Asian Journal of Chemistry; Vol. 34, No. 4 (2022), 767-773

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2022.23568

REVIEW

Organophosphorus Pesticide as Nerve Agent: Inhibition and Reactivation of AChE: A Review

U. DAS

Department of Chemistry, Sarsuna College, Kolkata-700061, India

Corresponding author: E-mail: ujjwalsccs@gmail.com

Received: 6 October 2021; *Accepted*: 19 November 2021; *Published online*: 10 March 2022; AJC-20716

In last five decades, organophosphorus compounds have become significant because of the widespread uses as pesticides abutting a clear threat to people from the potential use of chemical weapon as nerve agents in the cold wars and terrorist activities. Sarin, tabun, soman, cyclosarin belong to organophosphorus compounds called nerve agents due to their biological interaction as powerful inhibitor of enzyme acetylcholinesterase (AChE) and rendered its neurological function, the underlying mechanism is discussed. The identification and detection of such toxic nerve agents using spectrometry, sensors including enzymatic assays is the prime target of recent researchers. Typically some antidotes like oxime derivatives are most common and are the light of hope for nerve gas exposure. The modern techniques applied for the discovery of new antidote and their way of action by reactivating the blocked nerve enzyme and the hidden mechanism of reactivation of AchE are explained.

Keywords: Nerve agent, Organophosphorus, Warfare, Acetylcholinesterase, Antidote.

INTRODUCTION

A large number of organophosphorus compounds are extensively used in the agricultural and related industry around the world as pesticides and insecticides [1,2]. In the modern and sustainable agriculture for the sake of high and continuous production of agro-products due to their high demand, it is necessary to optimization of resources of soil including minerals, nutrients and also use of pesticides, which become a regular practice primarily to controlling weeds and pests [3]. Because some pests are present on the surface or inside the crops or in the upper layer of soil are known to harm the agricultural crops and cause yield reduction. Although a huge number of pesticides including organophosphorus compounds are used regularly to enhance the agricultural yield and minimise the time of growth of crops. It is important to mention that a massive number of biological functions like photosynthesis, metabolism, chromosome synthesis, biocatalytic activity and coenzyme [4,5] systems, where phosphorous compounds play an essential role. Generally, phosphorous adopted a combination of +3 and +5 oxidation states in these compounds and their derivatives and presenting optical isomerism possessing chiral centre. Interestingly, they have different importance in term of sustainable agriculture since all enantiomers are not equally active as pesticides due to their different chemical activity with stereochemical rearrangement. Various studies have revealed that the organophosphorus pesticides can alter the enzyme activity of pests including tiny organisms and the impact of pesticides to biological lives. In this standpoint a new methodology with a mechanistic understanding appears helpful to explore the toxicity of organophosphorus compounds [6,7] and their biointeraction with enzymes present in central nervous system of living creatures. Among numerous categories of pesticides organophosphorus halide and psuedohalide compounds are very effective in terms of neurological toxicity. These chemicals kill creatures by inhibiting cholinesterase and acetylcholinesterase (AChE), present in nerve cells and the brain and play a vital role in nerve action. The inactivation of these enzymes fatally disrupts the nervous system. Much interest and weightage are given for such halide compounds specially fluorinated analogues such as sarin, cyclosarin, soman and some of the derivatives since they are included in the chemical warfare [8-10] agents utilizing their destructive property like nerve agents [11,12]. Nowadays some of these organophosphorus compounds

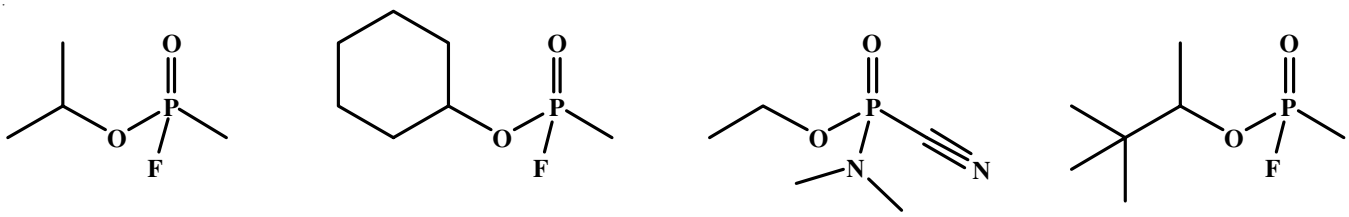
are extensively use in the chemical combat as lethal weapons using the toxic properties rather than in other positive or beneficial interests. Comparing with the deadly nerve gases, sarin is the most toxic and fast-acting chemical weapons [13]. This type of warfare is distinct from nuclear warfare and biological warfare, rather considered as weapons of mass destruction. Organophosphorus compounds must be handled with greater care since they are extremely toxic even deadly to mammals including humans. Several oxime functionalized compounds called antidotes commonly act as reactivators of acetylcholinesterase, deactivated by organophosphorus nerve agents, are the reversible inhibitors of AChE [14-18]. Though all such oxime based antidotes are not fully effective to treat the neuro-deactivated condition due to some limitations.

In this circumstance, synthesis approaches to the new antidotes by creating modification of oxime functionality or amalgamation of hetero functional moity with oxime and their derivatives are the focal theme for researchers to reactivate the blocked enzyme by nerve chemicals. Simultaneously, the exploration of the synthetic route of halogenated organophosphorus compounds [19] by which disclose the hidden biochemistry of interaction between enzyme-nerve agents antidotes can be encountered. The findings of enhanced lethal effect *in vivo* and *in vitro*, sensing methods and other remedies adopted from various sources were admitted. Also the mechanistic approach of interaction of organophosphorus nerve agents with neuro enzymes, which developing toxicity results the potential long-term health effects even fatal when high dose is administrated and the possible recovery pathway from the inhibited nerve enzyme due to application of antidotes and their farther scopes are described in concise form.

Organophosphorus nerve agents: A range of structural diversity are observed in organophosphorus pesticides generally are amides, thiols or esters of phosphonic, phosphinic, phosphoric or thiophosphoric acids having cyanide, phenoxy or thiocyanate group in side chains and in many cases halides, specially fluorides are also the other substituent. These volatile compounds are most important due to their structural cum stereochemical feature and way of action as killer nerve agents [20]. There are mainly four series of nerve gas among them the G-series agents include tabun (GA), sarin (GB), soman (GD) and cyclosarin (GF), which were developed by Germans and are of most significant (Fig. 1). The V- series agents for example VX also an effective nerve agent having organosulfur group are differ from G-agents in terms of their lower volatility, superior environmental persistency and higher toxicity [21]. Other organophosphorus pesticides including malathion, dichlorvos, azinphosmethyl, chlorpyrifos unlike nerve gases were more popular and extensively used, since they are easily degrade in the environment and effective than persistent organochlorine insecticides like DDT, aldrin and dieldrin. Much interest paid and focused on the organophosphorus halogenated nonsulfur compounds especially sarin, since they possess a rich covalently binding property with biomolecules and are extensively used chemical as nerve agents. Including sarin other nerve agents are dispersing hurriedly because of air currents due to their high volatility. Commonly, nerve agents are akin to certain kinds of pesticides used as insect killers called organophosphates in terms of how they work and what kind of destructive effects they cause. Sarin is a colourless, transparent, odourless, tasteless, extremely fatal and occurring mutually as a volatile liquid and vaporized as a gas in the pure form belongs to this group.

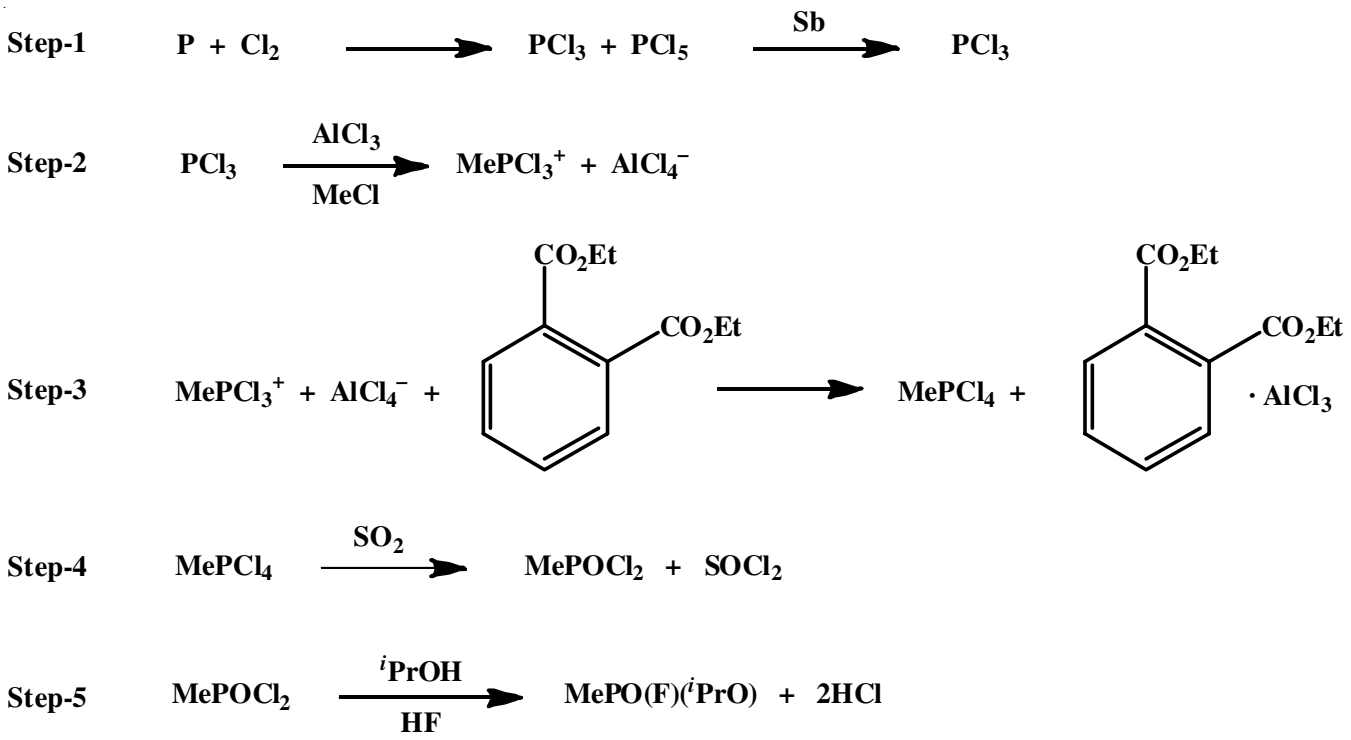
Their high volatility and physically silent nature made them short-lived threat for people unconcerned that they get exposed. Most of the organophosphorus nerve agents are synthesize in numerous multistep routes involving some hazardous chemicals like HF, PCl_3, PCl_5, $SOCl_2$, SO_2, *etc*. [19]. The synthetic route concerning the direct transformation of phosphorus trichloride to methylphosphonic dichloride and utilising the Friedel-Craft type reaction followed by fluorination using HF or NaF to methylphosphonic difluoride and finally to sarin and its derivatives applying *i*PrOH in base-CH_2Cl_2 medium (**Scheme-I**).

Biochemical activity of nerve agents: Organophosphorus compounds are structurally more important since their effectiveness and modes of action as pesticides depends on the structure they adopted. Stereoisomer of organophosphorus compounds play an essential role in the context of the range of toxicity of the compounds and it is found that generally P(-) enantiomers are more toxic in nature. For example, like other member sarin is a chiral molecule with respect to the tetrahedral central phosphorus atom (Fig. 1) [22]. It possess two enantiomers P(-) and P(+), among them the P(-) optically active S configuration being more active enantiomer hence more toxic, can show effective binding affinity to the important enzyme AChE [23] found in synaptic cleft of nervous system. Acetylcholinesterase enzyme is composed of α-helical and β-sheet domains which belong to the superfamily of the α/β hydrolase fold enzymes more specifically serine hydrolase [24]. High pH is one of the important factors for studying the biological impact since they get decomposes rapidly in this condition to phosphonic acid derivatives.



Sarin (GB) **Cyclosarin (GF)** **Tabun (GA)** **Soman (GD)**

Fig. 1. Structure of G-series nerve gas agents



Scheme-I: Route to synthesize sarin (organophosphorus fluorinated nerve agent)

Study of biological activity including interaction with neuroenzymes and mechanism of action with neurochemicals are the hot topic of research [11]. Organophosphorus nerve gases are readily hydrolyzed either fully or partially to the respective range of components as alkylmethyl phosphonates for instance isopropylmethyl phosphonate, pinacolylmethyl phosphonate, ethylmethylphosphonate, *etc*. and eventually they converted to phosphoric acid or its derivatives [25] *via* methylphosphonate intermediate (Fig. 2) in aqueous medium and then get absorbed in soil, hence pH of the corresponding soil changed to acidic region.

The partially hydrolyzed product of nerve agents or some active product during the bio degradation process like $POCl_3$ and its derivatives $HOP(O)Cl_2$ and $(HO)_2P(O)Cl$ are effectively bind with the serine residue of AChE and inhibition resulted with neuromuscular block (Fig. 3) confirmed by ^{31}P NMR.

Mechanisms of toxicity as nerve agent: In general nerves communicate with muscles, glands and other nerves by releasing biochemicals and neurotransmitters at their connection sites called synapses. The enzyme AChE is emanated into the synaptic cleft and degrades the neurotransmitter acetylcholine. This acetylcholine performed their function at the neuromuscular junction and made link to vital parts of the body by which the nerve signals are transmitted between neurons. Normally, acetylcholine is released from the neuron to stimulate the muscle, after which it is degraded by acetylcholinesterase to stop further stimulation of the nerve, muscle or gland and allowing them to relax. Interestingly, acetyl group is detached from the choline moiety during acylation of acetylcholine proceeds through forming a covalent bond to a particular serine amino acid residue of the enzyme and again in the deacylation step, acetyl group is hydrolyzed from that serine residue is followed. Most of the organophosphorus nerve chemicals bear a POX_3 or $PO(OH)X_2$ type of moiety which is a powerful covalent irreversible inhibitor of enzyme acetylcholinesterase [26,27] (Fig. 4) showing anti-cholinesterase action and also serum butyrylcholinesterase (BuChE) inhibition. This inhibition hits the nervous system at neuromuscular junctions by interfering with the re-absorption of neurotransmitters and death will be the result due to the incapability to control the breathing muscles. Neuromuscular block is observed in pests like harmful insects and also the micro-organism exists in soil, amphibian as well as in mammals.

Not only had the lower class of life, mammals including humans are also exposed by organophosphorus nerve agents who are associated to the agro-industries and almost same type of nerve agent biomolecule interaction can be observed with some complex way. The toxicity level in human due to exposure of organophosphorus compounds nerve agents in terms of LD_{50}, LC_{50} and LCt_{50} are reviewed and summarized in Table-1 [28,29]. Organophosphorus nerve chemicals like sarin, the electrophillic phosphorus of it attacked by the particular serine amino acid residue of enzyme and forming a covalent bond irreversibly in the active site of the enzyme designated as serine-sarin adduct [30,31]. Halides or pseudohalides (fluoride in case of sarin) act as the leaving group and generate phosphoester which is robust and biologically inert [32,33]. As a result of inhibition of AChE, the neurotransmitter acetylcholine accumulates in the synaptic cleft and over-stimulates the muscarinic and nicotinic receptors [34] and causes hyperactivity of the cholinergic nerves, muscles and glands, eventually leads to their desensitization. If such nerve chemicals like sarin is not removed from AChE within a few hours of exposure, AChE

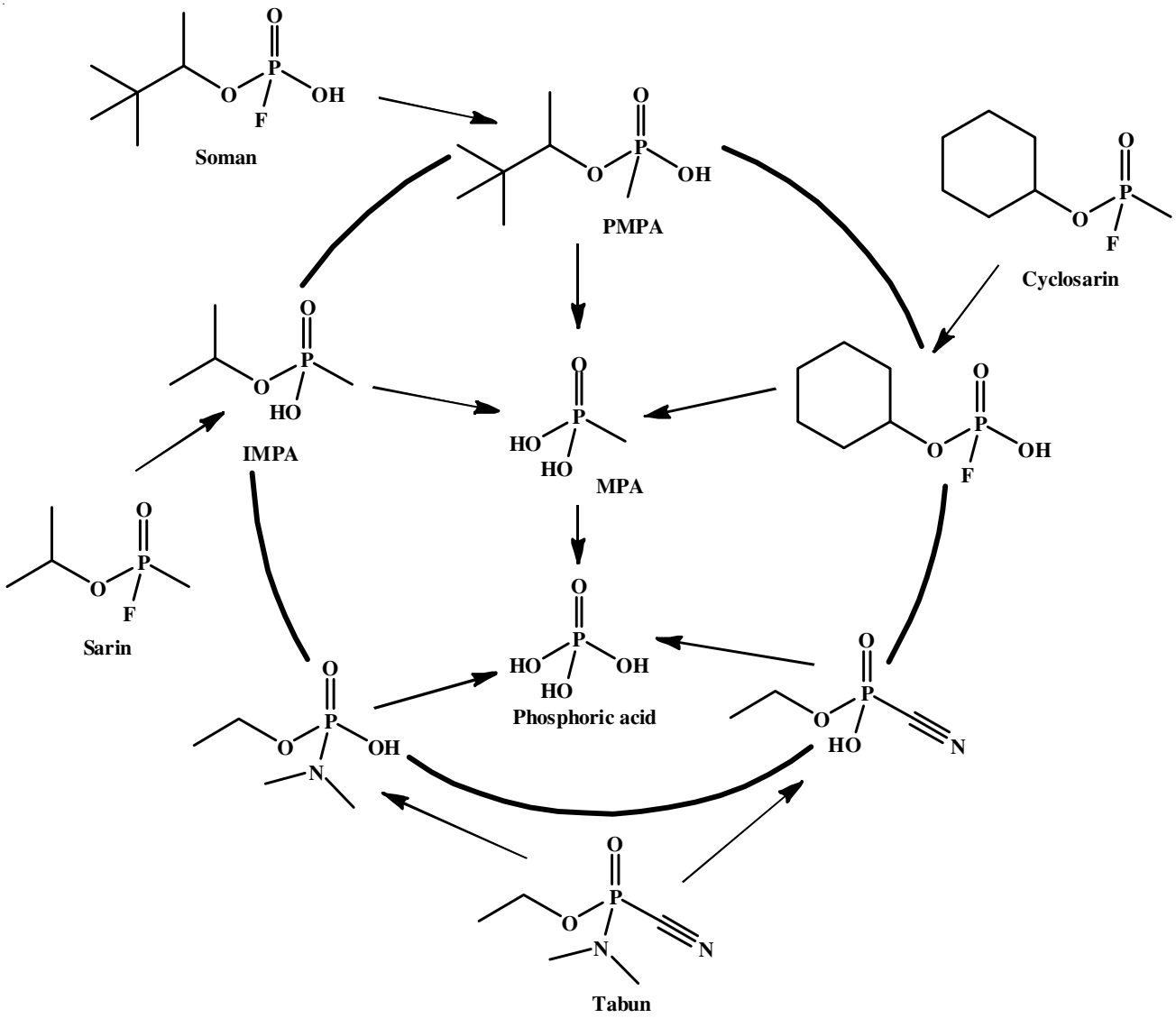


Fig. 2. Hydrolysis scheme of nerve agents

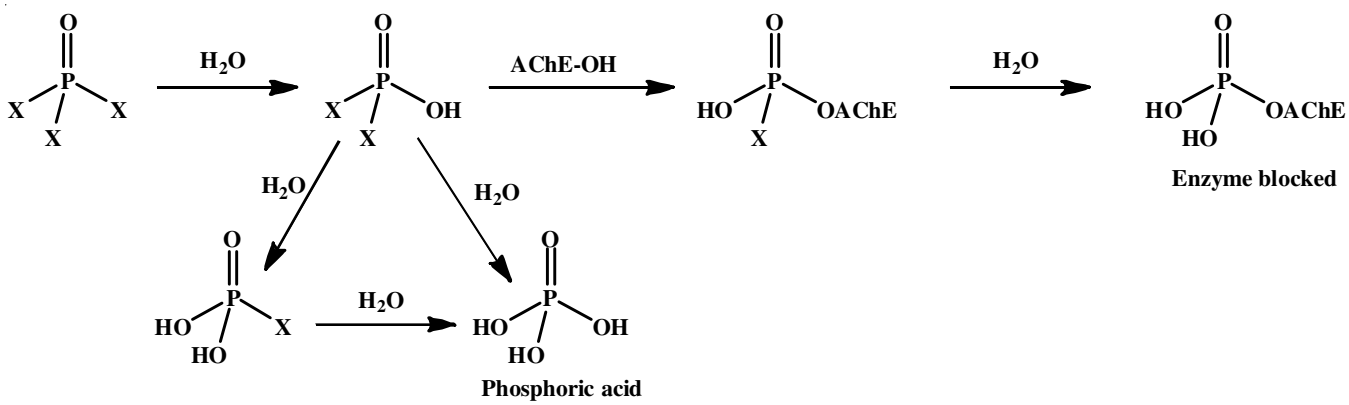


Fig. 3. Proposed mechanism of hydrolysis of OP compound and then neuro-enzyme block by the active nerve agents, X= halide, pseudohalides, alkoxide

will undergo a dealkylation process known as "aging" [35] in which the phosphorylated AChE becomes resistant to hydrolysis and is considered irreversibly bound to sarin become inert adduct and thus, irreversibly inhibited (Fig. 4). Organophosphorus nerve agents can be lethal nevertheless at very low concentrations, with death following in few moments after direct inhalation due to suffocation from lung muscle paralysis, unless some antidotes are applied. People can be exposed to

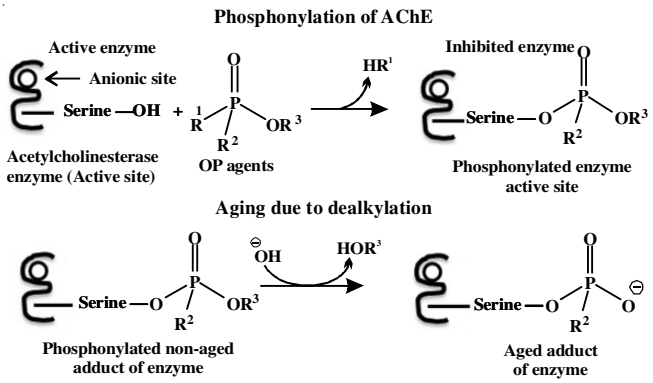


Fig. 4. Mechanism of inhibition of acetylcholinesterase (AChE) (phosphonylation) and aging (dealkylation) by nerve agents [R^1 = halides/psuedohalides, R^2 = alkyl]

TABLE-1
MEASURED TOXICITY DATA OF COMMON NERVE AGENTS

Nerve agents	Toxicity of nerve agents		
	LD_{50} (mg/person)	LC_{50} (ppm)	LCt_{50} (mg min/m^3)
Tabun (GA)	1000	2.0	150-400
Sarin (GB)	1700	1.2	60-100
Soman (GD)	350	0.9	35-70
Cyclosarin (GF)	30	Unknown	35
VX	10	0.3	10-50

the vapour even if they do not come in contact with the liquid form of chemicals through skin or eye contact or by breathing air. They can be affected by touching or drinking contaminated water by toxins since it is highly miscible into water called poison water. It may be exposed from contaminated food or even through clothing also.

Detection: Nowadays at first identification, detection [20, 36] and then determination as well as elimination of organophosphorus compounds including nerve agents have became important mission in the view point of their potential use in the terrorists warfare. The most common ways for detecting organo-phosphorus pesticides are chromatographic methods like liquid chromatography (LC) or high performance liquid chromatography (HPLC) coupled with different detectors such as mass spectrometry (MS). Few researchers [37-40] reported the detection process utilize spectrometry, molecular imprinting coupled with luminescence and fluorescent study for this purpose. In the case of the direct method, the assay is based on the spectrophotometric or electrochemical measurement is the advanced method of recognition. Fluorescence based sensors biosensors/chemosensors [41-45] offer significant advantages over other conventional methods for detection of organophosphorus compounds and many other advanced tools are developing for detection devices and modern approaches also included in the detection and analysis procedure.

Remedy of nerve agent exposure: Removal of organophosphorus pesticides from the exposed area of the body as soon as possible and providing supportive medical care are the most important treatment of exposure. Some most effective medicines like atropine, biperiden and pralidoxime are available for organophosphorus nerve agent's treatment [46] but they do not re-establish the functionality of AChE and thus, are not considered as antidote for this purpose. A diverse range of pyridinium salts functionalized oxime derivatives are used as antidotes to treat the nerve agent's exposure efficiently. Antidote like pralidoxime or pyridine-2-aldoxime methiodide (2-PAM) [47], which can be easily synthesized by reaction of pyridine-2-aldehyde with hydroxylamine followed by addition of methyl iodide [48] is renowned as first drug used very effectively in acetylcholinesterase reactivator [49]. They actually oriented such a way that generate an interaction with the anionic site of the enzyme using it's positive nitrogen centre and reactivates the inhibited enzyme through a nucleophillic attack from oximate anion(antidote) to the phosphorus atom of the covalently bound inhibitor followed by the detachment of nerve agent-antidote adduct generating free AChE (Fig. 5) [50-59]. In this perception, amalgamation of amidine group into the organic oxime drug molecules enhances the binding interaction to the biomolecules as enzyme adduct by generating positive charge and hence the electron draining power on amidine moiety, which increases the nucleophillic ability of nearby oxime group attached to it, is responsible for reactivation of blocked enzyme by nerve agents [60,61]. Compare to the normal oxime antidotes or the pro-drug therapy, application of amidine-oxime reactivators have the advantages as (i) improved chemical stability, (ii) greatly improved lipophilicity, (iii) increased *in vitro* reactivation efficacy and (iv) significant protection from the in vivo toxic effects of organophosphorus pesticides, made them superior combined antidotes in this field [62].

After rigorous study and experiments on the various animal models, it is found that a large number of incompatible data on the efficacy of different oximes in the treatment of different nerve agents exposure. Benzodiazepines and midazolam is much effective drug for seizures due to organophosphorus intoxication and authenticated by animal model study [63-66]. Many other bis-quaternary oximes such as HI-6, Hlö-7, MMB-4, obidoxime, *etc*. are more promising antidotes [67-71] and encouraged for recent efforts in the field of organophosphorus antidote research. However, the main difficulty is that broad-spectrum antidotes *i.e*. applied for regeneration of maximum

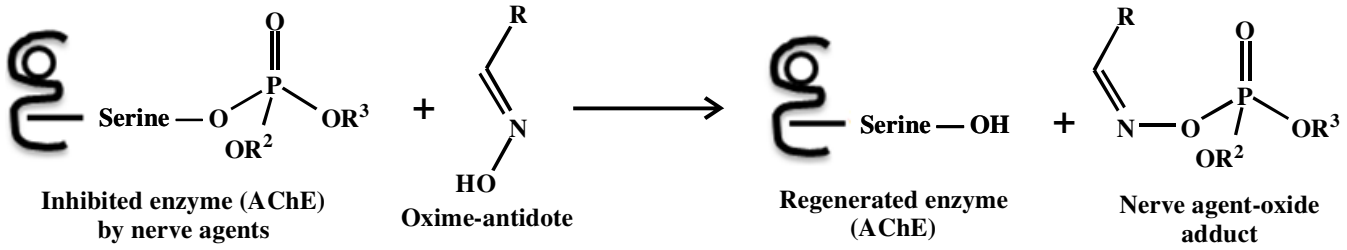


Fig. 5. Mechanism of regeneration of nerve agent-inhibited acetylcholinesterase (AChE) by general oxime as antidote

nerve agent's inhibition have not been identified [72,73] and their safety profile is not up to the mark as that of 2-PAM [58]. There is some reports of unconventional application of pro-drug of 2-PAM, which is oxidized *in vivo* (CNS) producing functionally active quaternary oxime are effective for reactivation of blocked enzyme [74-76] though the prime disadvantages are the synthesis difficulties [77] and poor stability of such pro-drugs. There are many modern approaches to dealing with nerve agent exposure as catalytic antibody therapy [78,79] and prophylactic gene therapy [80] are much successful.

Conclusion

Although the organophosphorus compounds are developed for the agro-industrial use as pesticides, but they are frequently employed as chemical warfare of terrorist attack utilizing their destructive property as nerve agents. In this review article, the chemical property and mechanisms of bio-interaction, way of action, toxicity, the potential health effects on exposure, currently available treatments for intoxication and the future directions and the scope of modern research of organophosphorus pesticides nerve agents are summarized. There are many tools for sensitive detection of trace of organophosphorus chemicals such as fluorescence method, sensing, chromatography are beneficial. A promising novel technology for the miniaturized detectors utilizing micro-fluidics and nanotechnology, are easy to operate and can be applied for onsite. Here, the different parameters of mechanism of action of AChE inhibition (phosphonylation and aging) by nerve agents and its physiological aspects as well as the biochemistry involved there are systematically reviewed. Again, the current available treatment options and the modern research directions for their progress including the development of novel drugs as antidote are compulsory for medical countermeasures. It must be undeniable that the design and development of new drug is promising field of research to counter the adverse effect of organophosphorus compounds once it is exposed to mankind. Finally, there must be a mutual understanding between the policies and protocols to minimize the use of harmful organophosphate pesticides for agronomic problems as well as their application as chemical warfare must be monitored and banned globally.

ACKNOWLEDGEMENTS

The infrastructural support from Sarsuna College, Kolkata, India is gratefully acknowledged. The author heartily acknowledges Smt. Sucheta Sarkar for her support in this work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. J. Kang, V.H. Zettel and N.I. Ward, *J. Nutr. Environ. Med.*, **5**, 325 (1995); https://doi.org/10.3109/13590849509007239
2. M. Sakata, Organophosphorus Pesticides, Springer-Verlag: Berlin Heidelberg (2005).
3. N.A. Milosevia and M.M. Govedarica, *Matica Srpska Novi Sad.*, **102**, 5 (2002).
4. M. Thakur, I.L. Medintz and S.A. Walper, *Front. Bioeng. Biotechnol.*, **7**, 289 (2019); https://doi.org/10.3389/fbioe.2019.00289
5. N. Turton, R.A. Heaton, F. Ismail, S. Roberts, S. Nelder, S. Phillips and I.P. Hargreaves, *Neurochem. Res.*, **46**, 131 (2021); https://doi.org/10.1007/s11064-020-03033-y
6. B. Balali-Mood, Chemistry and Classification of Organophosphorus Compounds In: Basic and Clinical Toxicology of Organophosphorus Compounds, Springer: London, UK (2014).
7. S.V. Kumar, Md. Fareedullah, Y. Sudhakar, B. Venkateswarlu and E.A. Kumar, *Arch. Appl. Sci. Res.*, **2**, 199 (2010).
8. T. Okumura, N. Takasu, S. Ishimatsu, S. Miyanoki, A. Mitsuhashi, K. Kumada, K. Tanaka and S. Hinohara, *Ann. Emerg. Med.*, **28**, 129 (1996); https://doi.org/10.1016/S0196-0644(96)70052-5
9. T. Suzuki, H. Morita, K. Ono, K. Maekawa, R. Nagai, Y. Yazaki, H. Nozaki, N. Aikawa, Y. Shinozawa, S. Hori, S. Fujishima, K. Takuma and M. Sagoh, *Lancet*, **345**, 980 (1995); https://doi.org/10.1016/S0140-6736(95)90726-2
10. T. Okumura, T. Hisaoka, A. Yamada, T. Naito, H. Isonuma, S. Okumura, K. Miura, M. Sakurada, H. Maekawa, S. Ishimatsu, N. Takasu and K. Suzuki, *Toxicol. Appl. Pharmacol.*, **207**, 471 (2005); https://doi.org/10.1016/j.taap.2005.02.032
11. S. Costanzi, J.-H. Machado and M. Mitchell, *ACS Chem. Neurosci.*, **9**, 873 (2018); https://doi.org/10.1021/acschemneuro.8b00148
12. C. Michaelidou, P. Piera and S.A. Nicolaou, Eds.: T. Albanis, Evaluation of Combination Toxic Effects and Genotoxicity of Pesticides for Environmental Protect and Sustainability, Proceeding of the 1st European Conference on Pesticides and Related Organic Micropollutants in the Environment, Ioannina, Greece (2000).
13. P.I. Plaza, E. Martínez-López and S.A. Lambertucci, *Sci. Total Environ.*, **687**, 1207 (2019); https://doi.org/10.1016/j.scitotenv.2019.06.160
14. M. Calic, A. Bosak, K. Kuca and Z. Kovarik, *Chem. Biol. Interact.*, **175**, 305 (2008); https://doi.org/10.1016/j.cbi.2008.04.010
15. Z. Kovarik, M. Calic, A. Bosak, G. Šinko and D. Jelic, *Croat. Chem. Acta*, **8**, 47 (2008).
16. R. Odzak, M. Calic, T. Hrenar, I. Primozic and Z. Kovarik, *Toxicology*, **233**, 85 (2007); https://doi.org/10.1016/j.tox.2006.08.003
17. Z. Kovarik, N. Ciban, Z. Radic, V. Simeon-Rudolf and P. Taylor, *Biochem. Biophys. Res. Commun.*, **342**, 973 (2006); https://doi.org/10.1016/j.bbrc.2006.02.056
18. Z. Kovarik, M. Calic, G. Šinko and A. Bosak, *Arh. Hig. Rada Toksikol.*, **58**, 201 (2007); https://doi.org/10.2478/v10004-007-0013-7
19. R. Norlin and G. Lindberg, *J. Labelled Comp. Radiopharm.*, **46**, 599 (2003); https://doi.org/10.1002/jlcr.699
20. O. Yagmuroglu and B. Subasi, *Open Access J. Sci.*, **4**, 47 (2020); https://doi.org/10.15406/oajs.2020.04.00151
21. A. Watson, D. Opresko, R. Young, V. Hauschild, J. King and K. Bakshi, Eds.: R.C. Gupta, Organophosphate Nerve Agents, In: Handbook of Toxicology of Chemical Warfare Agents, Academic Press Inc.: London, Chap. 6 (2009).
22. H.P. Benschop and L.P.A. De Jong, *Acc. Chem. Res.*, **21**, 368 (1988); https://doi.org/10.1021/ar00154a003
23. Z. Kovarik, Z. Radic, H.A. Berman, V. Simeon-Rudolf, E. Reiner and P. Taylor, *Biochem. J.*, **373**, 33 (2003); https://doi.org/10.1042/bj20021862
24. Y. Bourne, Z. Radic, G. Sulzenbacher, E. Kim, P. Taylor and P. Marchot, *J. Biol. Chem.*, **281**, 29256 (2006); https://doi.org/10.1074/jbc.M603018200
25. M. Kataoka, K. Tsuge, H. Takesako, T. Hamazaki and Y. Seto, *Environ. Sci. Technol.*, **35**, 1823 (2001); https://doi.org/10.1021/es001529z
26. C.B. Millard, G. Kryger, A. Ordentlich, H.M. Greenblatt, M. Harel, M.L. Raves, Y. Segall, D. Barak, A. Shafferman, I. Silman and J.L. Sussman, *Biochemistry*, **38**, 7032 (1999); https://doi.org/10.1021/bi9826781
27. A. Hörnberg, A.-K. Tunemalm and F. Ekström, *Biochemistry*, **46**, 4815 (2007); https://doi.org/10.1021/bi0621361

28. S.W. Wiener and R.S. Hoffman, *J. Intensive Care Med.*, **19**, 22 (2004); https://doi.org/10.1177/0885066603258659
29. V. Rembovskiy, A. Radilov, E. Savelieva, S. Kuznetsov, I. Mindukshev, D. Prokofieva, N. Krivorotova, N. Khlebnikova, R. Jenkins, N. Goncharov and V.X. Russian, Rds.: R.C. Gupta, Handbook of Toxicology of Chemical Warfare Agents, Academic Press, Ed.: 2 (2015).
30. J. Wang, S. Roszak, J. Gu and J. Leszczynski, *J. Phys. Chem. B*, **109**, 1006 (2005); https://doi.org/10.1021/jp040574g
31. A.W. Tuin, M.A.E. Mol, R.M. van den Berg, A. Fidder, G.A. van der Marel, H.S. Overkleeft and D. Noort, *Chem. Res. Toxicol.*, **22**, 683 (2009); https://doi.org/10.1021/tx8004218
32. H.Y. Tan, W.K. Loke, Y.T. Tan and N.-T. Nguyen, *Lab Chip*, **8**, 885 (2008); https://doi.org/10.1039/b800438b
33. S. Upadhyay, M.K. Sharma, V.K. Rao, B.K. Bhattacharya, D. Sharda and R. Vijayaraghavan, Eds.: M. Stoytcheva, Pesticides – Strategies for Pesticides Analysis, InTech, Croatia, pp. 315-333 (2011).
34. P. Taylor, Eds.: J.G. Hardman, L.E. Limbird and A.G. Gilman, Anticholinesterase Agents, In: Goodman & Gilman's The Pharmacological Basis of Therapeutics, McGraw-Hill: New York, Ed.: 10 (2001).
35. P. Masson, F. Nachon and O. Lockridge, *Chem. Biol. Interact.*, **187**, 157 (2010); https://doi.org/10.1016/j.cbi.2010.03.027
36. T.-M. Shih and J.H. McDonough, *Arch. Toxicol.*, **74**, 165 (2000); https://doi.org/10.1007/s002040050670
37. J. Gündel and J. Angerer, *J. Chromatogr. B Biomed. Sci. Appl.*, **738**, 47 (2000); https://doi.org/10.1016/S0378-4347(99)00499-5
38. J.F. García-Reyes, I. Ferrer, M. Thurman, A. Molina-Díaz and A. Fernandez-Alba, *Rapid Commun. Mass Spectrom.*, **19**, 2780 (2005); https://doi.org/10.1002/rcm.2127
39. A.L. Jenkins, O.M. Uy and G.M. Murray, *Anal. Commun.*, **34**, 221 (1997); https://doi.org/10.1039/A704220E
40. C.M. Rudzinski, A.M. Young and D.G. Nocera, *J. Am. Chem. Soc.*, **124**, 1723 (2002); https://doi.org/10.1021/ja010176g
41. A.A. Ciucu, C. Negulescu and R.P. Baldwin, *Biosens. Bioelectron.*, **18**, 303 (2003); https://doi.org/10.1016/S0956-5663(02)00173-2
42. E.E. Ferapontova, V.G. Grigorenko, A.M. Egorov, T. Börchers, T. Ruzgas and L. Gorton, *Biosens. Bioelectron.*, **16**, 147 (2001); https://doi.org/10.1016/S0956-5663(01)00134-8
43. J-M. Lin, Chemiluminescence-Basic Principle and Applications. Chemical Industry Press: Beijing, p. 33 (2004).
44. F.N. Kok, F. Bozoglu and V. Hasirci, *Biosens. Bioelectron.*, **17**, 531 (2002); https://doi.org/10.1016/S0956-5663(02)00009-X
45. S. Upadhyay, G.R. Rao, M.K. Sharma, B.K. Bhattacharya, V.K. Rao and R. Vijayaraghavan, *Biosens. Bioelectron.*, **25**, 832 (2009); https://doi.org/10.1016/j.bios.2009.08.036
46. M.A. Dunn and F.R. Sidell, *JAMA*, **262**, 649 (1989); https://doi.org/10.1001/jama.1989.03430050065028
47. T. Zorbaz, D. Malinak, N. Marakovic, N.M. Hrvat, A. Zandona, M. Novotny, A. Skarka, R. Andrys, M. Benkova, O. Soukup, M. Katalinic, K. Kuca, Z. Kovarik and K. Musilek, *J. Med. Chem.*, **61**, 10753 (2018); https://doi.org/10.1021/acs.jmedchem.8b01398
48. G. Petroianu, *Pharmazie*, **68**, 916 (2013).
49. I.B. Wilson and B. Ginsburg, *Biochim. Biophys. Acta*, **18**, 168 (1955); https://doi.org/10.1016/0006-3002(55)90040-8
50. K. Kuca, D. Jun and K. Musilek, *Mini Rev. Med. Chem.*, **6**, 269 (2006); https://doi.org/10.2174/138955706776073510
51. F. Worek, H. Thiermann and T. Wille, *Arch. Toxicol.*, **94**, 2275 (2020); https://doi.org/10.1007/s00204-020-02797-0
52. S.B. Bharate, L. Guo, T.E. Reeves, D.M. Cerasoli and C.M. Thompson, *Bioorg. Med. Chem.*, **18**, 787 (2010); https://doi.org/10.1016/j.bmc.2009.11.052
53. J. Smythies and B. Golomb, *J. R. Soc. Med.*, **97**, 32 (2004); https://doi.org/10.1258/jrsm.97.1.32
54. E. Dolgin, *Nat. Med.*, **19**, 1194 (2013); https://doi.org/10.1038/nm1013-1194
55. S. Mukherjee and R.D. Gupta, *J. Toxicol.*, **2020**, 3007984 (2020); https://doi.org/10.1155/2020/3007984
56. M.A. Gallo and N.J. Lawryk, Organic Phosphorus Pesticides, In: Handbook of Pesticide Toxicology, Academic Press: Chestnut Hill, MA, p. 917 (1991).
57. J.B. Sullivan and J. Blose, Organophosphate and Carbamate Insecticides, Hazardous Materials Toxicology-Clinical Principles of Environmental Health, Williams & Wilkins: Baltimore: USA, p. 1015 (1992).
58. M.A. Howland, Antidotes in Depth: Pralidoxime, Goldfrank's Toxicologic Emergencies, McGraw-Hill: New York, Ed.: 7, p. 1361 (2002).
59. K. Musilek, M. Dolezal, F. Gunn-Moore and K. Kuca, *Med. Res. Rev.*, **31**, 548 (2011); https://doi.org/10.1002/med.20192
60. J.C. Jaen, V.E. Gregor, C. Lee, R. Davis and M. Emmerling, *Bioorg. Med. Chem. Lett.*, **6**, 737 (1996); https://doi.org/10.1016/0960-894X(96)00102-3
61. T. Sauvaitre, M. Barlier, D. Herlem, N. Gresh, A. Chiaroni, D. Guenard and C. Guillou, *J. Med. Chem.*, **50**, 5311 (2007); https://doi.org/10.1021/jm070536w
62. J. Kalisiak, E.C. Ralph, J. Zhang and J.R. Cashman, *J. Med. Chem.*, **54**, 3319 (2011); https://doi.org/10.1021/jm200054r
63. B. Boskovic, *Fundam. Appl. Toxicol.*, **1**, 203 (1981); https://doi.org/10.1016/S0272-0590(81)80059-0
64. M.R. Murphy, D.W. Blick and M.A. Dunn, *Aviat. Space Environ. Med.*, **64**, 110 (1993).
65. R.M. Dawson, *J. Appl. Toxicol.*, **14**, 317 (1994); https://doi.org/10.1002/jat.2550140502
66. E. Gilat, M. Goldman, E. Lahat, A. Levy, I. Rabinovitz, G. Cohen, R. Brandeis, G. Amitai, D. Alkalai and G. Eshel, *Arch. Toxicol.*, **77**, 167 (2003); https://doi.org/10.1007/s00204-002-0425-8
67. K. Sakurada, K. Matsubara, K. Shimizu, H. Shiono, Y. Seto, K. Tsuge, M. Yoshino, I. Sakai, H. Mukoyama and T. Takatori, *Neurochem. Res.*, **28**, 1401 (2003); https://doi.org/10.1023/A:1024960819430
68. D.E. Lorke, H. Kalasz, G.A. Petroianu and K. Tekes, *Curr. Med. Chem.*, **15**, 743 (2008); https://doi.org/10.2174/092986708783955563
69. G. Cassel, L. Karlsson, L. Waara, K.W. Ang and A. Göransson-Nyberg, *Eur. J. Pharmacol.*, **332**, 43 (1997); https://doi.org/10.1016/S0014-2999(97)01058-3
70. H. Kalász, S.M. Nurulain, G. Veress, S. Antus, F. Darvas, E. Adeghate, A. Adem, F. Hashemi and K. Tekes, *J. Appl. Toxicol.*, **35**, 116 (2015); https://doi.org/10.1002/jat.3048
71. T. Zorbaz, A. Braïki, N. Marakovic, J. Renou, E. de la Mora, N.M. Hrvat, M. Katalinic, I. Silman, J.L. Sussman, G. Mercey, C. Gomez, R. Mougeot, B. Pérez, R. Baati, F. Nachon, M. Weik, L. Jean, Z. Kovarik and P.-Y. Renard, *Chem. Eur. J.*, **24**, 9675 (2018); https://doi.org/10.1002/chem.201801394
72. P.M. Lundy, A.S. Hansen, B.T. Hand and C.A. Boulet, *Toxicology*, **72**, 99 (1992); https://doi.org/10.1016/0300-483X(92)90089-W
73. R.H. Inns and L. Leadbeater, *J. Pharm. Pharmacol.*, **35**, 427 (2011); https://doi.org/10.1111/j.2042-7158.1983.tb04316.x
74. E. Shek, T. Higuchi and N. Bodor, *Med. Chem. (N.Y.)*, **19**, 113 (1976); https://doi.org/10.1021/jm00223a019
75. E. Shek, T. Higuchi and N. Bodor, *Med. Chem. (N.Y.)*, **19**, 108 (1976); https://doi.org/10.1021/jm00223a018
76. J.C. DeMar, E.D. Clarkson, R.H. Ratcliffe, A.J. Campbell, S.G. Thangavelu, C.A. Herdman, H. Leader, S.M. Schulz, E. Marek, M.A. Medynets, T.C. Ku, S.A. Evans, F.A. Khan, R.R. Owens, M.P. Nambiar and R.K. Gordon, *Chem. Biol. Interact.*, **187**, 191 (2010); https://doi.org/10.1016/j.cbi.2010.02.015
77. N. Bodor, E. Shek and T. Higuchi, *J. Med. Chem.*, **19**, 102 (1976); https://doi.org/10.1021/jm00223a017
78. M.A. Dunn, B.E. Hackley and F.R. Sidell, Eds.: F.R. Sidell, T.E. Takafuji and D.R. Franz, Pretreatment for Nerve Agent Exposure, In: Textbook of Military Medicine: Medical Aspects of Chemical and Biological Warfare, Office of the Surgeon General, U.S. Army: Falls Church, VA, USA, p. 181 (1997).
79. Y.X. Ci, Y.X. Zhou, Z.Q. Guo, K.-T. Rong and W. Chang, *Arch. Toxicol.*, **69**, 565 (1995); https://doi.org/10.1007/s002040050213
80. J. Cowan, C.M. Sinton, A.W. Varley, F.H. Wians, R.W. Haley and R.S. Munford, *Toxicol. Appl. Pharmacol.*, **173**, 1 (2001); https://doi.org/10.1006/taap.2001.9169