG; CAS-No. 78-53-5)	*O*-ethyl	*O*-ethyl	Ethyl	Ethyl
Diisopropyl-amiton (CAS-No. 219662-56-3)	*O*-ethyl	*O*-ethyl	Isopropyl	Isopropyl
Dimethyl-VX (CAS-No. 20820-80-8)	Methyl	*O*-ethyl	Methyl	Methyl
Diethyl-VX (VM; CAS-No. 2177086-5)	Methyl	*O*-ethyl	Ethyl	Ethyl
VX (CAS-No. 50782-69-9)	Methyl	*O*-ethyl	Isopropyl	Isopropyl
Dimethyl-VE (CAS-No. 98543-25-0)	Ethyl	*O*-ethyl	Methyl	Methyl
VE (CAS-No. 21738-25-0)	Ethyl	*O*-ethyl	Ethyl	Ethyl
Diisopropyl-VE (CAS-No. 73835-17-3)	Ethyl	*O*-ethyl	Isopropyl	Isopropyl
Russian VX (VR; CAS-No. 159939-87-4)	Methyl	*O*-isopropyl	Ethyl	Ethyl
Chinese VX (CVX; CAS-No. 468712-10-9)	Methyl	*O*-*n*-butyl	Ethyl	Ethyl

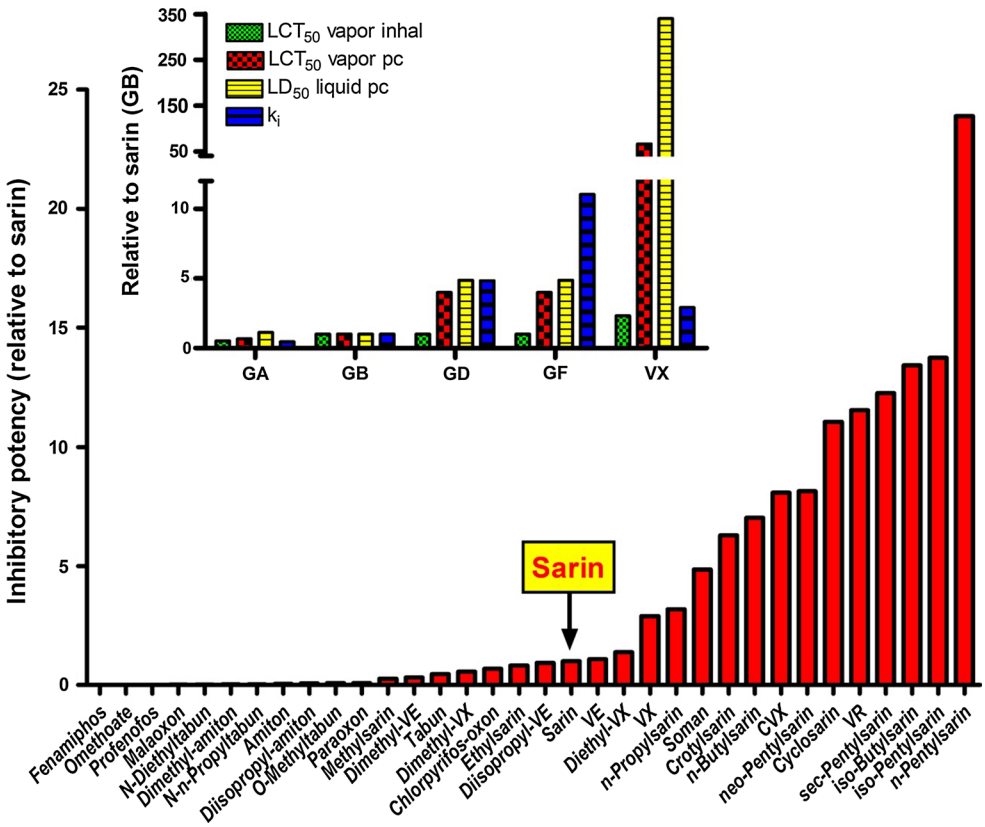


Fig. 4 Inhibitory potency of OP pesticides, nerve agents and nerve agent analogues toward human AChE in vitro Data are from Aurbek et al. (2006, 2010), Worek et al. (2007), Bartling et al. (2007), Worek et al. (2004, 2009) and from unpublished data. The respective second-order inhibition rate constants, k_i, were referred to sarin (k_i 4×10^7 $M^{-1}min^{-1}$; set at 1). The *inset* presents the relation between the in vitro inhibitory potency and human toxicity estimates referred to sarin (cf. Table 6). GA tabun, GB sarin, GD soman, GF cyclosarin

Table 6 Selected physical and chemical properties of important OP nerve agents Data were derived from (Marrs 2007; Watson et al. 2015; U.S. Army Chemical School 2005)

Parameter	VX	Tabun (GA)	Cyclosarin (GF)	Soman (GD)	Sarin (GB)
Volatility (mg/m^3 at 25 °C)	0.00044	0.037	0.044	0.27	2.1
Hydrolysis half-life (pH 7 and 20 °C)	400–1000 h	8.5 h	~42 h	~80 h	80 h

Table 7 Human toxicity estimates of OP nerve agents

Exposure route	Tabun (GA)	Sarin (GB)	Soman (GD)	Cyclosarin (GF)	VX
LCT_{50} vapor inhalation ($mg/min/m^3$)	70	35	35	35	15
LCT_{50} vapor percutaneous ($mg/min/m^3$)	15000	10,000	2500	2500	150
LD_{50} liquid percutaneous (mg/man)	1500	1700	350	350	5

Data are given for inhalation and percutaneous vapor and for percutaneous liquid exposure (National Research Council—Committee on Toxicology 1997)

be 70–500 times less toxic via this exposure route. Hence, knowledge of physicochemical properties of potential threat agents is essential for the assessment of preferential routes of exposure.

Bioavailability, distribution and metabolism of OP nerve agents and to less extent of OP pesticides have been reviewed extensively by multiple authors (cf. Benschop and de Jong 2001; John et al. 2015). These data are essential for physiology-based pharmacokinetic–pharmacodynamic modelling (PBPK-PD) of OP (Benschop and de Jong 2001; John et al. 2015; Timchalk et al. 2007; Sweeney et al. 2006; Covington et al. 2016). Investigation of interactions between OP and blood and tissue constituents, of transfer rates of OP from blood to tissues and of detoxification rates by endogenous enzymes provides important information for the estimation of onset and duration of poisoning after OP incorporation (Fig. 5). Again, research is mainly focused on a small number of nerve agents and pesticides

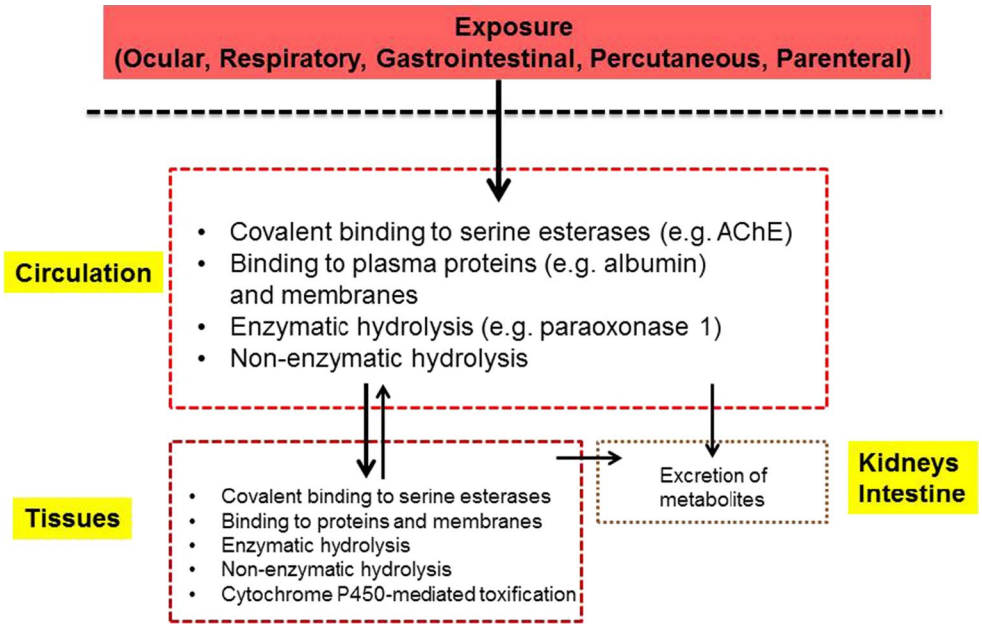


Fig. 5 Toxicokinetics of OP. Scheme of important interactions of OP in the body

and data on many other pesticides and almost all nerve agent analogues are not available in the open literature. However, existing data on the toxicokinetics of selected G- and V-agents may provide insight into the potential behavior of tabun, sarin and VX analogues in mammalian organisms. There is convincing evidence that tabun, sarin, soman and cyclosarin are detoxified in vivo within a rather short time, i.e., half-life of less than 1 h (Benschop and de Jong 2001; Tenberken et al. 2010; Reiter et al. 2007). In contrast, VX and VR are highly stable in living organisms and limited toxicokinetic data indicate a persistence of toxicologically relevant concentrations for more than 12 h (van der Schans et al. 2003; Reiter et al. 2008, 2015).

The exposure route is decisive for the velocity of agent absorption into the systemic circulation (Benschop and de Jong 2001). Inhalation and intravenous administration, serving as a model for the inhalation route, of G- and V-agents result in a rapid increase of OP concentration in the circulation, while percutaneous exposure to V-agents and OP pesticides is characterized by a delay of several hours until OP can be quantified in blood (Feldmann and Maibach 1974; Reiter et al. 2008; van der Schans et al. 2003; Wester et al. 1983). Hence, percutaneous OP poisoning may result in a rather significant lag time between exposure and onset of clinical signs. These properties are an important factor for the development of appropriate concepts for decontamination and drug treatment (Mikler et al. 2011).

Available data on OP toxicokinetics allow an assumption on the potential persistence of tabun, sarin and VX analogues in vivo. It may be assumed that structural analogues of tabun and sarin should have a short half-life in blood after inhalation exposure (Benschop and de Jong 2001; Jose et al. 2015; Garner and Jones 2014), while VX analogues should persist for prolonged time in the body (van der Schans et al. 2003; Bouchard et al. 2003; Feldmann and Maibach 1974).

Medical aspects

The adequate care of OP casualties requires an initial rapid and effective skin decontamination, especially in case of percutaneous exposure to OP nerve agents and pesticides (Roberts and Maynard 2007; Zilker 2005; Joosen et al. 2013; Chilcott 2007). Immediate skin decontamination can preserve survival and can prevent the occurrence of signs of poisoning. However, the effectiveness of decontamination decreases dramatically with time (Joosen et al. 2013; Braue et al. 2011; Bjarnason et al. 2008; Hamilton et al. 2004). A major disadvantage of commercial skin decontamination kits, e.g., Reactive Skin Decontamination Lotion (RSDL) Kit or M291 Skin Decontamination Kit, is the need to know the exposure location since it is hardly possible to perform whole body decontamination with such kits, and limited information on the effectiveness of commercially available skin decontamination kits against a broader range of OP. In consequence, available skin decontamination kits allow a provisional, nevertheless potentially lifesaving, on-site decontamination which must be followed by timely undressing and whole body decontamination.

Besides decontamination, treatment with specific antidotes is essential to prevent mortality and incapacitation of OP casualties (Vale et al. 2007; Watson et al. 2015; Thiermann et al. 2016). Since decades, antimuscarinics,

Table 8 Experimental approaches for improved treatment of poisoning by OP

Therapeutic approaches	Experimental options
AChE reactivators	Broad-spectrum oximes[a,b] Non-charged oximes[c] BChE-specific oximes[d] Non-oxime reactivators[e,f,g]
Anticonvulsants	Midazolam, procyclidine[h,i]
Antinicotinics	Bispyridinium compounds[j,k]
Small molecule scavengers	Cyclodextrins[l,m]
Stoichiometric bioscavengers	Human AChE[n] Human BChE[o]
Catalytic bioscavengers	Paraoxonase 1 (PON1)[p] Bacterial phosphotriesterase (PTE)[q] OpdA[r]

Selection of options being under investigation

[a] Musilek et al. (2011)

[b] Worek and Thiermann (2013)

[c] Sit et al. (2011)

[d] Kovarik et al. (2010)

[e] Bhattacharjee et al. (2015)

[f] Katz et al. (2015)

[g] Bierwisch et al. (2016)

[h] Shih et al. (2007)

[i] Myhrer et al. (2015)

[j] Price et al. (2016)

[k] Seeger et al. (2012)

[l] Letort et al. (2016)

[m] Worek et al. (2014c)

[n] Cohen et al. (2001)

[o] Mumford et al. (2013)

[p] Rochu et al. (2007)

[q] Nachon et al. (2013)

[r] Jackson et al. (2014)

primarily atropine, are used as basic therapeutic drugs (McDonough and Shih 2007). Atropine antagonizes the OP effects at muscarinic synapses and can thus reverse or diminish the stimulation of smooth muscles and glands, the decreased heart rate and the impaired function of central respiratory centers. The effect of atropine is only symptomatic and requires appropriate blood and tissue concentrations for prolonged time (Thiermann et al. 2011). Atropine has no therapeutic effect on nicotinic receptors. For that reason and to restore the activity of inhibited AChE atropine has to be supplemented by oximes. The first clinically used pyridinium oxime pralidoxime (2-PAM) was introduced some 60 years ago, followed by the bis-pyridinium oximes obidoxime, trimedoxime (TMB-4) and HI-6 (Eyer and Worek 2007). Up to now, several thousand oxime structures were published and their ability to reactivate OP-inhibited AChE and to counteract the toxic OP effects was investigated in numerous in vitro and in vivo studies. In summary, the available data demonstrate a variable reactivating potency and therapeutic efficacy which is dependent on the structure of the oxime and the OP as well as their concentration (Worek and Thiermann 2013). In general, AChE inhibited by sarin, cyclosarin, VX, VR, CVX and pesticides bearing a dimethoxy or diethoxy residue is susceptible toward reactivation by oximes while AChE inhibited by soman, due to rapid aging, tabun and different pesticides, e.g., fenamiphos and ethoprophos, is rather resistant. Up to now, no broad-spectrum oxime covering the whole spectrum of classical nerve agents and most relevant pesticides was identified. An additional limitation of present oximes, i.e., charged hydrophilic compounds, is the very limited ability to penetrate the blood brain barrier and to reactivate brain AChE (Kalasz et al. 2015). Only very limited information on the ability of oximes to reactivate human AChE inhibited by nerve agent analogues was published. It appears that inhibition by a variety of sarin and VX analogues (Tables 3, 5) results in phosphylated human AChE which can be reactivated by established oximes (Bartling et al. 2007; Worek et al. 2009) while close tabun analogues, *N*,*N*-diethyltabun and *N*,*N*-di-*n*-propyltabun (Table 4), turned out to be completely resistant toward reactivation by oximes (Worek et al. 2007, 2015).

The inadequate effectiveness of presently available antidotes initiated intensified research on alternative therapeutic approaches (Table 8). These include non-charged oximes to improve the reactivation of brain AChE, non-oxime reactivators and specifically designed oximes to reactivate OP-inhibited BChE in order to transfer endogenous or injected BChE into a catalytic scavenger (Katz et al. 2015; Mercey et al. 2012; Sit et al. 2014). Ongoing research tries to identify more effective anticonvulsants as replacement of the widely used diazepam and to provide compounds (e.g., antinicotinics) being active at the neuromuscular junction in order to restore OP-impaired neuromuscular function in case of oxime resistance (McDonough 2002; Weissman and Raveh 2008; Ring et al. 2015; Price et al. 2016).

The perception of limited efficacy of standard atropine + oxime therapy, being able to increase survival but hardly to prevent incapacitation, shifted the focus to options directed to avoid the onset of toxic signs. This led to research on small molecules and proteins acting as stoichiometric or catalytic (bio)scavengers which should be able to detoxify incorporated OP before it can reach target tissues (Masson 2015; Nachon et al. 2013; Lenz et al. 2007; Letort et al. 2016; Masson 2016). It could be shown that the prophylactic and therapeutic, i.e., after OP exposure but prior to the onset of signs of poisoning, administration of a small molecule scavenger, an oxime-substituted β-cyclodextrin, the stoichiometric scavenger human BChE and of catalytic enzymes, PON1 and PTE mutants,

at appropriate doses almost prevented incapacitation and preserved survival of animals poisoned by selected nerve agents (cyclosarin, VX) (Mumford et al. 2011; Worek et al. 2014a, b, c).

Research on improved therapies of OP poisoning in the last decades was focused almost exclusively on a very limited number of nerve agents (tabun, sarin, soman, cyclosarin, VX) and an even lower number of pesticides. Information on the effectiveness of oximes and other therapeutic options against nerve agent analogues and many pesticides is almost missing in the open literature. Only a small number of studies investigated other OP and give some insight into the potential of present and future therapies (Rice et al. 2015; Cherny et al. 2013; Daczkowski et al. 2015; Goldsmith et al. 2016; Bartling et al. 2007; Worek et al. 2007, 2009).

In consequence, the focus of research activities on a very limited number of OP must be considered as a major issue. The inadequate knowledge of the potential efficacy of standard treatment against poisoning by a large number of OP as well as the missing consideration of these agents for the design of catalytic small molecule and bioscavengers could result in an inadequate therapeutic efficacy of present and future OP therapies.

Impact of changing threats on defense against organophosphorus compounds

The rather well-defined threat to our societies by state actors prior to the implementation of the CWC is going to change fundamentally. Cold war scenarios of chemical war will lose their importance, while asymmetric threats will develop as major issue. Moreover, the increasing interest of terrorist groups in chemical warfare agents may ultimately result in a markedly broader spectrum of threat agents (Pita and Anadon 2015). Although no open source information is available confirming terrorist use of OP nerve agents after the attacks in Japan in 1994 and 1995, there is some evidence that Islamic terrorists may have attacked girl schools in Afghanistan with the OP pesticide malathion (Pita and Anadon 2015). Although not yet verified, the assumed use of the chemical warfare blister agent sulfur mustard by Islamic State terrorists gives a clear indication on the interest and potential capability of this group (European Parliamentary Research Service 2015; OPCW Technical Secretariat 2015). Hence, there is an increasing risk by terrorists to get hold of pesticide stocks or even to synthesize nerve agents or nerve agent analogues on a small scale.

Information on the synthesis of OP pesticides, nerve agents and nerve agent analogues is available in the public domain and gives in part detailed instruction on synthetic procedures (Sartori 1951; Saunders 1957; Schrader 1963; Holmstedt 1951; Black and Harrison 1996; Timperley 2015). OP synthesis by terrorists will depend on intention, chemical skills, technical capabilities and availability of precursors. This may result in the production of OP analogues apart of the classical nerve agents.

Research on defensive procedures against OP nerve agents was focused on a small number of agents in the past decades. Only limited physicochemical, toxicological and medical information on nerve agent analogues is available in the open literature (cf. Aquilonius et al. 1964; Binenfeld 1966; Berry and Davies 1966; Hall et al. 1977; de Jong and Benschop 1988). In consequence, adequate information on the toxicity, chemical and biological stability of nerve agent analogues and the efficacy of antidotes is rather scarce. Moreover, these agents may provoke new challenges for material and skin decontamination and detection. Early detection is fundamental for the initiation of appropriate medical countermeasures. Enzyme-based detection tickets may indicate the dissemination of a broad spectrum of OP and may be suitable for spot detection, while ion mobility spectrometry (IMS) mobile detectors may fail if the respective spectrum is not included into the device database or volatility is too low for sensitive detection (McKone et al. 2000; Mäkinen et al. 2010).

The potentially enlarged spectrum of threat agents has an additional impact on the ongoing development of novel antidotes. The in vitro and in vivo effectiveness of AChE reactivators and catalytic (bio)scavengers is usually tested with a limited number of classical OP nerve agents, and promising candidates may be of inadequate efficacy against nerve agent analogues (Mercey et al. 2012; Worek and Thiermann 2013; Nachon et al. 2013). Only the primary target, human AChE and to less extent human BChE, has the potential to detoxify all OP analogues if their main mechanism of action is cholinesterase inhibition. Hence, cholinesterase-based stoichiometric scavengers, atropine and potentially antinicotinics can be considered as generic antidotes covering the whole spectrum of OP threat agents.

Conclusions

The implementation of the CWC, prohibiting the development, production, storage and use of chemical weapons by 192 nations (United Nations Treaty Collection 1997) and the ban of highly toxic OP pesticides, especially class I pesticides according to the WHO classification (International Programme on Chemical Safety 2010), by many countries constitutes a great success of the international community. However, the increased interest of terrorist groups in toxic chemicals and chemical warfare agents presents new

challenges to our societies (Pita and Anadon 2015; European Parliamentary Research Service 2015).

Selection of different residues attached to the central phosphorus atom allows the synthesis of innumerable, potentially toxic OP pesticides, nerve agents and nerve agent analogues (Holmstedt 1963; Timperley 2015). Research on defensive procedures against OP nerve agents was focused on a small number of agents in the past decades. Only limited physicochemical, toxicological and medical information on nerve agent analogues is available in the open literature. This implies potential gaps of our capabilities to detect, to decontaminate and to treat patients if nerve agent analogues are disseminated. Moreover, focus on a limited number of classical nerve agents during research and development of novel medical countermeasures may lead to an inadequate efficacy of new treatment options.

In summary, we may face new, up to now disregarded, threats by toxic OP which calls for increased awareness and appropriate preparedness of military and civilian CBRN defense, a broader approach for new physical and medical countermeasures and an integrated system of effective detection, decontamination, physical protection and treatment.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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