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Organophosphorous Compounds- Toxicity and Detection Approach

Sanjay Upadhyay, Mukesh K. Sharma, Vepa K. Rao,
Bijoy K. Bhattacharya, Dileep Sharda and R. Vijayaraghavan
*Defence Research & Development Establishment,
Jhansi Road, Gwalior-474002
India*

1. Introduction

The first organophosphorus (OP) compound tetraethyl pyrophosphate (TEPP) was synthesized by de Clermont in France. Later, von Hofmann synthesized methyl-phosphoryl dichloride (Fest & Schmidt, 1982). OP compounds are widely used in the agriculture industry around the world as pesticides and insecticides. Phosphorous compounds play a central role in the living organism; it is pertinent to mention photosynthesis, metabolism, and involvement in coenzyme systems etc. It can have a variety of oxidation states 3 and 5, generally OP compounds based on their derivatives of phosphorous. Organophosphate triesters, phosphonates, phosphonofluoridates and phosphonothioates comprise a broad class of chemical neurotoxins (Fig 1). The hydrolysis of OP compounds follows several patterns, depending upon the type of ester, the solvent, the pH range or upon catalytically active additives.

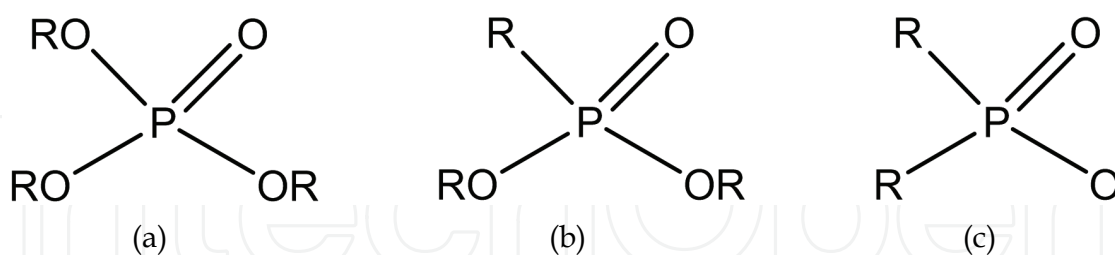


Fig. 1. Structures of (a) Phosphate, (b) Phosphonates and (c) Phosphinates

However, the high toxicity of the OP compounds had not been recognized until the 1930s, when Lange and Krüger described effects, which they noticed during synthesis of some OP with the P-F bond (Holmstedt, 1963). German Chemists subsequently became interested in synthesizing insecticides. G. Schrader, in 1936, synthesized highly toxic OP insecticide ethyl-N,N-Dimethylphosphoramidocyanidate (tabun) and isopropyl methylphosphonofluoridate (sarin) in 1937 (Robinson & Leitenberg, 1971). Schrader synthesized the toxic OP compounds in search of better insecticides. Nerve agents are also OP compounds such as sarin (GB), tabun (GA), soman (GD) and VX are categorized as chemical warfare (CW) agents. During World War II, the Germans possessed large quantities of tabun and sarin although they

were not used in that conflict. Nerve agents are divided into two main groups: the G-agents and V-agents. The G-agents are nonpersistent (sarin, soman, & tabun) and cause casualties primarily by inhalation. Sarin is highly volatile compared to tabun and soman. The V-agents are persistent (VX) they can therefore cause casualties by both inhalation and absorption through the skin.

In 1944, G.Schrader synthesized the parathion series of OP compounds. The first member of parathion series is O,O-diethyl O-4-nitrophenyl phosphate (paraoxon). These compounds have excellent insecticidal properties, but on the other hand they are highly toxic to mammals. Schrader therefore sought to synthesize esters with as low toxicity as possible to ensure maximum safety for all users. Therefore, he changed the ethyl group to methyl esters i.e. parathion methyl (O,O-dimethyl O-4-nitrophenyl phosphorothioate (Fig.2).

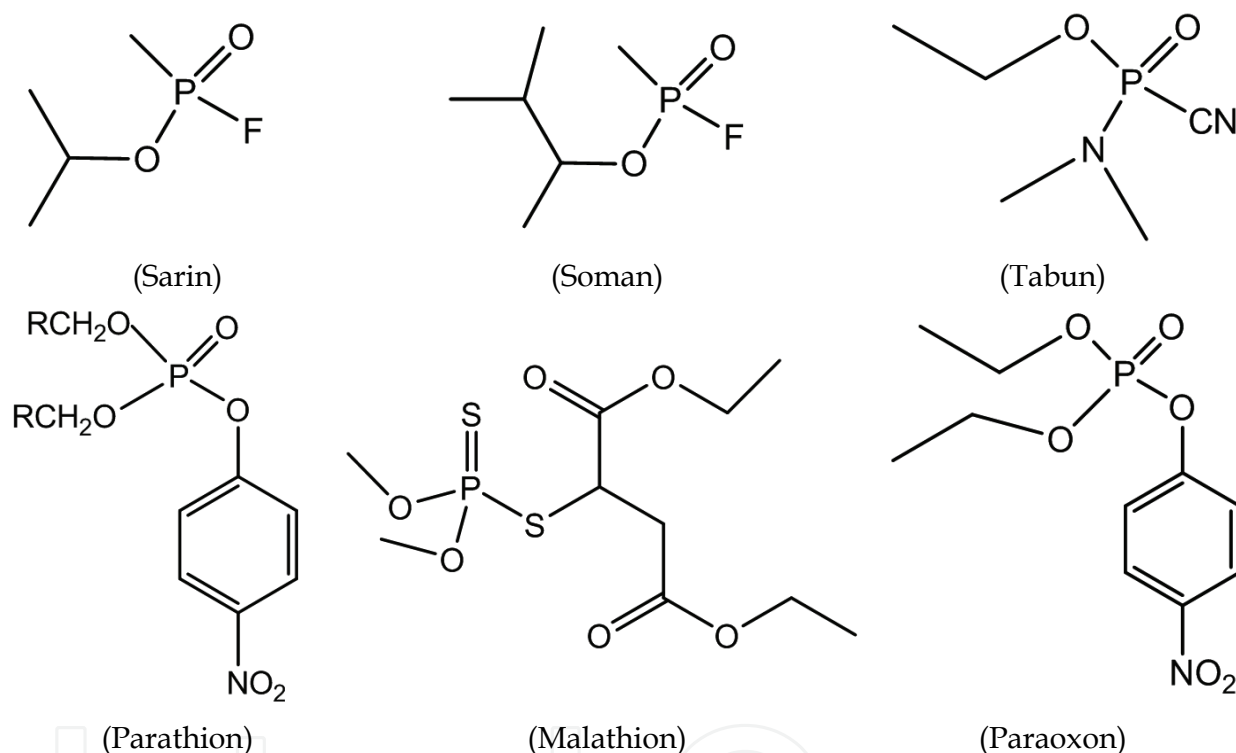


Fig. 2. Structures of OP compounds- Nerve agents and Pesticides

OP pesticides and insecticides are extensively used by farmers all over the World (Gilliom et al., 1999). The general chemical structure of these types of deadly OP compounds consist of a tetrasubstituted phosphorous (V) center, an oxygen or sulfur atom double bonded to the phosphorous, a leaving group, and two substituents that vary widely depending on the subclass. Due to their widespread presence, great environmental concerns have recently arisen around this type of pollution (Fig 3). These effective broad-spectrum compounds used against insect and arthropod, pests, are highly toxic to humans by different routes of exposures, such as dermal absorption, ingestion or inhalation. These contaminants pose serious to fatal health hazards, such as asthma, birth defects and deaths. Therefore, environmental monitoring is required to protect the public and the environment from possible organic toxins released into the air, soil, and water.

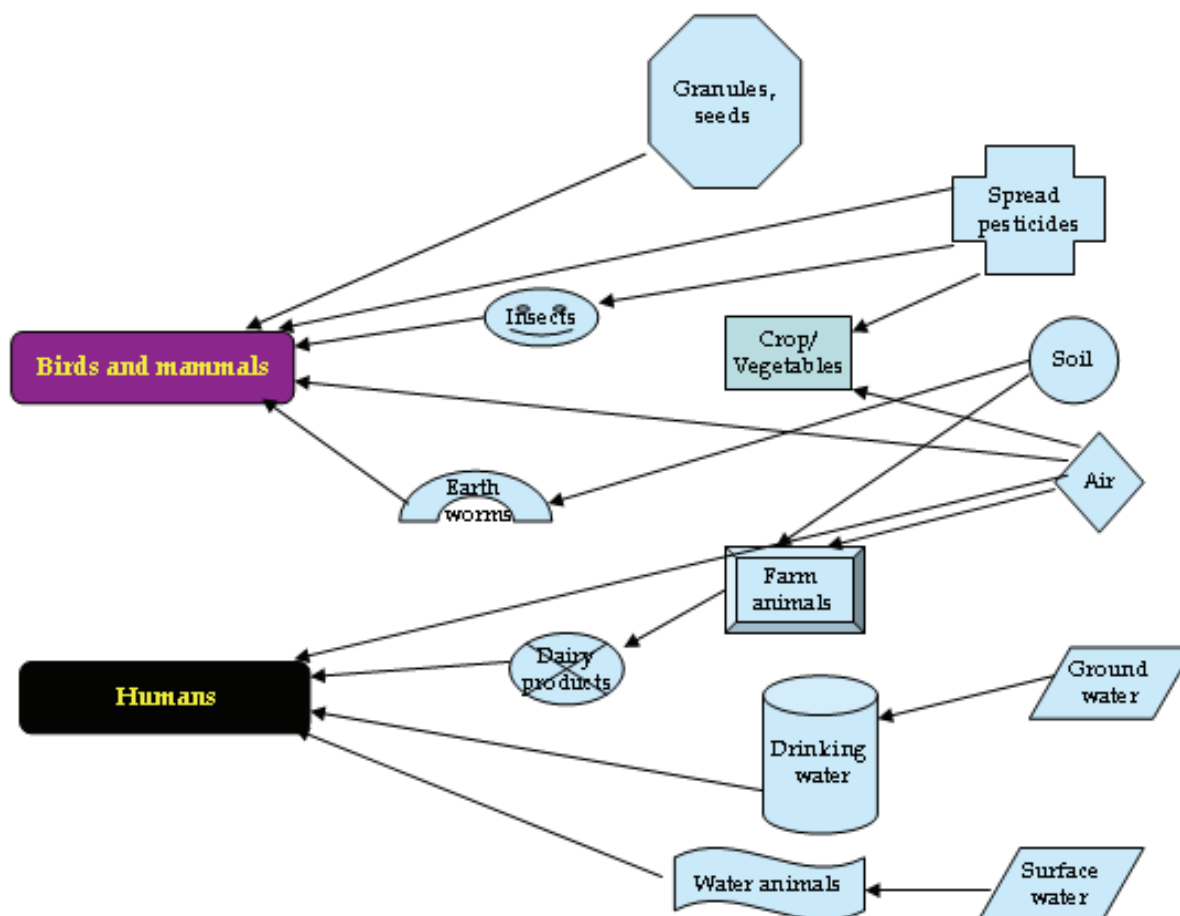


Fig. 3. Possible routes of environmental exposure of OP Pesticides/nerve agents to humans and wildlife

2. Mode of action of OP compounds on acetylcholinesterase enzyme

The toxicity or mode of action of OP compounds can be attributed to the inhibition of the enzyme acetylcholinesterase (AChE). AChE is a globular protein and its three-dimensional structure is known. Its physiological substrate is acetylcholine. The active site of AChE consists of two subsites, anionic and esteratic sites. The anionic site is represented by a glutamate ion. The esteratic site has serine moiety and histidine as well as tyrosine residues (Schumacher et al., 1986). This enzyme is essential for the central nervous system, and being present in both humans and insects. The normal function of AChE is the hydrolysis of acetylcholine neurotransmitter in the synaptic membrane to prevent its accumulation, and as a result forming acetylated enzyme and releasing choline. The high percentage of released choline is transported back into the nerve ending for reconversion to acetylcholine and storage (Fig. 4). This degradation process results in a lowered level of acetylcholine, and ultimately the termination of nerve impulses.

OP compounds covalently block the active site of serine residue of AChE by undergoing nucleophilic attack to produce a serine-phosphoester adduct. This irreversible inactivation leads to an excess accumulation of acetylcholines in the peripheral and central nervous system causing cholinergic manifestations. At high doses, there is depression of the respiratory centre in the brain, followed by peripheral neuromuscular blocked causing respiratory paralysis and

death (Baigar, 2004; Somani, 1992; Vijayaraghavan et al., 2010). The pharmacologic effects and toxicity of these OP compounds are dependent on their stability, rate of absorption by various routes, distribution ability to cross the blood-brain barrier, rate of reaction with AChE.

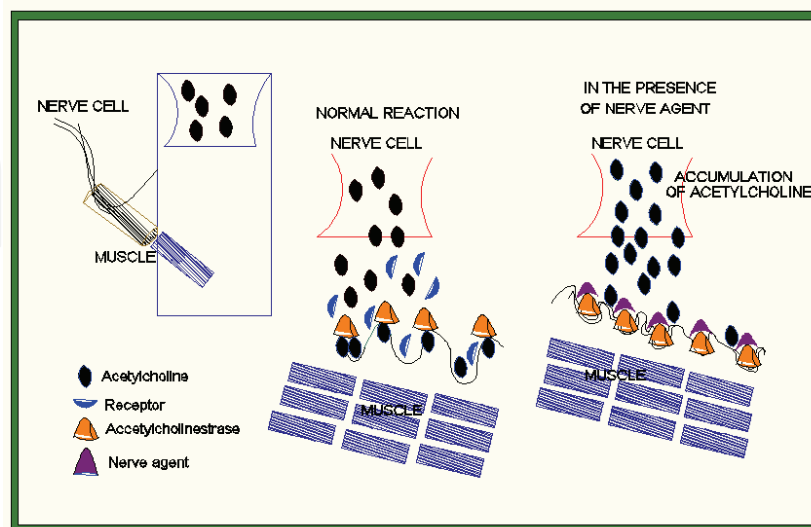


Fig. 4. Action of OP compounds on acetylcholinesterase

3. Toxicity of OP compounds and treatment

The nerve agents (also known as nerve gases) are organophosphorus compounds (OP). All OP compounds do not qualify as war gases due to their differential toxicity. Some of the OP compounds are less toxic to humans and are used as insecticides. Agents that fall in the nerve agent category are tabun, sarin, soman and VX. The absorption of these agents into the system is through inhalation, and if the skin is also exposed, they can be absorbed appreciably.

The effects of nerve agents are the result of the action on the muscarinic and nicotinic receptors within the central nervous system. They include constriction of the pupil (meiosis), increased production of saliva, running nose, increased perspiration, urination, defecation, bronchosecretion, bronchoconstriction, decreased heart rate and blood pressure, muscular twitches and cramps, cardiac arrhythmias, tremors and convulsions. The most critical effects are paralysis of the respiratory muscles and inhibition of the respiratory center. Ultimately death is due to respiratory paralysis. If the concentration of the nerve agent is high, death is immediate (Baigar, 2004; Munro et al., 1994; Somani, 1992; Vijayaraghavan et al., 2010)).

LD₅₀ is the dose that may kill 50% of the population exposed. LC_{t50} is the product of concentration of a vapour or an aerosol and the time for which one is exposed, that may kill 50 % of the population. Toxicological parameters of nerve agents and OP pesticides are shown in Table 1.

4. Treatment of OP compounds poisoning

The treatment of nerve agent poisoning requires to be done under the supervision of medical personnel (Marrs et al. 2006; Thiermann et al.2007) The treatment schedule can be classified as:

- i. Termination of further intoxication
- ii. Artificial respiration or oxygen therapy, and
- iii. Antidote therapy

5. Termination of further Intoxication

Like any other poison, the first and the foremost step is removal of the subject from the contaminated environment and removal of the toxicant from the skin.

NerveAgents/ Insecticides/	LD ₅₀ (bare skin) mg	LD ₅₀ (oral) mg	LCT ₅₀ (inhalation) mg.min.m ⁻³
Tabun	200-1000	25-50	100-200
Sarin	100-500	5-20	50-100
soman	50-300	5-20	25-50
VX	5-15	3-10	5-15
Dichlorvas	>7000	300-6000	500-1000
Malathion	>25000	400-40000	-
Parathion	1470	70	-
Methidathion	> 100000	1400	-
Fenthion	>23000	>15000	-
Mevinpos	>300	>250	-

Table 1. Toxicity data of nerve agents and insecticides for a 70 Kg man (Median lethal dose).

6. Artificial respiration or oxygen therapy

Artificial respiration is very important since it assists the patient in breathing, and should be initiated as early as possible either manually or by mechanical respirators. Artificial respiration must continue until natural breathing of the patient is restored.

7. Antidote therapy

The principle of antidote therapy is based on the effects of the nerve agents as shown in the Table 2.

Effects	Treatment	Drugs
Excess of acetylcholine	Antagonists of acetylcholine	Cholinolytics
Cholinesterase Inhibition	Reactivation of cholinesterase	Oximes
Convulsions	Anticonvulsants	Diazepam

Table 2. Treatment of OP compounds exposure.

8. Cholinolytics

These drugs are very important to block the excess action of acetylcholine. They are competitive inhibitors of muscarinic receptors. As atropine has been studied extensively in this group, it is invariably used. Atropine should be administered immediately and

repeatedly starting with an initial dose of 2 mg intravenously, till it is adequate (atropinisation), as indicated by dryness of mucosa of nose and mouth, and an increase in heart rate. The administration of atropine has to be continued for several days or weeks, depending on the severity of intoxication (2-4 mg per week for moderate exposure). The dosage of atropine should not hinder the performance of a non-intoxicated individual. Side effects of 2 mg atropine in a normal individual are increased heart rate, drying of secretions, mydriasis (dilatation of pupil) and paralysis. Most of the effects are reversible. Inhibition of sweating in a non-nerve agent poisoned individual is hazardous and is temperature dependent.

9. Oximes

Oximes are used as cholinesterase reactivators, thereby restoring the inhibited AChE. The oximes in common use are pralidoximechloride (2-PAM) and obidoxime (toxogonin). But, these oximes are not effective for soman poisoning. For this H-series oximes are preferred e.g. HI-6.

The oximes should be administered in combination with atropine. The dose of pralidoxime chloride is 15 - 25 mg.kg⁻¹ by slow intravenous injection. Autoinjectors like Combopen type contain 600 mg of pralidoxime chloride in 2 ml solution. Commercially available vials containing 1 g of pralidoxime can be dissolved in 3 ml of sterile water or saline and 2 ml administered intramuscularly. The usual dose of obidoxime is 300 mg. Since these oximes are quickly excreted, a second or third dose may be needed at regular intervals.

10. Diazepam

Nerve agent poisoning leading to severe convulsions and may cause brain damage in severely exposed patients. Diazepam is used as an adjunct to reduce the convulsions. The usual dose of diazepam is 5 - 10 mg.

11. Self treatment

It is important that the antidotes should be administered very quickly in the field itself in the form of first aid. This is done by the use of autoinjectors. These autoinjectors contain a cholinolytic (atropine) and an oxime (2-PAM or obidoxime). The autoinjectors are simple to use and are for intramuscular injection only. Reusable autoinjectors are also available (Autoject Injectors) for atropine sulphate and pralidoxime chloride in which the drug cartridges can be replaced. This is ideal for mass casualty management.

12. Prophylaxis against nerve agent poisoning

There are no accepted prophylactic antidotes for nerve agent poisoning, i.e., drugs administered before exposure to the agent. Physostigmine or pyridostigmine, a reversible cholinesterase inhibitor, has been tried with some success in the prophylaxis of nerve agent poisoning. Pyridostigmine bromide has been introduced as a pretreatment drug. The dose is 30 mg, three times a day. Though it may give some protection against nerve agent poisoning it has side effects.

13. Detection of OP compounds

In recent years the determination of OP compounds and nerve agents have become important because of the widespread use of OPs as pesticides and clear threat to people from the potential use of nerve agents by terrorists. Several organizations regulate the maximum levels of permissible pesticide residues in drinking water and in food for human and animal consumption. Among them, notable are Food and Agricultural Organization (FAO) of the United Nations, the World Health Organization (WHO), the European Union (EU), the US Environmental Protection Agency (EPA) and the US National Institute for Occupational Safety and Health (NIOSH). The Organization for the Prohibition of Chemical Weapons (OPCW) regulates the use of CW agents, through the implementation of the provisions of Chemical Warfare Convention (CWC).

Therefore, there is need to develop fast, sensitive, and field-deployable screening technology for quick response. The most common ways for detecting OP pesticides are chromatographic methods coupled with different detectors and spectrometry (Gundel & Angerer, 2000; Hernandez et al., 2005). This method is sensitive and reliable but can not carried out in field, it is expensive and time consuming too. In addition to this, variety of approaches have been investigated for sensors, including enzymatic assays (Russell et al., 2003), molecular imprinting coupled with luminescence (Jenkins et al., 1997; Rudzinski et al., 2002), colorimetric methods (Wallace et al., 2005), surface acoustic waves (Nieuwenhuizen & Harteveld, 1997), fluorescent organic molecules (Yamaguchi et al., 2005; Zhang & Swager, 2003), interferometry (Sohn et al., 2000) and enzyme biosensors based on inhibition of cholinesterase activity (Evtugyn et al., 1996; Trojanowicz, 2002).

14. Enzyme inhibition based biosensors

Enzyme-based biosensors have emerged during past few years and based on the principle of inhibition of AChE and electrochemical or optical based detection. Analytical devices based on the determination of inhibition of AChE have been widely used for the detection of OP compounds (Pavlov et al., 2005; Schulze et al. 2003; Tran-Minh et al. 1990). The screen-printed biosensors were used for the determination of methamidophos pesticides. The inhibition of AChE is measured by direct or indirect measurement of its activity. In the case of the direct method, the assay is based on the spectrophotometric or electrochemical measurement of thiocholine produced from the following reaction:

AChE



The rate of inhibition (%) is calculated before and after incubation with OP compounds as $100 \times (I_0 - I_i) / I_0$ where I_0 is current before inhibition and I_i is current after inhibition (Amine et al. 2006).

In the development of biosensors immobilization of enzymes is the critical step in maintaining enzyme activity, stability and shelf life of electrode. Various techniques are used such as physical entrapment, microencapsulation, covalent binding, adsorption and cross-linking. AChE was encapsulated in sol-gel film on a glass cap that could be fixed on an optical fiber (Doong & Tsai, 2001). Sol-gel technology provides an attractive way for the immobilization of biological entities including full cell, enzyme, protein and antibody or antigen due to the inert low temperature process (Pandey et al. 2000). Recently, the

nanoparticles and carbon nanotube (CNT) have received considerable attention to increase the sensitivity of the biosensor due to their high conductivity, catalytic and electrical properties (Pavlov et al., 2005). AChE was immobilized on silica sol-gel assembling gold (Au) nanoparticles for the inhibition study with OP compounds. Due to the large quantities of hydroxyl groups in the sol-gel composite provide a biocompatible environment for AChE enzyme. Immobilized AChE catalyze the hydrolysis of acetylthiocholine chloride and produce thiocholine which is again oxidized to produce signal (Fig.5). The Au nanoparticles catalyze the electro-oxidation of thiocholine. After incubation with OP compounds, peak current decreases and it shows the inhibition of immobilized enzyme and the inhibition is directly proportional to the concentration of OP compounds. In another approach reaction of thiocholine with 7-diethylamino-3-(4'-maleimidylphenyl)-4-methylcoumarin (CPM) generates fluorescent product which can also be monitored for detection purpose based on inhibition approach (Parvari et al. 1983).

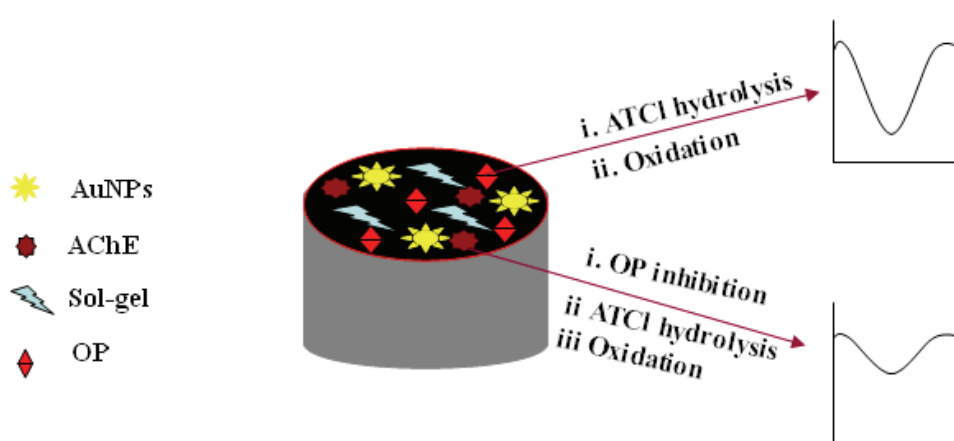
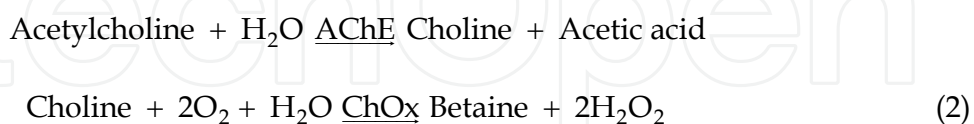


Fig. 5. Acetylcholinesterase biosensor principle for the detection of OP compounds based on AuNPs immobilized in silica sol-gel process

The detection of OP compounds have also been developed on the basis of two-enzyme approach. Acetylcholinesterase (AChE) and choline oxidase (ChOx) are used to recognize acetylcholine and choline. In this reaction, choline is acted upon by ChOx and resulted hydrogen peroxide (H_2O_2) is determined. Finally, oxidation of H_2O_2 is monitored amperometrically.



Various sensor based on bienzymatic approach have been developed for the detection of OP compounds (Ciucu et al., 2003, Ferapontova et al., 2001, Lin et al., 2004, Ferapontova et al., 2001, Kok et al., 2002, Upadhyay et al. 2009). The ferrophthalocyanine (FePC) modified carbon paste electrodes are used for the construction of bienzymatic amperometric biosensor which is operated at low potential (0.35 V) for the detection of OP pesticides (Ciucu et al. 2003). They reported detection limit up to 10^{-10} M of paraoxon and carbofuran. A disposable CNT based amperometric biosensor developed for the detection of these compounds (Lin et al., 2001). Here, also the biosensor is comprised of co-immobilization of CNT with AChE/ChOx enzymes on a screen printed electrode. In this method multiwall

CNT (MWCNT) showed a significant catalytic effect for the reduction and oxidation of H_2O_2 , leading to the development of an effective biosensor for the assay of chlorpyrifos, fenitrothion and methyl parathion with detection limit up to $0.05 \mu M$. These improved characteristics are attributed to the catalytic effect to H_2O_2 and the large surface area of MWCNT material. The application of bimetallic nanoparticles (Bi-MNPs) is also reported (Fig. 6) for the sensitive detection of OP pesticides and nerve agents (Upadhyay et al., 2009). A novel sensitive amperometric biosensor based on electrodeposition of gold-platinum bimetallic nanoparticles onto 3-aminopropyltriethoxy silane modified glassy carbon electrode for the detection of paraoxon ethyl, aldicarb, and sarin has been developed. The AChE and ChOx are coimmobilized on the Au-PtNPs modified electrode by cross-linking through glutaraldehyde. The key idea for using Au-PtNPs modified glassy carbon electrode is to improve the electrocatalytic activity of H_2O_2 on the modified electrode. Inhibition of enzyme depends upon the preincubation time of enzyme with inhibitors. It is observed that the inhibition level of enzyme increases or remaining enzyme activity decreases with increasing incubation time. The detection limit and linear working range of biosensors were reported at 30-40% inhibition level $150-200 \text{ nM}$, $40-50 \text{ nM}$ and $40-60 \mu M$ for paraoxon ethyl, sarin and aldicarb respectively. It can be reached below this range but to avoid the interference, 30-40% enzyme inhibition level was considered as optimum. This result showed that the biosensor has good analytical characteristics for these inhibitors due to electrochemical catalytic efficacy of Au-Pt NPs.

Biosensor based on flow injection amperometric detection of OP compounds/nerve agents has been developed. AChE is immobilized on the negatively charged CNT surface by alternatively assembling a cationic poly(diallyldimethylammonium chloride) (PDDA) layer and followed by self assembly of the negatively charged AChE layer. Under optimum conditions, the biosensor is used to measure as low as 0.4 pM paraoxon with a 6-min incubation time (Liu & Lin, 2006). In some other method AChE is immobilized on the pH sensitive redox polymer (polyaniline), which is coated on the vertically aligned thiol terminated ss-DNA-SWCNT on gold electrode for the detection of methyl parathion and chlorpyrifos. The key step of this biosensor is AChE-acetylcholine enzymatic reaction which causes small changes of local pH in the vicinity of an electrode surface. The pesticides are determined through inhibition of enzyme reaction (Viswanathan et al., 2009).

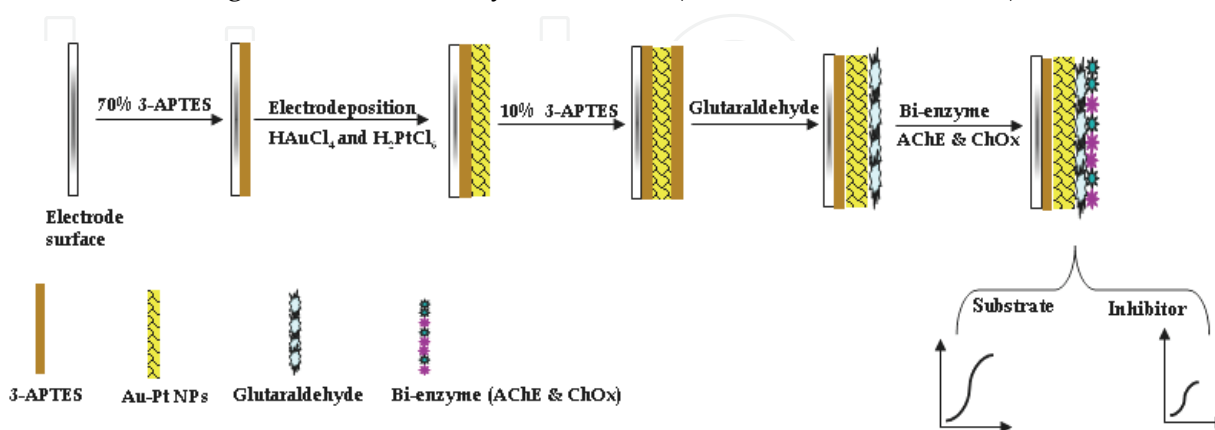


Fig. 6. Preparation of the bienzyme biosensors on Au-Pt NP modified electrode.

The reactivation of AChE enzyme after the inhibition by OP compounds has been investigated by using pyridine-2-aldoxime methyl iodide (2-PAM) and 4-formylpyridinium

bromide dioxime (TMB-4) (Amine et al., 2006, & Upadhyay et al., 2009). It has been found that the enzyme activity retains nearly 60% of initial activity with TMB-4, where as in the case of 2-PAM, the enzyme activity retention dropped to less than 50% of initial activity. Thus, it is recommended that the pesticide inhibited enzyme should be reactivated within 10 min to achieve the maximum reactivation of enzyme. In case of nerve agents owing to aging of inhibited AChE, a fraction of enzyme will irreversibly inhibited. In case of bienzymatic approach the relative proportion of AChE/ChOx will change. Therefore, it is not easy to get 100% recovery of enzyme after inhibition.

15. Fluorescence based detection of pesticides and Organo-phosphorous compounds

Fluorescence-based sensors (biosensors/chemosensors) offer significant advantages over other conventional methods for detection of OP compounds. The principal advantages of fluorescence are its high single-molecule sensitivity and in most of the cases it shows almost instantaneous response. Fluorescence methods are capable of measuring concentrations of analytes 10^6 times smaller than absorbance techniques (Martinez et al. 2003). A variety of analytical techniques have been developed which exploit changes in fluorescence properties of a molecule in different environments, whether those changes are quenching (Chen et al. 2000), and surface modified fluorescence (Kummerlen et al. 1993; Lichlyter et al. 2003). Molecular beacons provide an example of the use of surface modified fluorescence for the detection of DNA with sensitivity down to the mid nanomolar level (Bonnet and Libchaber 1999). The requirements for a successful sensor are: (1) high specificity in binding between recognition molecule and target, and (2) ability to easily manipulate the distance between nanoparticle and fluorophore in response to the target molecule concentration.

One of the most convenient and simple means of chemical detection is the generation of an optical signal, for example, changes in absorption or emission bands of the chemosensor in the presence of the target analyte. The principle behind sensor operation is based on nanoparticle- associated optical biosensors for the direct detection of organophosphate chemical warfare agents and pesticides is shown in Fig 7a and Fig 7b. As shown in Fig. 7a gold nanoparticle is covalently bound to an enzyme molecule. A fluorophore decoy, being a weak competitive inhibitor of organophosphorus hydrolase (OPH) with a similar chemical structure to the substrate (analyte of interest), is introduced to the solution and is bound to the OPH active site. If the gold particle attached via amino- or sulfhydryl groups to the OPH is at the certain distance from the decoy (size ranging from 10 to 40 nm), enhancement of fluorescence will be observed. If the nanoparticle is at a distance of greater than about 40 nm from the fluorophore, then fluorescence will be unaffected by the presence of the gold, leading to a reduction in fluorescence signal. Once the decoy is bound to the OPH active site, then it is possible test for the presence of the analyte of interest (which is a substrate of OPH). If the substrate is present, then the analyte will displace the decoy because of its much higher affinity for the OPH active site, and the fluorescence signal of the sample will change. As seen in Fig. 7b, for the case of an enhancement-based sensor, the analyte (indicated by S), will displace the decoy bound to the enzyme active site. As the decoy moves away from the gold nanoparticle, its fluorescence intensity will change. The change in fluorescence intensity is related to the concentration of analyte present in the solution.

Rogers et al. used a pH-sensitive fluorescent dye, consisting of AChE linked to the pH-sensitive compound fluorescein isothiocyanate (FITC). This biosensor was found to be very sensitive (capable of detecting nanomolar (nM) concentrations of paraoxon when exposed to the solution containing the analyte for ten minutes), and it demonstrated some selectivity toward different OP compounds (Rogers et al. 1991). A number of biosensors have been developed based on fluorescence polarization immunoassays (FPIA) (Kolosova et al. 2003; Kolosova et al. 2004; Lee et al. 2005; Tang et al. 2008). A rapid, fiber-optic biosensor assay for the direct detection of organophosphates was developed to provide continual remote monitoring and spectral fluorescent notification. In this study, the bio-recognition element, organophosphate hydrolase (OPH), was conjugated with both biotin and a fluorescence marker i.e. carboxynaphthofluorescein (CNF). Avidin was attached to the polystyrene waveguide surface of a fluorescent detector, and the OPH-CNF-biotin biosensor conjugate was bound to the avidin. The recognition element (OPH) and reporter (CNF) molecules were designed to entertain OP samples with concentrations of neurotoxin as low as 0.05 μM . Quantitative detection could be determined from 1 to 800 μM for paraoxon and from 2 to 400 μM for DFP (Viveros et al. 2006).

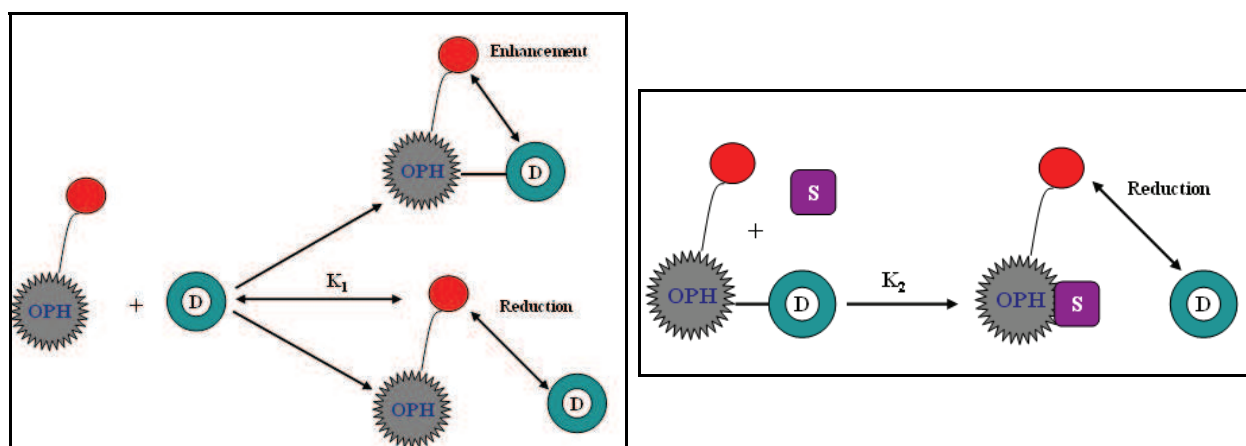


Fig. 7. (a) Decoy-enzyme interaction for enhancement in the absence of substrate. Decoy binds to enzyme-nanogold conjugate (organophosphorus hydrolase-OPH), leading to a surface enhanced fluorescence of the decoy; (b) Analyte (S) displacement of decoy (D) from OPH-gold complex (OPH), leading to decrease fluorescence signal from the decoy.

Gold nanoparticle based surface enhanced fluorescence (NSEF) spectroscopy for rapid and sensitive screening of organophosphorus agents (OPA) was reported. In this technique, the fluorescent from Eu^{3+} ions that are bound within the electromagnetic field of gold nanoparticles exhibit a strong enhancement. In the presence of OPA, Eu^{3+} ions are released from the gold nanoparticle surface and thus a very distinct fluorescence signal change was observed with the high sensitivity of 1 μM (Samuel et al. 2008). Dale et al presented a small molecule sensor that provides an optical response to the presence of an organophosphorus (OP)-containing nerve agent mimic. Exposure to an OP nerve agent mimic triggers phosphorylation of the primary alcohol followed rapidly by an intramolecular substitution reaction as the amine displaces the created phosphate. The quaternized ammonium salt produced by this cyclization reaction no longer possesses a lone pair of electrons, and fluorescence readout is observed as the nonradiative PET quenching pathway of the fluorophore is shut down (Dale et al., 2006). Anandakathir et al. reported the synthesis of

stilbene-based fluorophore, 3,4-dihydroxy-4'-aminostilbene (DHAS) for the detection of chemical warfare agents such as organophosphorus nerve gases. The interaction of DHAS with nerve agent simulant, diethyl chlorophosphate (DCP) was investigated in solution and vapor phase by fluorescence spectroscopy (Anandakathir et al. 2009).

16. Immunological determination of pesticides and Organo-phosphorous compounds

Bioanalytical assays based on enzymatic or immunochemical principles have been proposed as promising alternatives, as they are highly sensitive, selective, specific, rapid and reliable. A selective enzyme-linked immunosorbent assay (ELISA) for the insecticide chlorpyrifos was developed using the sera of highest specificity. This shows an I_{50} of 160 ppb with a detection limit of 10 ppb (Cho et al., 2002). Sensitive, simple and rapid enzyme linked immunosorbent assay (ELISA) methods have been reported for the determination of four organophosphorus pesticides diazinon, fenthion, malathion and chlorpyrifos in extra virgin olive oil. The limits of detection for the pesticides in olive oil are from 46 ng ml⁻¹ for diazinon to 10 ng ml⁻¹ for fenthion (Garcia et al., 2006). Teller et al developed a combined piezoelectric/amperometric sensor based on the modular assembly of different recognition elements. Acetylcholinesterase was chemically modified by benzoyllecgonine-1,8-diamino-3,4-dioxaoctane (BZE-DADDO), thus providing an additional recognition element for anti-cocaine antibodies or butyrylcholinesterase, respectively. It was possible to determine cocaine in dynamic range of 10⁻⁷ to 10⁻⁹ mol/L using polyclonal antibody. At the same time the in-situ inhibition of the adsorbed BZE-AChE by the organophosphate chlorpyrifos-oxon could be monitored by amperometric activity measurement (Teller et al., 2008). Liang et al developed immunoassay method for the O,O-dimethyl organophosphorus pesticides, including malathion, dimethoate, phenthoate, phosmet, methidathion, fenitrothion, methyl parathion and fenthion. Three haptens with different spacer-arms were synthesized. The haptens were conjugated to bovine serum albumin (BSA) for immunogens and to ovalbumin (OVA) for coating antigens. The IC₅₀ values, under optimum conditions, were estimated to be 30.1 µg/L for malathion, 28.9 µg/L for dimethoate, 88.3 µg/L for phenthoate, 159.7 µg/L for phosmet, 191.7 µg/L for methidathion, 324.0 µg/L for fenitrothion, 483.9 µg/L for methyl parathion, and 788.9 µg/L for fenthion (Liang et al., 2008). A nanoparticle-based electrochemical immunosensor has been reported for the detection of phosphorylated acetylcholinesterase (AChE), which is a potential biomarker of exposure to organophosphate (OP) pesticides and chemical warfare nerve agents. Zirconia nanoparticles (ZrO₂ NPs) were used as selective sorbents to capture the phosphorylated AChE adduct, and quantum dots (ZnS@CdS, QDs) were used as tags to label monoclonal anti-AChE antibody to quantify the immunorecognition events. The voltammetric response of the immunosensor is highly linear over the range of 10 pM to 4 nM phosphorylated AChE, and the limit of detection is estimated to be 8.0 pM. The immunosensor also successfully detected phosphorylated AChE in human plasma (Liu et al., 2008).

17. Microfluidics based detection

Rapid detection of OP compounds/agents is required to take a quick decision or efficient decontamination for a particular site. Miniaturization of analytical devices is attracting considerable interest due to the potential for greatly enhancing the speed of analytical

separations or characterizations. Over the last decade, micrototal analysis system (μ TAS) or Lab-on-a-chip for various purposes have been developed that aim for the rapid high throughput analysis of molecules, such as DNA and proteins, point-of-care testing and microchip for the fast screening of OP compounds (Figeys & Pinto, 2000; Vilknér et al., 2004; Wang et al., 2002). Microfluidic system is a potential platform for biochemical/chemical analysis with numerous advantages including low sample/reagent consumption, high sample throughput and total analysis on the same platform (Vilknér et al., 2004).

An integrated microfabricated device that performs automated enzymatic assays was developed. Active and precise microfluidic control of reagent transport throughout the interconnected channel network was achieved using electrokinetic-induced motion. They controlled the reagent dilution and mixing by regulating the applied potential at the terminus of each channel, using voltages derived from an equivalent circuit model of the microchip. Assay of enzyme (β -galactosidase) was monitored by using β -D-galactopyranoside resorfuin a substrate that is hydrolyzed to resorfuin, which is a fluorescence product (Hadd et al., 1997). The separation and sensitive electrochemical detection of OP compounds have been developed by using on-chip micellar electrokinetic chromatographic (MEKC) techniques (Wang et al., 2001). In this study they microfabricated capillary electrophoresis glass chips with planar thick film amperometric detectors for the separation and detection of toxic OP compound. The integrated microsystem offers rapid (~ 2.5 min.) simultaneous measurements of micromolar levels of OP compounds. The detection of regenerated sarin in human blood samples has been developed in a lab-on-a-chip device. This device should allow early detection of sarin exposure in human being. The device is based on continuous-flow microfluidics with sequential stages for lysis of whole blood, regeneration of free nerve agent from its complexes with blood cholinesterase, protein precipitation, filtration, enzyme-assisted reaction and optical detection (Tan et al., 2008). The reactor for nerve gas regeneration is designed as a micromixer based on chaotic advection with herring-bone structures. The reaction chamber is located on the other side of the main device layer, with herring-bone patterns to improve the transport of reagents to the glass surface with immobilized enzyme. Detection of sarin in whole blood spiked with a low level sarin concentration, 200nM is achieved. They also reported the kinetics of inhibition reaction to estimate the required flow rate and inhibition time. In the present system the pumping and valving processes were carried out outside the lab-on-a-chip. The device is suitable for other applications in occupational hygiene in agriculture. Microfluidics system for OP compound detection holds great promise for a timely warning and alarm in the emergency case and its also can be carried any where for on site detection. Microfluidics provide better platform with combination of nanoparticles to enhance the sensitivity and selectivity on the chip.

18. Protective measures against OP compounds/nerve agents

The threat of OP compounds or commonly used pesticides and chemical manufacturing by products act as anticholinesterases, posing an occupational low-dose exposure hazard to workers in a variety of professions as well as public (Aas, 2003). Protective equipment which is used by an individual to achieve physical protection is termed as individual protection equipment (IPE). These include gas masks and protective clothing such as trousers, jacket, over boots and gloves. These physical protections create an artificial barrier between the OP

compounds and the subject (human being), and they have provision for breathable air. The barrier has to provide protection against liquids, aerosols or gases and should possess the essential characteristics of air tightness and non-permeability to gases. The basic materials are used in these barrier include spherical carbon coated fabric, activated charcoal, polyurethane foam and for aerosol High efficiency Particulate Aerosol (HEPA) filter media is composed of glass fibers of diameter 1.0 to 10 microns. The aerosol particles from the contaminated air captured over the surface of the filter medium by van-der wal's force. This type of protection comes under physical protection. Medical countermeasures have been discussed earlier in the section of toxicity of OP compounds. Three drugs atropine, pralidoxime chloride and diazepam are used to treat nerve agent exposure. Atropine is very important to block the excess action of acetylcholine. It is a competitive inhibitor of muscarinic receptors. Atropine should be administered immediately and should be repeated starting with an initial dose of 2 mg intramuscularly or intravenously.

19. Conclusions and future perspectives

We have described a brief summary about OP compounds/nerve agents and mode of action toxicity of these compounds and various detection approach. Despite the considerable research activity towards the development of detection system for monitoring the OP compounds in the lab and on site it is not easy to discriminate various OP compounds in the same sample. Current research activity involves numerous efforts for improving the analytical performances of the biosensing systems in order to be able to monitor a wide range of pollutants in environmental and food samples. The use of nanoparticles/CNT leads to a greatly improved electrochemical detection due to its electrocatalytic activity. For more sensitive detection fluorescence method is also beneficial. However, with the increasing threat of terrorism, and large scale use of OP pesticides the roles of detectors are also increasing in civil emergency responses. In these instances, the detectors are used to monitor the presence of these compounds in the atmosphere, provide an indication of their levels in order to determine the necessary level of protection. Due to the structural similarity of OP compounds, it is also of paramount importance that the designed sensors must be fabricated such that they are highly selective towards specific OP compounds. The Microfluidics and nanotechnology offer a promising technology for the miniaturized detectors which can be used for onsite and easy to operate. By using this sensitivity, real time detection, response time, and selectivity can be improved. It requires very low sample volume and other reagents and the interference can also be minimized. The engineered variants of enzymes could be another approach in biosensor design for the discrimination and detection of various enzyme-inhibiting compounds when used in combination with chemometric data analysis using artificial neural network. New opportunities are considered with the application of novel enzymes or enzyme sources as well as biocomponents with necessary enzyme activity. Combined with traditional biosensors and test kits this biosensing can be applied as alarm monitors of environmental pollution. In this combination nanoparticles will play a very important and effective role to increase the sensitivity and selectivity. When we introduce nanoparticle in the combined system all the physical parameters will change and it creates a new phenomenon. The development of antidote is also necessary for medical countermeasures. For this new drug development research is promising to counter the effect of OP compounds once it is exposed to any person.

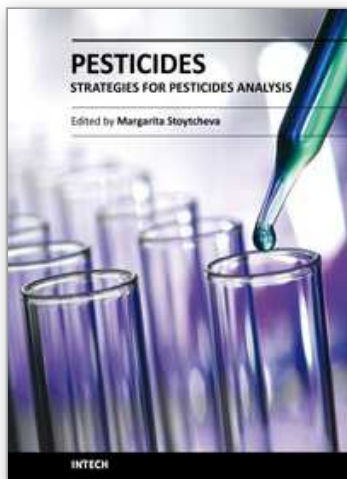
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Pesticides - Strategies for Pesticides Analysis

Edited by Prof. Margarita Stoytcheva

ISBN 978-953-307-460-3

Hard cover, 404 pages

Publisher InTech

Published online 21, January, 2011

Published in print edition January, 2011

This book provides recent information on various analytical procedures and techniques, representing strategies for reliability, specificity, selectivity and sensitivity improvements in pesticides analysis. The volume covers three main topics: current trends in sample preparation, selective and sensitive chromatographic detection and determination of pesticide residues in food and environmental samples, and the application of biological (immunoassays-and biosensors-based) methods in pesticides analysis as an alternative to the chromatographic methods for "in situ" and "on line" pesticides quantification. Intended as electronic edition, providing immediate "open access" to its content, the book is easy to follow and will be of interest to professionals involved in pesticides analysis.

How to reference

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Sanjay Upadhyay, Mukesh K. Sharma, Vepa K. Rao, Bijoy K. Bhattacharya, Dileep Sharda and R.Vijayaraghavan (2011). Organophosphorous Compounds-Toxicity and Detection Approach, Pesticides - Strategies for Pesticides Analysis, Prof. Margarita Stoytcheva (Ed.), ISBN: 978-953-307-460-3, InTech, Available from: <http://www.intechopen.com/books/pesticides-strategies-for-pesticides-analysis/organophosphorous-compounds-toxicity-and-detection-approach>

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Slavka Krautzeka 83/A
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Phone: +385 (51) 770 447
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中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
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