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Organophosphate pesticides: a review on classification, synthesis, toxicity, remediation and analysis

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Pesticides are toxic organic compounds extensively used in agriculture to control undesirable pests, enhance crop yields and extend shelf life of food crops. The widespread utilization of pesticides is a major contributor to pollution in soil, air and water. A class of artificial chemicals known as organophosphorus pesticides (OPPs) is predominantly employed in public health campaigns, agriculture, pest management, landscaping, and vector control (e.g., mosquito management). Although they were first promoted as safer alternatives for persistent organochlorine pesticides, their effects on human health, bioaccumulation potential, and environmental persistence have caused serious concerns. Due to their high toxicity and low persistence, many agriculturalists regularly use OPPs for various crops such as fruits and vegetables. The regular utilization of pesticides has led to deleterious influences to the ecosystem and neurological disorders to humans. The toxicity of OPPs arises from their capacity to block the enzyme acetylcholinesterase (AChE) at the cholinergic synapses inside the nervous system. The significant rise in the use of OPPs in agriculture necessitates precise assessment of their levels to safeguard food supplies and the ecology. Current studies on the history, classification, chemical characteristics, environmental behavior, health impacts, clinical manifestation and mitigation techniques of OPPs, as well as several approaches for their remediation and assessment are summarized in this review.

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Introduction

The Environmental Protection Agency (EPA) defines pesticides as compounds or mixtures of substances used to destroy, prevent, repel, or mitigate pests. They are extensively utilized in agriculture to manage plant diseases and pests, enhance crop yields, elevate crop quality, and prolong the shelf life of food

products. Agriculturalists thoroughly make use of pesticides, resulting in significant application of these chemicals in farming regions globally. Moreover, the variety of pesticides is extensive, with over 1000 different pesticides utilized globally to safeguard food and crops against pests. Approximately, 5.2 billion pounds are expended every year on insecticides.^{1,2}

The majority of pesticides do not directly target pests; their application can adversely harm non-target creatures, including plants, animals, pollinators (e.g., bees) and humans. Reports indicate that merely 0.1% of pesticides effectively reach the target organisms, while the remainder contaminates the surrounding environment. Residues are detectable in soil, water, air, food, crops, and human blood. Therefore, pesticides have been categorized as carcinogenic pollutants in many countries.^{3,4}

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This review sheds light on pesticides and their classification including OPPs, routes of exposure to OPPs (the circumstances leading to exposure and absorption), and a list of OPPs active ingredients and their chemical structures. In addition, the synthesis of OPPs, their poisoning and environmental issues are also summarized herein. Furthermore, the mechanism of acetylcholinesterase (AChE) enzyme inhibition induced by OPPs, and different remediation approaches of OPPs are reviewed.

Several methodologies exist for the detection of OPPs including liquid chromatography/mass spectrometry (LC-MS), gas chromatography/mass spectrometry (GC-MS), liquid chromatography/tandem mass spectrometry (LC-MS/MS), gas chromatography/tandem mass spectrometry (GC-MS/MS), nuclear magnetic resonance (NMR) spectroscopy, high-performance liquid chromatography (HPLC), electrochemical detection techniques, and sensors.^{5,6} The conclusion of this review article collects a selection of the most recently reported methodologies for the assay and removal of OPPs.

The unique contribution of the present review is that it provides a comprehensive summary and an updated coverage that encompasses the latest advances over the period 2010–2019. In addition, the review is useful for readers whose research is focused on cross-disciplinary investigations as it provides critical insight on the chemistry, classes, toxicity, mechanism of action, synthesis, properties, methods of remediation and methods of analysis of OPPs.

Research gaps

Research gaps in OPPs studies remain significant despite extensive investigation. Key gaps include insufficient longitudinal data on chronic low-dose exposure effects, such as neurobehavioral deficits and developmental disorders, which limits

understanding of long-term human health impacts. There is also a disparity in geographic and linguistic representation, with underreporting from regions heavily reliant on OPPs like South Asia and Latin America, compounded by inconsistent healthcare access affecting data quality and generalizability. Methodological weaknesses such as selection bias toward severe poisoning cases, inadequate blinding, and heterogeneous inclusion criteria limit the robustness of clinical and toxicological studies. Environmental fate mechanisms—specifically degradation pathways, metabolite toxicity, and interaction with emerging contaminants like microplastics—require further elucidation to inform risk assessment and remediation. Additionally, improved analytical techniques to detect OPPs and related metabolites at trace levels in diverse matrices are needed to provide accurate exposure assessment. Addressing these gaps would enhance preventive strategies, therapeutic interventions, and policy decisions to mitigate OPP-related risks effectively.^{7–10}

Classification of pesticides

Pesticides can be categorized in several manners. Pesticides are primarily categorized based on:

The target organisms they address

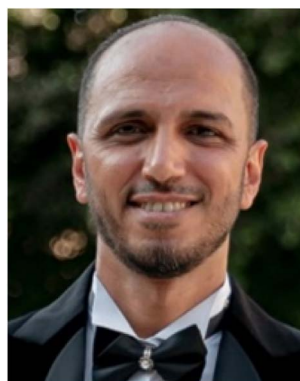
There are four principal classes, namely, insecticides (for insects), fungicides (for fungi/molds), herbicides (for plants) and rodenticides (for rodents), alongside several minor classes, including acaricides or miticides (for mites), molluscicides (for snails and other mollusks), algicides (for algae), bactericides (for bacteria), nematocides (for nematodes), piscicides (for fishes), and virucides (for viruses).^{11,12}



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Mechanisms of action in the target organism

Pesticides include stomach poisons (pesticides that enter the pest's digestive system through the mouth resulting in death), fumigants (pesticides that kill target pests by generating vapors) and contact poisons (pesticides that affect target pests upon direct contact).¹³

Chemical composition

Based on the chemical nature of the active ingredients, pesticides are classified into four primary classes; organophosphorus, organochlorines, carbamates, pyrethrin and pyrethroids.¹⁴

Methods of application

Agriculture use and several sectors for pest management, public health, domestic, such as spray dispersion, liquid formulations, aerial spraying, granular formulations and soil injection. In vast applications, such as forestry or large agricultural regions, aerial spraying utilizing drones is implemented. In confined environments, such as greenhouses or storage facilities, certain OPPs function as fumigants, causing insects to die through inhalation.¹⁵

Toxicity of the pesticide

The World Health Organization (WHO) recommends that pesticides be classified into five classes based on their corresponding LD₅₀ values for rats (Table 1).²

Among pesticides, organophosphorus compounds are thoroughly employed in agriculture as insecticides, herbicides and pesticides, owing to their rapid biodegradability, great efficacy in insects' control, and lower environmental persistence than organochlorine compounds. The OPPs are usually safer and having a milder impact compared to their organochlorine counterparts. These compounds constitute the main components of chemical nerve agents.^{16,17}

Pesticides exhibit minimal decomposition at room temperature and in direct sunlight. Highly toxic pesticide residues in raw agricultural products are transferred to the human body through the consumption of contaminated food, posing potential long-term risks to human health.¹⁸

For instance, chlorpyrifos (CPF), a member of the OPPs, is also applied in agricultural practices. Recent reports indicate that CPF is classified as a neurotoxin and a human carcinogen.

A team of National Cancer Institute (NCI) researchers recently reported on the first epidemiologic study to carefully evaluate cancer among chlorpyrifos applicators. Their results suggest a possible link between the insecticide and lung cancer.¹⁹ Consequently, it is essential to monitor CPF concentrations in food products.²⁰ The Codex Alimentarius Commission (CAC) has established maximum residue limits (MRLs) for pesticides in food commodities to safeguard consumers from the fatal consequences of pesticide exposure. The MRLs represent the highest permissible levels of pesticide residues (measured in mg kg⁻¹) in food products following the application of pesticides in accordance with established agricultural practices. Table 2 presents relevant data on MRLs.

Exposure to organophosphate pesticides (OPPs) can occur *via* inhalation, dermal absorption, ingestion, and ocular contact. The absorption rate of OPPs through inhalation, typically occurring during the dipping of animals, spraying of crops, or direct contact with contaminated entities, surpasses that of dermal absorption.²¹ Typically, accidental pesticide ingestion or administration for suicidal purposes result in high oral doses, which ultimately lead to acute poisoning and mortality. OPPs are exposed to workers during the manufacture, transport, blending, loading, and application of pesticides, as well as during the harvesting of crops that have been sprayed with pesticides. The dermal route has the highest potential to pesticide exposure, while the respiratory route contributes only slightly when aerial pesticides or aerosol applications are employed.²²

Pesticides continue to significantly contribute to acute human poisonings due to their rapid distribution and accumulation in the liver, kidneys, and adipose tissues. Insecticides are the most acutely toxic among pesticides. Herbicides typically exhibit moderate to low acute toxicity, with paraquat, a widely utilized herbicide for grass and weed control, serving as a notable exception. Fungicides demonstrate low acute toxicity, whereas rodenticides are highly toxic to rats but do not display comparable toxicity in humans. Numerous studies conducted in developing countries have demonstrated that insecticides, particularly OPPs and paraquat, are frequently the primary cause of acute human poisonings.²³

History of organophosphate pesticides

The history of organophosphorus (OP) pesticides extends over nearly two centuries, characterized by notable scientific,

Table 1 Classification of pesticide compounds according to the WHO recommendations¹⁵

Pesticide class	Level of toxicity	LD ₅₀ for rats (mg per kg body weight)		Examples
		Oral	Dermal	
Class I _a	Extremely hazardous	<5	<50	Parathion
Class I _b	Highly hazardous	5–50	50–200	Eldrin and dichlorvos
Class II	Moderately hazardous	50–2000	200–2000	DDT and chlordane
Class III	Slightly hazardous	>2000	>2000	Malathion
Class IV	Unlikely to present acute hazards in normal use	≥5000	—	Carbamate and cycloprothrin



Table 2 Maximum residue limits of a number of OPPs in food set by the CAC

Pesticide	MRLs (vary according to the product, mg kg ⁻¹)	Pesticide	MRLs (vary according to the product, mg kg ⁻¹)	Common commodities	Codex year of adoption
Acephate	0.01–50.0	Malathion	0.01–20.0	Citrus fruits, sugarcane, vegetables, rice	2015–2023
Azinphos-methyl	0.05–10.0	Parathion-methyl	0.05–1.00	Apples, pears, grapes, potatoes	2015–2020
Chlorpyrifos	0.01–5.00	Phorate	0.05–0.10	Citrus, coffee, leafy vegetables, cereals	2023
Diazinon	0.01–5.00	Phosmet	0.05–0.20	Apples, pears, grapes, tomatoes	Recent decade
Dimethoate	0.02–2.00	Terbufos	0.05–0.30	Fruits (citrus, grapes), vegetables, cotton	2015–2021

agricultural, and military developments. Organophosphate chemicals were identified in the early 19th century. Phosphoric acid derivatives were produced by chemists such as Franz Anton Vögel, Wladimir Moschnin, and Philippe de Clermont.²⁰ In 1820, Lassaing synthesized phosphate esters, which were subsequently advanced by Michaelis, a German chemist, in the late 19th and early 20th centuries. The latter phases of this investigation coincided with Arbuzov, a Russian chemist, who introduced the Michaelis–Arbuzov reaction for the formation of the P–C bond. This reaction is a highly appealing approach for synthesizing alkyl phosphonates from phosphites.^{21,22}

Organophosphates are chemical compounds synthesized through the esterification of alcohol and phosphoric acid. Organophosphates featuring a carbon–phosphoryl bond (C–P(O)) possess extensive and notable uses in agricultural chemistry.²³ These chemicals were initially synthesized by Schrader, a German scientist, soon before and during World War II. Initially employed as agricultural insecticides, they were subsequently deployed as nerve agents or chemical warfare agents. They act as acetylcholinesterase inhibitors, consequently influencing neuromuscular transmission.²⁴

Schrader noted insecticidal action in certain organophosphorus chemicals. He was researching a category of substances known as organophosphates, which lethally affect insects by blocking the enzyme AChE. Consequently, he identified several potent pesticides, including bladon or tetraethyl pyrophosphate (TEPP), the inaugural commercial organophosphorus insecticide, which was promoted as a nicotine substitute for aphid control.^{25,26}

Schrader's research facilitated the creation of further organophosphate chemicals, such as octamethylpyrophosphoramide (OMPA) in 1942 and parathion (E605) in 1944. Parathion was distinguished by its stability and insecticidal efficacy, gaining extensive commercialization post-World War II, while Adrian concurrently identified that organophosphates block cholinesterases by alkylphosphorylation at

the esteratic site. Schrader inadvertently discovered tabun, a highly lethal organophosphorus nerve poison.

In parallel with Schrader, British scientists, McCombie and Saunders, were also working on OPs, and subsequently patented dimefox and diisopropyl fluorophosphate (DFP). During those years some of the OPs synthesized by Schrader turned out to be extremely toxic to mammals. In 1938 the German government declared all research on OPs to be “secret”, and the development of OPs followed 2 parallel strategies: one was to synthesize chemicals that were less toxic to mammals and effective as insecticides; the other was to develop compounds of high human toxicity and high volatility, to be used as poison gases instead of chlorine, mustard gas or phosgene. Compounds like tabun (1936), sarin (1938), and soman (1944) and cyclosarin (1949) were developed in that period for potential use as chemical warfare nerve agents, (their chemical structures are depicted in Fig. 1).

The most significant advancement in OPPs was achieved in 1944 *via* synthesizing the *O,O*-diethyl-*p*-nitrophenyl phosphorothionate (a.k.a. parathion) by Schrader. Although parathion is extremely toxic to insects and mammals, slight modifications in its chemical structure have resulted in the creation of less toxic pesticides, including chlothion (1952), fenthion (1958), and fenitrothion (1959).

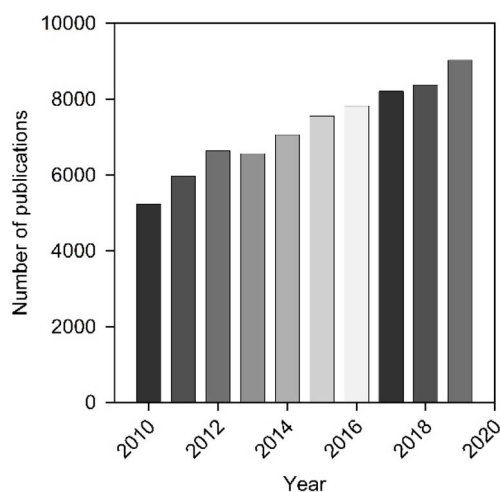


Fig. 2 The results of a google scholar-based search over the past decade (from 2010 to 2019) using the keyword “organophosphate pesticides” indicating the increased researchers’ interests in OPPs.

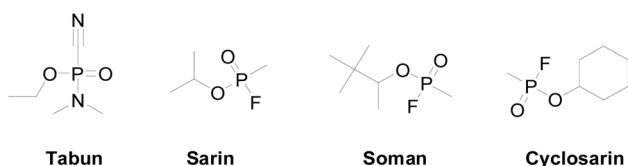


Fig. 1 Chemical warfare nerve agents.

Currently, research devoted to OPPs has grasped the attention of researchers. Fig. 2 depicts the number of published articles-identified in the google scholar database with the keyword “organophosphate pesticides” over the period from 2010 (5230 publications) to 2019 (9020 publications).²⁷

Chemical structure and physicochemical properties of organophosphate pesticides

OPPs are organic ester derivatives of phosphorus, typically amide or thiol derivatives of phosphoric, phosphonic, phosphinic, or thiophosphoric acids, featuring extra cyanide side chains, thiocyanate, and phenoxy groups. They are either biogenic or synthetic substances that possess a chemically and thermally inert covalent carbon-to-phosphorus (C–P) linkage. Consequently, the majority of OPPs exhibit greater resistance to chemical disintegration, thermal hydrolysis, and photolytic destruction than other organophosphates characterized by more reactive O–P, N–P, or S–P linkages.²⁸

The general structure of OPPs consists of a phosphorus atom linked to a terminal oxygen/sulphur atom by a double bond (*i.e.*, a phosphoryl group), together with two hydrophobic groups and a leaving group, typically a halide attached to the central phosphorus as shown in Fig. 3.²⁹

In Fig. 3, R₁ and R₂ represent alkyl or aryl groups linked to the phosphorus atom either directly (resulting in phosphinates), or through an oxygen/sulphur atom (yielding phosphorothioate). The X group is called the “leaving group” due to its detachment from phosphorus by the hydrolysis of the ester linkage. The variation of the leaving group depends upon the type of the OPP (Table 3).^{26,30} The thiophosphoryl (P=S) containing structure is occasionally termed a thion, while the phosphoryl (P=O) structure is known as an oxon. Organophosphates with a thiophosphoryl functional group represent a significant category of these commonly utilized insecticides. They pertain to the more reactive phosphoryl organophosphates, which encompass chemical warfare and nerve agents, such as, sarin, VX, and soman. Phosphothioates (*e.g.*, parathion, diazinon and fenitrothion) exhibit greater hydrophobicity than phosphates (*e.g.*, tetrachlorvinphos, dichlorvos and mevinphos), and are stored in fat, and may cause delayed toxic symptoms after exposure to phosphothioate pesticides.^{19,31} In phosphoramidates, the phosphorus atom is linked to the carbon atom through an NH group. OPPs are highly reactive

compounds that exhibit diverse biological activities, potencies as AChE inhibitors, and physicochemical characteristics, including lipid-solubility and volatility, depending on the structures of the substituents R₁, R₂, and X.³² OPPs are formulated in the form of liquid concentrate or water-soluble granules. All of them are rapidly oxidized and hydrolysed in the environment and in alkaline media, to mono- or disubstituted phosphoric or phosphonic acid. Most OPPs have slight water solubility and have a high oil–water/octanol–water partition coefficient, low vapour pressure and comparatively low volatility except dichlorvos. Nerve gases, such as sarin, have low molecular weights, with simple R₁ and R₂ substituents and leaving groups (*e.g.*, fluoro and cyano), which are usually directly bonded to the phosphorus atom without an intermediate oxygen or sulphur atom. The resulting molecules exhibit both lipid solubility and volatility.^{31,33} OPPs undergo hydrolytic breakdown, yielding water-soluble compounds typically regarded as non-toxic. The hazardous threat is primarily short-term, in contrast to the persistent organochlorine pesticides.²

Classification of organophosphate pesticides

OPPs have been categorized based on their chemical structures (the chemical nature of the atoms surrounding the central phosphorus atom). They are divided into at least 13 types, including phosphates, thiophosphates (phosphorothioate), phosphorodithioates, S-substituted phosphorothioate, phosphoramidates, phosphoramidothioates, S-substituted phosphoramidothioates, phosphonates, phosphinates, phosphonothioates, S-substituted phosphonothioates, phosphorofluoridates, and phosphonofluoridates.^{33,35} The classification of OPPs based on their chemical structures, and some common OPPs are shown in Table 3.

Synthesis of organophosphate pesticides

OPPs are synthesized primarily through chemical reactions involving phosphorus-based precursors and alcohols or other organic compounds. The synthesis methods can be categorized mainly into industrial routes and laboratory-scale procedures, each designed to produce distinct organophosphate compounds with pesticidal properties.

Industrial routes

Esterification of phosphoric acid. Esterification reactions between phosphoric acid (or its anhydride P₂O₅) and alcohols produce mono- and diesters. This method requires high temperatures and often results mostly in monoesters due to the reactivity limitations of phosphoric acid, which can dehydrate into less reactive polyphosphoric acids. This route is less common industrially but used for specific organophosphate derivatives like surfactants.³⁶

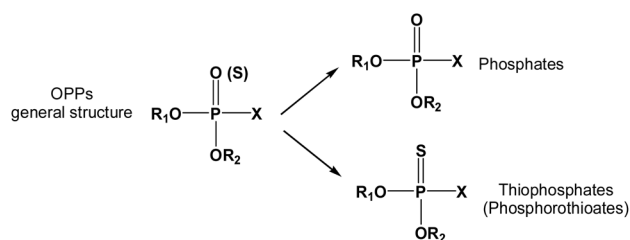


Fig. 3 General chemical structures of OPPs.





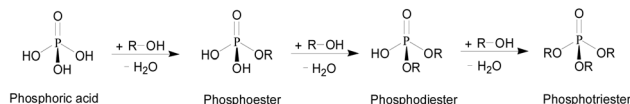
Table 3 Classification of OPPs according to their chemical structures^{33,34}

Type of ops	General structure	Additional information	Trade names	Persistent uses
Oxo-phosphoryl compounds	$\begin{array}{c} \text{O} \\ \parallel \\ \text{R}_1-\text{P}-\text{X} \\ \\ \text{R}_2 \end{array}$	R ₁ and R ₂ may be <i>O</i> -alkyl (phosphates) or <i>S</i> -alkyl (phosphorothioates); if no <i>O</i> or <i>S</i> is binding to R ₁ or R ₂ then they should be considered phosphonates or phosphinates (see below)	No specific trade names	General category including synthetic insecticides, herbicides with continued agricultural use due to efficacy Widely used in crop protection in horticulture, cereals, and vegetables for insect pest control
Thio-phospharyl compounds	$\begin{array}{c} \text{S} \\ \parallel \\ \text{R}_1-\text{P}-\text{X} \\ \\ \text{R}_2 \end{array}$			
Phosphate				
<i>O</i> , <i>O'</i> -Dialkyl-phosphate	$\begin{array}{c} \text{O} \\ \parallel \\ \text{R}_1\text{O}-\text{P}-\text{OX} \\ \\ \text{OR}_2 \end{array}$	Dichlorvos-Chlorfenvinphos-Mevinphos-Monocrotophos-Tetrachlorvinphos	Dichlorvos (Vapona), chlorfenvinphos (MCW-770), mevinphos (Phosdrin), monocrotophos (Azodrin), tetrachlorvinphos (Stirofos)	Used as contact insecticides, especially dichlorvos for stored product pests and disinfestation
Thiophosphate (phosphorothioate)				
<i>O</i> , <i>O'</i> -Dialkyl thiophosphate	$\begin{array}{c} \text{S} \\ \parallel \\ \text{R}_1\text{O}-\text{P}-\text{X} \\ \\ \text{OR}_2 \end{array}$	Bromophos-chlorpyrifos-chlorpyrifos methyl-diazinon-parathion-fenitrothion-fenthion-(pirimiphos-methyl)-triazophos	Chlorpyrifos (Lorsban, Dursban), chlorpyrifos methyl, diazinon (Diazinon), parathion (Niran), fenitrothion, fenthion, pirimiphos-methyl, triazophos	Broad-spectrum insecticides like chlorpyrifos and diazinon persist in agricultural pest management worldwide
<i>O</i> , <i>O'</i> -Dialkylphosphorodithioate	$\begin{array}{c} \text{S} \\ \parallel \\ \text{R}_1\text{O}-\text{P}-\text{SX} \\ \\ \text{OR}_2 \end{array}$	(Azinphos-methyl)-dimethoate-disulfoton-malathion-thiometon-phorate-terbufos	Azinphos-methyl (Guthion), dimethoate (Cygon), disulfoton (di-syston), malathion (Malathion), thiometon, phorate (Thimet), terbufos (Counter)	Systemic insecticides such as malathion and azinphos-methyl widely used in fruit, vegetable, and cotton crops
<i>O</i> -Alkyl, <i>S</i> -alkyl phosphorodithioate	$\begin{array}{c} \text{S} \\ \parallel \\ \text{R}_1\text{O}-\text{P}-\text{OX} \\ \\ \text{OR}_2 \end{array}$	Protiopho-sulpropho-phosmet	Prothiophos (Kroton), sulprofos, phosmet (Imidan)	Used in various field crops for soil and foliar pest control with moderate to high persistence
Phosphorothioate (S-substituted)				
<i>O</i> , <i>O'</i> -Dialkyl phosphorothioate	$\begin{array}{c} \text{O} \\ \parallel \\ \text{R}_1\text{O}-\text{P}-\text{SX} \\ \\ \text{OR}_2 \end{array}$	(Demeton- <i>S</i> -methyl-VG) (a nerve agent-omethoate)	Demeton- <i>S</i> -methyl (Metasystox), omethoate, VG (nerve agent)	Includes highly toxic agents, some used in niche agricultural applications or banned/restricted in many countries
<i>O</i> -Alkyl, <i>S</i> -alkyl phosphorothioate	$\begin{array}{c} \text{O} \\ \parallel \\ \text{R}_1\text{S}-\text{P}-\text{OX} \\ \\ \text{OR}_2 \end{array}$	Profenofos	Profenofos (Curacron)	Used in commercial fruit and vegetable pest control with persistence concerns leading to regulations
Phosphoroamidate				
<i>O</i> , <i>O'</i> -Dialkylphosphoramidate	$\begin{array}{c} \text{O} \\ \parallel \\ \text{R}_1\text{O}-\text{P}-\text{NR}_2 \\ \\ \text{OR}_2 \end{array}$	Crufoamat-fenamiphos Methamidopho-acephate	Crufoamate, fenamiphos Methamidophos (Monitor), acephate (orthene)	Used as nematocides and insecticides primarily on vegetables and some field crops



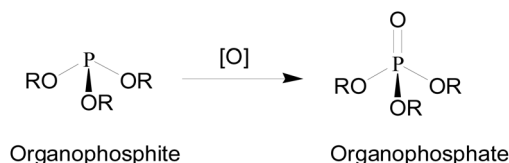
Table 3 (Contd.)

Type of ops	General structure	Additional information	Trade names	Persistent uses
O-Alkyl, S-alkylphosphoramidothioate (S-substituted)	$\begin{array}{c} \text{O} \\ \\ \text{R}_1\text{O}-\text{P}-\text{NR}_2 \\ \\ \text{SR}_2 \end{array}$			Used in commercial orchards and vegetable production; highly toxic with some use restrictions
O,O'-Dialkylphosphoramidothioate	$\begin{array}{c} \text{S} \\ \\ \text{R}_1\text{O}-\text{P}-\text{NR}_2 \\ \\ \text{OR}_2 \end{array}$	Isofenpho-propetamphos	Isofenphos (Vapam), propetamphos	Applied to vegetables, fruits, and grains for efficient pest control; persistence varies
Phosphonate and phosphinate				
O-Alkyl, alkyl phosphonate	$\begin{array}{c} \text{O} \\ \\ \text{R}_1\text{O}-\text{P}-\text{OX} \\ \\ \text{R}_2 \end{array}$	Triclorphon (phosphonates = one of the R groups is not bound to the P thorough an O atom)	Trichlorfon (Dipterex)	Use mainly as herbicides (e.g., glufosinate) with persistent soil activity influencing weed control strategy
DialkylPhosphinates	$\begin{array}{c} \text{O} \\ \\ \text{R}_1-\text{P}-\text{OX} \\ \\ \text{R}_2 \end{array}$	Glufosinate	Glufosinate (Finale, Liberty)	Used as selective herbicides with moderate persistence in crop production systems
Phosphonofluoridate				
O-Alkyl, alkyl phosphonofluoridate	$\begin{array}{c} \text{O} \\ \\ \text{R}_1\text{O}-\text{P}-\text{F} \\ \\ \text{R}_2 \end{array}$	Soman-Sarin-Cyclosarin or GF- (chemical weapons, nerve agents)	Soman, sarin, cyclosarin (chemical warfare agents)	Chemical warfare agents, no agricultural use
Phosphorofluoridate				
O,O'-Dialkyl phosphorofluoridate	$\begin{array}{c} \text{O} \\ \\ \text{R}_1\text{O}-\text{P}-\text{F} \\ \\ \text{OR}_2 \end{array}$	DFP (Diisopropylphosphorofluoridate)	DFP (diisopropylfluorophosphate, a nerve agent)	Chemical warfare agents, no agricultural use
Phosphonothioate				
O-Alkyl, alkyl phosphonothioate	$\begin{array}{c} \text{S} \\ \\ \text{R}_1\text{O}-\text{P}-\text{OX} \\ \\ \text{R}_2 \end{array}$	Leptophos-EPN	Leptophos, EPN	Used in limited agricultural contexts; some compounds restricted due to persistence and toxicity
O-Alkyl, alkyl phosphonothioate (S-substituted)	$\begin{array}{c} \text{O} \\ \\ \text{R}_1\text{O}-\text{P}-\text{SX} \\ \\ \text{R}_2 \end{array}$	VX (chemical warfare agent)	VX (chemical warfare agent)	Chemical warfare agents, no agricultural use



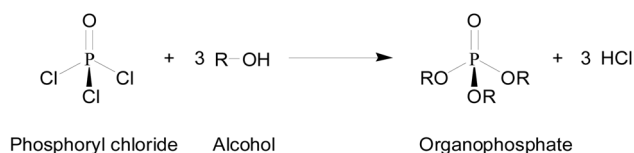
Oxidation of phosphite esters

Organophosphites (phosphite esters) can be oxidized to give organophosphates³⁷ (see the following equation).



Alcoholysis of POCl₃

The most dominant industrial method involves the reaction of phosphorus oxychloride with alcohols in an alcoholysis process. This reaction forms organophosphate esters while generating hydrochloric acid as a by-product. Catalysts such as aluminium trichloride or magnesium chloride are commonly used to enhance reaction rates. Control of reaction conditions, including temperature and base addition, is important to prevent side reactions like formation of organochlorides and lower esters. This route is favoured industrially due to its efficiency and scalability.³⁸ This reaction is known as alcoholysis of phosphoryl chloride.



Laboratory and specialized synthesis

Nucleophilic displacement on phosphorus halides. In laboratory synthesis, organophosphorus compounds are often produced by nucleophilic displacement reactions involving phosphorus halides and organometallic reagents (such as Grignard reagents) or metal phosphides. This allows the introduction of various organic substituents onto the phosphorus atom.

Addition reactions of phosphines. Various organophosphorus compounds, including phosphines, can be synthesized by nucleophilic addition of phosphines to alkenes or alkynes, either with or without strong bases or radical initiators. Oxidation of phosphines is common to obtain stable phosphine oxides used in downstream syntheses.

Special considerations

Phosphorothioate formation. Many commercial OPPs contain phosphorothioate groups (P=S bonds) that require bioactivation to their oxygen analogues for pesticidal activity. Synthesis often involves introducing sulphur atoms in place of oxygen in the phosphate esters.

Hydrolysis and safe disposal. Understanding the stability and hydrolysis mechanisms of organophosphates is important in synthesis and handling, with alkaline hydrolysis pathways studied extensively to guide degradation and disposal strategies.

Biological effects and mechanism of toxicity

The continued stimulation of ACh receptors accounts for the symptoms of OPPs poisoning, which involve nicotinic, muscarinic, and central nervous system effects.^{19,30} Common clinical features of the parasympathetic nervous system muscarinic effects can be memorized by the mnemonics SLUDGE

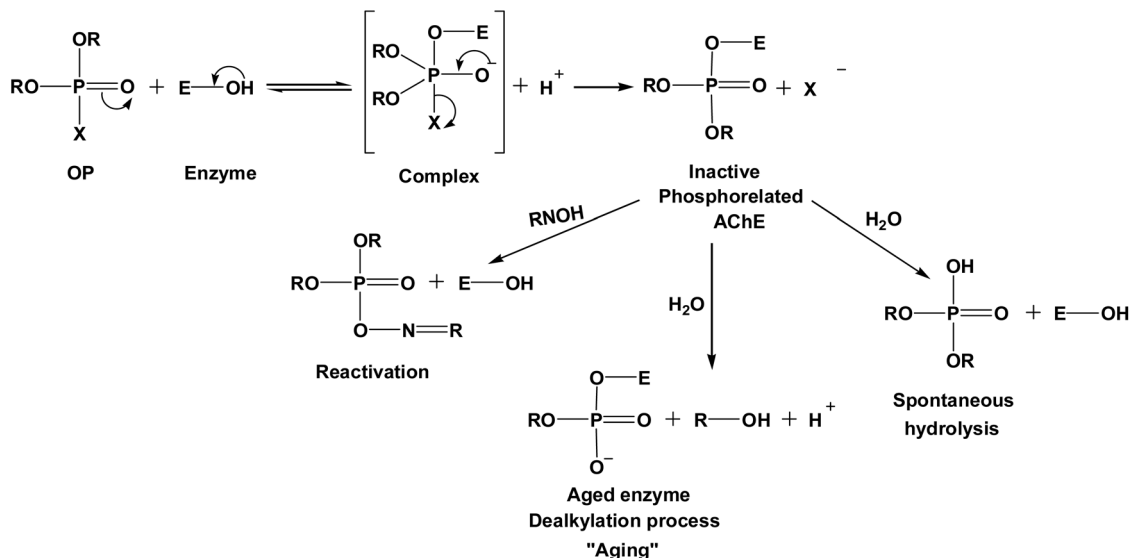


Fig. 4 Mechanism of inhibition of AChE by organophosphates; spontaneous hydrolysis, reactivation, and aging of the phosphorylated enzyme.



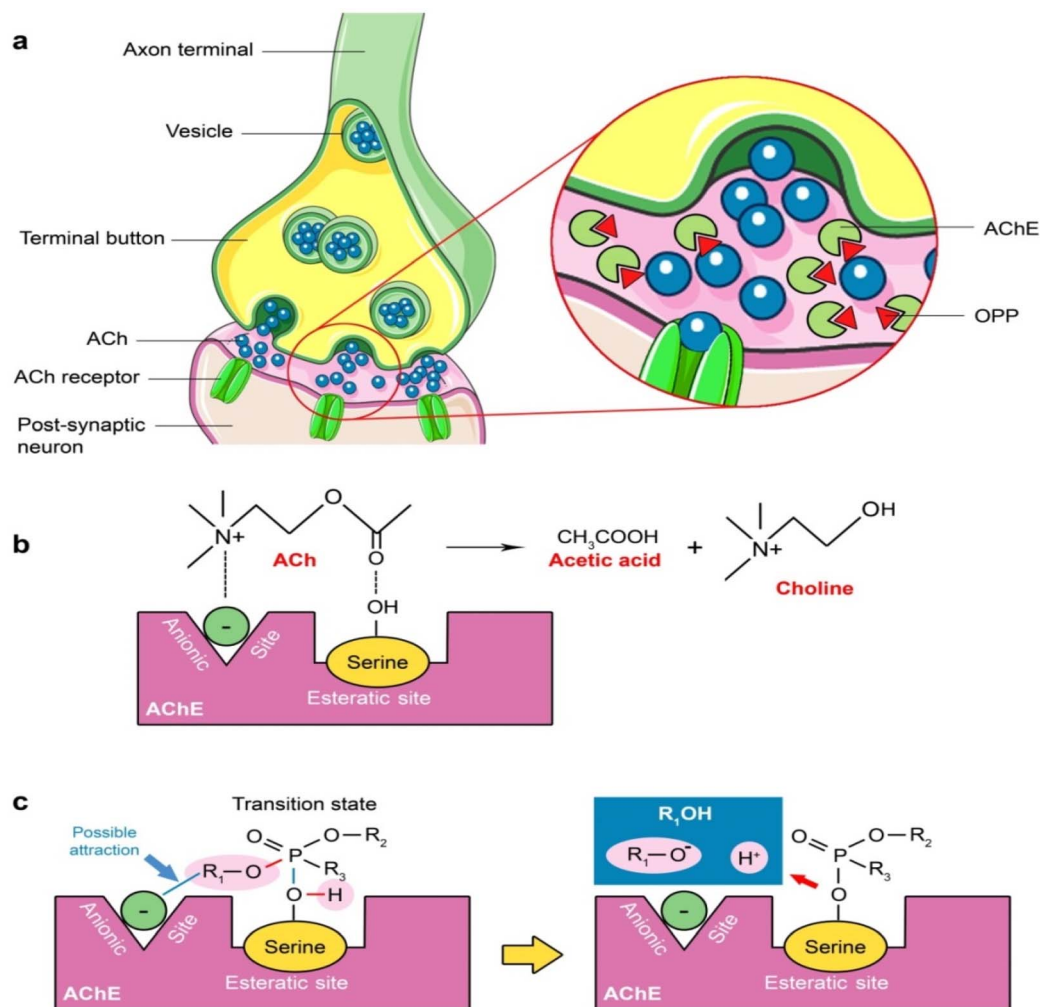


Fig. 5 (a) Inhibition of AChE by OPPs, (b) hydrolysis of ACh by AChE, (c) transition state in the inhibition of AChE by OPPs.

syndrome, which includes Salivation, Urination, Lacrimation, Diarrhea, Gastrointestinal cramps, and Emesis or DUMBELLS syndrome, which encompasses Diarrhea, Urination, Miosis/muscle weakness, Bradycardia, Bronchorrhea/bronchospasm, Emesis, Lacrimation, Lethargy and Salivation/seizures/sweating. Nicotinic effects at the autonomic synapses manifest as hypertension, dilated pupil, and tachycardia. In contrast, at somatic nerve endings, the predominant symptoms are weakness, muscle fasciculation, and paralysis. The central nervous system manifests through symptoms such as headache, restlessness, drowsiness, slurred speech, confusion, ataxia, psychosis, tremors, seizure, and delirium.^{39,40} The enzyme AChE is an effective cholinesterase that catalyzes the hydrolysis of ACh, a vital neurotransmitter in the central nervous system, into choline (Ch) and acetic acid (Fig. 4).⁴¹ It is a serine protease secreted in cholinergic synapses, associated with the postsynaptic cleft. The hydroxyl group of serine, one of the enzyme's amino acids, induces a nucleophilic attack on ACh to create an enzyme-ACh intermediate. The breakdown of this intermediate reproduces the active/free AChE enzyme, resulting in the hydrolysis of ACh into choline and acetic acid.⁴² The molecular mechanism of OPPs toxicity is based on the

irreversible phosphorylation of esterases. The pesticide attaches covalently to the hydroxyl group of serine at the active site, resulting in the formation of an organophosphorous intermediate with AChE. Upon phosphorylation, the enzyme loses its ability to hydrolyze ACh, resulting in excessive activation of muscarinic and nicotinic ACh receptors, and disrupted neurotransmission. The inhibition of the enzyme causes the buildup of ACh in the synaptic cleft, leading to over-stimulation of muscarinic and nicotinic ACh receptors, and disrupted neurotransmission (Fig. 5).⁴³ The typical three-stage reaction between AChE and OPPs throughout the inhibition process is outlined as follows (refer to Fig. 5):⁴¹ (i) initial formation of the enzyme-phosphate complex, (ii) subsequent phosphorylation of the enzyme, and (iii) gradual hydrolysis to the free enzyme.

The phosphorylated enzyme, which is more stable, has a lower rate of ACh hydrolysis and regeneration of the free active enzyme. The regeneration rate of the free enzyme for certain phosphorylated esterases can be extremely slow, to the point where the phosphorylated enzyme is completely inactive. Furthermore, enzyme aging is a process in which certain phosphorylated enzymes undergo a dealkylation reaction before regenerating as active enzymes. This aged enzyme is



irreversibly phosphorylated and cannot be regenerated through spontaneous hydrolysis or an oxime antidote.

The phosphorylated enzyme's aging occurs rapidly when the inhibitor is a powerful nerve agent such as sarin or soman, resulting in the permanent inactivation of AChE at a rapid pace.^{19,21,41}

Upon aging of the phosphorylated AChE, the enzyme is almost irreversibly inactivated and the only way of recovering its activity is the synthesis of a new enzyme, which may take days. Atropine, a muscarinic receptor antagonist, is the main OPPs poisoning antidote because it stops ACh from accumulations at the receptor sites. As mentioned above, oximes like pralidoxime, can also be used to treat OPPs poisoning. In some cases, diazepam is utilized in order to reduce anxiety and stops convulsions.^{18,44}

The side groups of organophosphates mostly determine their affinity to AChE, the time needed for hydrolysis and regeneration of the active enzyme, and the time of onset of the symptoms.⁴⁰

Without organophosphates, the AChE active center has two important sites: the esteratic and anionic sites. After ACh binds to the AChE anionic site, its acetyl group might connect to the esteratic site. Serine, histidine, and glutamine are three important amino acids in the esteratic site. These residues break down ACh into choline (Ch) and acetic acid, regenerating the active enzyme again.

Organophosphate pesticides inhibit the binding of the acetyl group of ACh to the esteratic site of AChE, so that ACh cannot be cleaved and will accumulate in the synaptic cleft. That keeps ACh receptors active, stopping nerve impulses from moving smoothly across the synaptic gap, leading to convulsions, loss of muscular coordination, and eventually death.⁴⁵

The primary treatment for organophosphate pesticide poisoning involves the administration of atropine, which works by blocking muscarinic acetylcholine receptors and mitigating the overstimulation caused by acetylcholinesterase inhibition. Alongside atropine, pralidoxime (2-PAM) is used to reactivate acetylcholinesterase before irreversible enzyme aging occurs, targeting the cause of toxicity. In cases of seizures caused by severe poisoning, benzodiazepines such as diazepam are used to control convulsions and provide neuroprotection. Supportive care, including airway management, oxygenation, and fluid replacement, is critical throughout treatment. Additional adjunct therapies like magnesium sulphate are being studied for their potential benefits. Immediate decontamination and continuous monitoring in an intensive care setting are essential to prevent complications and manage symptoms effectively.^{45–47}

Methods of organophosphate pesticide remediation

Pesticides can reach the soil through irrigation water, rains, and winds when they are applied to crops. Subsequently, they enter groundwater and surface water from the soil through infiltration processes, runoff, and wastewater treatment plants.⁴⁶ Remediation of pesticides from wastewater is one of the current

major environmental issues. Recently, the presence of pesticide residues in water has grown and has become a major topic of conversation. To minimize the potential health concerns, scientists have devised several ways to eliminate OPPs from polluted environmental samples. There are three main ways to remove OPPs, chemical, biological, and physical remediation.⁴⁷ The following sections will describe some of these strategies.

Biological remediation

Biological techniques of remediation (a.k.a. bioremediation methods) use plants or microorganisms to detoxify/degrade organic contaminants from the environment. Many microorganisms including bacteria, fungi, and protists can break down organic contaminants transforming them into harmless products (*e.g.*, CO₂ and H₂O). This is considered a low-cost and environmentally friendly method compared to its counterparts. Bioremediation, adds nutrients to the contaminated water in order to stimulate the growth of the suitable microorganisms that accelerate the biodegradation of the target pollutants.⁴⁸ Bioremediation methods have advantages over other treatment methods (physical or chemical methods).

These include minimal exposure of workers to the contaminants, environmental safety, long-term protection of public health, ability to be combined with other treatment technologies, possible reduction in the duration of the remediation process, and simultaneous treatment of contaminated waters.⁴⁹ There are three main types of bioremediations that can be summed up as follows:

Biostimulation. Where bacteria are stimulated to initiate the process of bioremediation. First, the contaminated water is mixed with special nutrients including other vital components either in the form of a gas or a liquid in order to increase the growth of microbes. Accordingly, bacteria and other microorganisms remove the contaminants quickly and efficiently.⁵⁰

Bioaugmentation. Where microorganisms are added to the contaminated soil/water. It is more successfully and commonly used to remove contaminants from the original sites, such as municipal wastewater. One major problem of this method is that it is hard to stop microbes from growing.⁵⁰

Intrinsic bioremediation. It is the degradation of organic pollutants by the naturally occurring microbial population without specific human intervention. This process is also known as “natural attenuation” which is used to degrade contaminants in soil, groundwater, and aquifer matrix.⁵¹ Microbes have been able to remove OPPs through bioremediation successfully. As scientists look into different bacteria that can use OPPs or their derivatives as a source of energy by making the enzymes that break down these molecules, this strategy has become the most popular one for the bioremediation processes to succeed, some essential factors are required including:⁵²

- (1) Presence of sustainable microbial populations and suitable kinds of organisms
- (2) Suitable environmental conditions for microbial growth (*e.g.*, presence of oxygen)



- (3) Availability of nutrients, such as nitrogen, phosphorus and sulfur
- (4) Appropriate temperature for microbial growth (0–40 °C)
- (5) Presence of water
- (6) Suitable pH range (pH 6.5–7.5)

The increasing diversity of bacteria that can break down OPPs and use their parts as a carbon, nitrogen, and phosphorus source through the common organophosphate degradation (OPD) gene is significant for the effective breakdown of OPPs in soil and water. Microbes gain energy from the contaminants that let them grow and reproduce. This process occurs by breaking chemical bonds and transferring electrons from the contaminants “electron donor” to an electron acceptor, like oxygen.²⁹

Aswathi *et al.* reported that *Pseudomonas nitroreducens* AR-3, which they got from pesticide-contaminated agricultural soil, could break down around 97% of CPF in 8 hours.⁵² Using three different types of bacteria from soil, *Pseudomonas peli*, *Burkholderia caryophylli*, and *Brevundimonas diminuta*, CPF was entirely removed after 8, 10, and 10 days at 20 mg L⁻¹ and 14, 16, and 16 days at 50 mg L⁻¹, respectively.⁵³ Gao *et al.* reported that on using the fungal strain *Cladosporium cladosporioides* Hu-01, the degradation rate of CPF (50 mg L⁻¹) is up to 90% with maximum hydrolase activity in 5 days.⁵³ *Bacillus cereus* was used for complete degradation of CPF at pH 7.0, 30 °C, and a CPF concentration of less than 150 mg L⁻¹, with a degradation of up to 78.85% of the total pesticide quantity.⁵⁴

Dichlorvos was degraded by employing four soil bacterial isolates; *Proteus vulgaris*, *Vibrio* sp., *Serratia* sp., and *Acinetobacter* sp. The researchers tested their ability to degrade dichlorvos in a medium supplied with different nutrients (NH₄NO₃, KH₂PO₄, and NPK (20:10:10, w/w/w) fertilizers which contain 20% nitrogen (N), 10% phosphorus (P₂O₅), and 10% potassium (K₂O)). It was found that the biodegradation of dichlorvos in soil amended with the inorganic fertilizer NPK is higher than those amended with NH₄NO₃ and KH₂PO₄. In another study, Pravin *et al.* studied the biodegradation of methyl parathion by using the marine bacterium *Nocardioopsis* sp., which led to the formation of *p*-nitrophenol (PNP) and diethylphosphate.⁵⁴

Adsorption and molecular imprinting

Adsorption is a surface phenomenon that depends on the surface area, number of accessible sites, porosity of the adsorbent, as well as various types of interactions. A number of parameters influences this process; the chemical features of adsorbate (ionic nature, functional groups, polarity, solubility, *etc.*), and the properties of the adsorption medium (pH, contact time, adsorbent dose, size, agitation speed, temperature, initial concentration and presence of other species, *etc.*). In most cases, the adsorption efficiency increases with increasing contact time, adsorbent dose, and agitation speed. However, the most favorable conditions are variable for different adsorbents.^{55,56} Adsorption is one of the most efficient and promising methods for removing hazardous substances from contaminated water due to its ease of use, simplicity of design and

inexpensiveness. Several adsorbents such as activated carbon, clays, zeolites, carbonaceous materials, agricultural wastes, polymeric, and inorganic adsorbents, have been used for the removal of OPPs.⁵⁷

Biosorption is a rapid sorption process resulting from physicochemical and ion exchange interactions occurring at the cell surface between a sorbate and live, dead, or inactive biomass. Biosorbents such as waste materials from agriculture and industries can be used as alternative adsorbents which are affordable and easily obtainable. Chitin, chitosan, peat, biomass agricultural waste (coir pith coco, rice husk biomass, sugar peat pulp, orange and banana peels) are commonly used as bioadsorbents. Chitosan's high sorption efficiency, porous structure, and high abundance, in addition to its biodegradability, biocompatibility, and biosafety makes it an ideal adsorbent for environmental remediation.⁵⁸ Sahithya *et al.* modified montmorillonite (MMT)-CuO composites by using three biopolymers, such as gum ghatti, chitosan, and poly(lactic acid) (PLA) for the remediation of monocrotophos (MCP) from water.⁵⁸ They found that the MMT-CuO-PLA composite showed the maximum removal (83.9%) of MCP. The maximum adsorption of MCP by MMT-CuO-PLA composite occurred due to the higher availability of functional groups. The equal contribution of PLA, MMT and CuO nanoparticles on the adsorbent's surface was assigned to the homogenous interaction of PLA with MMT-CuO composite thereby leading to a uniform dispersion of CuO nanoparticles.

Nowadays, the application of nanoparticles in the remediation of different environmental water pollutants is significantly progressing since they have advantages over conventional methods. Nanoparticles owe their potential to the high active surface area and surface reactivity compared to regular bulk materials.⁵⁹ Recently, wastewater treatment with nanomaterials (nano-adsorbents, nano-filters, nano-powders *etc.*) has been considered a good treatment method to reduce the potential risks of various emerging contaminants on the environment.⁶⁰ For instance, the adsorption of malathion on multi-walled carbon nanotubes (MWCNTs) was studied.⁶¹ The researchers found that MWCNTs could be effectively used to remove almost 100% of malathion from water at the optimized conditions.

Firozjaee *et al.* used low-cost sorbent chitosan/carbon nanotubes (CS/CNTs) to remove diazinon from aqueous environment.⁶¹ They synthesized CS/CNTs with 2.5% of MWCNTs which is a promising candidate for improving chitosan's physicochemical and mechanical properties. CNTs have been considered as ideal reinforcing fillers for chitosan to achieve high performance and multi-functions, because of its excellent mechanical strength, electrical and thermal properties, leading to increase in its diazinon removal efficiency.

The use of bimetallic Fe/Ni nanoparticles for the elimination of profenofos OPP from aqueous solutions was reported.⁶² The sorption kinetics presented that the removal rate of the profenofos from aqueous solutions depended on the adsorbent particle size, time to achieve the sorption equilibrium, solid/solution ratio and pH.

Wang *et al.* reported the use of wheat straw-derived biochar for the removal of CPF.⁶² The adsorbent was heated in a furnace



at 250, 350, 450, 550, 650 and 750 °C for two hours each. They show that wheat straw-derived biochar at 750 °C can effectively adsorb CPF and the maximum adsorption capacity is 16 mg g⁻¹. The driving force for CPF adsorption by wheat straw-derived biochar is most likely attributed to π - π stacking between aromatic rings in wheat straw-derived biochar surface and the aromatic ring of CPF.

Adsorption onto activated carbon is an advanced technique for treating contaminated water to get rid of toxic organic contaminants. There are different types of activated carbon materials, including powder active carbon (PAC), granular active carbon (GAC), carbon fibers, carbon cloth and carbon black. The most common types are PAC and GAC. PAC has advantages over GAC, such as low cost and finer particle size.⁵⁶ The research article authored by Pirsahab *et al.*⁶³ reported the adsorption of diazinon on granular activated carbon. Their findings indicated that elevated quantities of diazinon did not markedly enhance diazinon adsorption at a given activated carbon dosage. An increase in the quantity of granular-activated carbon enhances the removal of diazinon, attributable to a greater availability of adsorption sites. Furthermore, a robust association was identified between chemical oxygen demand (COD) measurements and diazinon concentrations. Consequently, COD measurement may serve as a substitute for the direct quantification of toxin levels.

Molecular imprinting is a technique employed to fabricate selective binding sites in synthetic polymers *via* a molecular template. Target molecules may serve as templates for the imprinting of crosslinked polymers. Upon the removal of the template, molecularly-imprinted polymer (MIP) cavities are created with precise dimensions, configurations, and spatial orientations of the functional groups at the recognition sites, which maintain selectivity and affinity to the target molecules.⁶⁴ The selectivity of the polymer is influenced by several parameters, including cavity morphology, size and rebinding contacts, covalent and non-covalent bond interactions, electrostatic interactions, and metal ion coordination.⁶⁵ MIPs have been utilized for the extraction of OPPs from aqueous solutions.⁶⁶

Chattrairat and Phromyothin synthesized molecularly imprinted superparamagnetic iron oxide nanoparticles (SPIONs) and kaolinite/SPIONs composite.⁶⁶ The magnetic MIPs (MMIPs) were synthesized using CPF as the template, ethylene glycol dimethacrylate as the crosslinker, azobisisobutyronitrile as the initiator for adsorption of chlorpyrifos from aqueous solutions. The adsorption capability of CPF onto MMIPs attained 100%. In addition, Abbasi Ghaeni *et al.* investigated the elimination of various OPPs, including malathion, dichlorvos, diazinon and glyphosate from aqueous solutions using a series of micro- and nano-MIPs.⁶⁷ They showed that all MIPs exhibited greater affinity than NIPs (non-imprinted polymers) for the extraction of OPPs from aqueous media and the purification of water from these hazardous substances.

The adsorption of CPF on MWCNTs-based MIP was reported.⁶⁸ MWCNTs-MIP were synthesized by selectively polymerizing MIP on the vinyl group-functionalized MWCNTs surface using CPF as the template. The maximum adsorption was attained at pH 7.0 with equilibrium reached after three hours.

Advanced oxidation processes (AOPs)

Advanced oxidation processes function at ambient pressure and temperature, or in close proximity to these conditions. They are ecologically sustainable as they do not generate substantial quantities of sludge transfer or pollutants between phases. AOPs can decompose all varieties of organic pollutants into innocuous byproducts or entirely mineralize them to produce H₂O, CO₂ and the respective inorganic salt. They are based on the generation of potent oxidizing chemical entities, primarily hydroxyl radicals, with a redox potential of 2.8 V, and they are achieved using a range of combinations of oxidants and catalysts. Ozone, H₂O₂ and UV based AOPs are prevalent due to their shown efficacy in oxidizing and mineralizing a diverse range of wastewater contaminants. The hydroxyl radical is reactive and initiates a sequence of oxidation reactions that yield mineralization products, including H₂O, CO₂ and an inorganic salt.⁴⁷ AOPs can be classified as photochemical or nonphotochemical processes. The majority of photochemical AOPs use direct photolysis using UV light, UV/TiO₂, UV/H₂O₂, photo-Fenton (the combination of H₂O₂ and Fe²⁺) and photo-Fenton-like (the combination of H₂O₂ and Fe³⁺) processes. Non-photochemical AOPs processes encompass Fenton process, ozonation, electrochemical oxidation.

Furthermore, AOPs can be categorized either as homogeneous or heterogeneous.⁶⁹ Homogeneous photocatalysis utilizes various oxidizing chemicals, including O₃, H₂O₂, Fenton reagent and NaOCl either alone or in conjunction with light exposure (UV, visible or solar). Conversely, heterogeneous photocatalysis employs semiconductor metal oxides such as catalysts (*e.g.*, TiO₂, WO₃, ZnO and ZrO₂) along with sulfides (*e.g.*, CuS, ZnS and FeS) under UV/solar light. These materials are non-toxic, cost-effective, chemically inert, readily accessible and exhibit strong photoactivity. Titania (TiO₂) has garnered significant interest of researchers over the years as an alternative approach for water purification.⁷⁰ Heterogeneous photocatalysis was determined to be more efficacious than homogeneous systems. Heterogeneous photocatalytic oxidation results complete elimination of pollutants and facilitates the partial degradation of non-biodegradable contaminants into biodegradable intermediates.⁴⁷

As examples of photocatalysis, a photocatalytic agent for the remediation of CPF using a metal free heterogeneous graphitic carbon nitride (g-C₃N₄) incorporated into chitosan as catalyst was investigated.⁷¹ The degradation of CPF using CS/g-C₃N₄ demonstrated an efficiency of approximately 85%. In addition, Rocha *et al.* reported the degradation of profenofos by *in situ* electrogenerated H₂O₂ and experiments were performed both with or without the Fe²⁺ catalyst.⁷¹ In the presence of 0.15 mmol L⁻¹ FeSO₄·7H₂O (electro-Fenton reaction), the elimination of profenofos reached 91% after 60 min, while the total organic carbon (TOC) decreased by 37%.

Gomez *et al.* studied the photocatalytic degradation of dichlorvos using zeolite/TiO₂ composite.⁷² A high TiO₂ content produced lower degradation due to the presence of larger TiO₂ particle aggregates on the zeolite matrix surface. Zeolite/TiO₂ composite exhibits appropriate characteristics for utilization as catalysts in the photocatalytic treatment of wastewater.



The remediation of the three pesticides, acephate, dimethoate and glyphosate, was investigated in contaminated water using UV radiation and TiO_2 immobilized on silica gel as a catalyst.⁷³ It was observed that dimethoate and glyphosate pesticides were entirely degraded within 60 min of irradiation, while complete disintegration of acephate occurred after 105 min of photocatalytic treatment.

Zerovalent iron (ZVI) is the frequently employed zerovalent metal for the remediation of groundwater and wastewater contaminated with OPPs. ZVI is commonly utilized as a reductant and can effectively degrade a variety of environmental contaminants. ZVI is non-toxic, economic, abundant, facile to manufacture, and its reduction process requires little maintenance. The removal mechanism of pollutants by ZVI involves the directional transfer of electrons from ZVI to the pollutants, which transforms the pollutants into less toxic or non-toxic species. Furthermore, ZVI can degrade and oxidize organic pollutants in the presence of dissolved oxygen (DO). The produced Fe^{3+} can react with H_2O_2 and HO_2 (hydroperoxyl radical), referred to as the “Fenton-like reaction”, resulting in the regeneration of Fe^{2+} . The regeneration of Fe^{2+} can also occur through reactions with organic radical intermediates.

Hydrolysis

Hydrolysis is the process of utilising water to decompose pesticides in diverse aqueous systems, and it is regarded as the most significant mechanism for pesticide degradation. Kunde *et al.* investigated the hydrolysis of triazophos in buffered aqueous solutions with pH levels ranging from 4 to 10, as well as in sodium hydroxide solutions with pH values exceeding 10.⁷³ It was discovered that triazophos readily undergoes hydrolysis in alkaline solutions. Huang and Mabury documented the hydrolysis of fenthion insecticides and their five oxidation metabolites in buffered aqueous solutions at pH 7 and pH 9, conducted at temperatures of 25, 50, and 65 °C. The researchers determined that the hydrolysis mechanisms involved the interaction of a water molecule and a hydroxide ion with the phosphorus atom to produce phenol derivatives, and with the carbon atom to yield dealkylation products.

Methods of analysis of organophosphate pesticides

The extensive variety of OPPs and their diverse chemical characteristics prompted scientists to create numerous analytical approaches for their detection in different samples. Among these technologies, chromatographic, spectroscopic, and electrochemical/optical sensing techniques are the most prevalent. This section summarises examples of standard analytical procedures employed to assay OPPs in Tables 4 and 5. The limit of detection (LOD), linear range, and target OPP are provided in each table to enable researchers to identify the appropriate analytical methodology for the OPP of interest. The following is a summary of the analytical methods used in the literature to analyse OPPs.

Chromatographic methods

Gas chromatography (GC) and high-performance liquid chromatography (HPLC) are widely used for quantitative and qualitative analysis of OPPs, often coupled with selective detectors or mass spectrometry (MS). GC with nitrogen-phosphorus detectors (NPD) or flame photometric detectors (FPD) enables sensitive detection of organophosphorus compounds. HPLC methods combined with solid-phase extraction and novel adsorbents allow efficient separation and determination in complex environmental and food samples.

These methods provide high sensitivity and selectivity for multiple pesticide residues in a single run.

The variation in analytical LODs for OPPs across chromatographic methods arises due to differences in the instrument used, the sample matrix complexity, and the sample preparation techniques applied. For example, IL-DLLME/HPLC typically yields LODs in the sub-microgram per liter range ($0.1 \mu\text{g L}^{-1}$) due to effective preconcentration (in this case, dispersive liquid-liquid micro-extraction) coupled with high-sensitivity HPLC detectors, making it ideal for trace-level environmental water analysis. Conversely, gas chromatography with flame photometric detection (GC-FPD) usually reports LODs in mg kg^{-1} or ng mL^{-1} ranges, varying with the pesticide and matrix. GC-FPD is widely used for food and soil residue analysis, where sample matrices are more complex and extensive cleanup steps are needed, which can affect sensitivity.

The sample matrix composition plays a critical role as complex matrices such as food or soil contain interfering substances that can suppress or enhance signals, thereby impacting LOD. Sample preparation methods like solid-phase extraction, QuEChERS, or multi-plug filtration clean-up help reduce matrix interferences and improve detection limits (Table 6).

Additionally, gas chromatography-mass spectrometry (GC-MS) offers confirmatory capabilities with improved selectivity and sensitivity (LODs often below $\mu\text{g kg}^{-1}$), but the evolution of advanced detectors like GC-MS/MS or SP/GC-MS lowers detection limits further, sometimes reaching single digit ng L^{-1} or ng kg^{-1} . Retention times also vary depending on the chromatographic conditions and analyte properties, affecting peak resolution and quantitation accuracy.

Spectroscopic methods

Spectroscopic techniques used include UV-visible spectrophotometry, nuclear magnetic resonance (NMR), X-ray analysis, and surface-enhanced Raman spectroscopy (SERS). UV-visible methods often rely on complex formation with reagents to detect OPPs in fruits and vegetables, providing simple, sensitive, and green approaches. SERS achieves ultrasensitive and non-destructive detection with minimal sample preparation and has been applied successfully to detect pesticides like methyl parathion on produce surfaces. NMR and mass spectrometry provide structural insight and confirmation of OPP residues.¹²⁴

Electrochemical methods

Electrochemical detection harnesses biosensors based on acetylcholinesterase (AChE) inhibition or biomimetic catalytic





Table 4 Methods of remediation of the most common five organophosphate pesticides

Organophosphate pesticide	Remediation methods	Efficiency	Reaction time	Conditions	Scalability	Byproducts	Ref.
Malathion	Bioremediation	High (up to 90% degradation)	Days to weeks	Ambient temp; aerobic/anaerobic soil	Moderate to high	Metabolites less toxic than parent	74 and 75
	Enzymatic degradation	Very high (specific cleavage)	Hours to days	Mild pH, temp; enzyme stability important	Moderate (enzyme immobilization aids)	Non-toxic products like phosphoric acid	75–77
	Chemical hydrolysis	Moderate to high	Hours	Alkaline pH, aqueous environment	High	Toxic intermediates possible	75 and 78
	Advanced oxidation (AOPs)	High	Minutes to hours	Acidic to neutral pH; UV or catalyst	Moderate	Mineralization to CO ₂ , water	77
Chlorpyrifos	Adsorption	High removal efficiency	Immediate to hours	Activated carbon or resin beds	High	Concentrated contaminated adsorbent	75
	Bioremediation	Moderate to high	Days to weeks	Soil, water, moderate temperature	Moderate	Metabolites less toxic but persistent	75, 79 and 80
	Enzymatic degradation	High	Hours to days	Mild conditions; enzyme-specific	Moderate	Inorganic phosphates	75 and 76
	Chemical oxidation/hydrolysis	Moderate to high	Hours to days	Variable; often catalytic	Moderate	Depends on oxidant; sometimes toxic	77 and 78
Diazinon	Adsorption	Moderate	Minutes to hours	Adsorbents used in water treatment	High	Spent adsorbents need disposal	75 and 81
	Microbial degradation	High	Days to weeks	Aerobic soil/water	Moderate	Less toxic metabolites	77 and 80
Parathion	Enzymatic remediation	High	Hours to days	Controlled, enzyme-stable	Moderate	Phosphoric acid derivatives	75 and 76
	Hydrolysis and photolysis	Moderate	Hours to days	UV light, alkaline to neutral pH	Moderate	Break-down products vary	77 and 78
	Biodegradation (bacterial consortia)	High	Days to weeks	Soil/water, aerobic/anaerobic	Moderate	Less toxic products	75 and 80
	Enzymatic hydrolysis	Very high	Hours to days	Enzyme-specific optimal conditions	Moderate	Non-toxic phosphates	75 and 76
Dichlorvos	Chemical hydrolysis and oxidation	Moderate to high	Hours	Alkaline or catalyzed	Moderate	Depends on reaction	77 and 78
	Microbial bioremediation	High	Days to weeks	Ambient soil/water	Moderate	Non-toxic metabolites	75 and 80
	Enzymatic degradation	High	Hours to days	Mild conditions, enzyme stability	Moderate	Phosphoric acid and derivatives	75 and 76
	Chemical methods	Moderate to high	Hours	Catalytic and chemical oxidants	Moderate	Mineralized products possible	77 and 78

Table 5 Common chromatographic methods for the determination of OPPs in various sample

Chromatographic method	Analytes (OPPs)	Retention time (min)	LOD	Linear range	Recommended application	Ref.
IL-DLLME/HPLC	Fenitrothion fenthion	~13	0.1 $\mu\text{g L}^{-1}$	0.01–100 $\mu\text{g L}^{-1}$	Trace level analysis	82
GC-FPD	Dichlorvos	5.490	0.007 mg kg^{-1}	0.01–1.0 mg kg^{-1}	in water/	83
	Monocrotophos	9.160	0.030 mg kg^{-1}		environmental	
	Phorate	9.440	0.005 mg kg^{-1}		samples	
	Dimetoate	9.860	0.040 mg kg^{-1}			
	Diazinon	10.43	0.060 mg kg^{-1}			
	Paraxon-methyl	11.22	0.050 mg kg^{-1}			
	Phosphomidon	12.03	0.050 mg kg^{-1}			
	Chlorpyrifos-methyl	12.44	0.020 mg kg^{-1}			
	Parathion methyl	12.67	0.100 mg kg^{-1}			
	Fenitrothion	13.61	0.030 mg kg^{-1}			
	Malathion	13.88	0.010 mg kg^{-1}			
	Chlorpyrifos	14.25	0.020 mg kg^{-1}		Rapid screening of	
	Fenthion	14.25	0.030 mg kg^{-1}		residues in food and	
	Parathion	14.43	0.010 mg kg^{-1}		soil	
	Chlorfenvinfos	15.95	0.040 mg kg^{-1}			
	Quinolphos	16.42	0.010 mg kg^{-1}			
	Fenamiph	17.86	0.020 mg kg^{-1}			
	Profenofos	18.58	0.030 mg kg^{-1}			
	Ethion	20.93	0.010 mg kg^{-1}			
	Trizophos	21.60	0.020 mg kg^{-1}			
	Edfinphos	21.99	0.010 mg kg^{-1}			
	Anilophos	24.15	0.050 mg kg^{-1}			
	Phosalone	25.74	0.020 mg kg^{-1}			
GC-FPD	Dicrotophos	6.89	1.36 ng mL^{-1}	0.80–8.0 ng mL^{-1}	Sensitive trace	83
	Dimethoate	7.62	0.39 ng mL^{-1}	0.40–4.0 ng mL^{-1}	residue analysis	
	Diazinon	8.19	0.38 ng mL^{-1}	2.5–25 ng mL^{-1}		
	Parathion-methyl	10.25	0.26 ng mL^{-1}	2.0–20 ng mL^{-1}		
	Malathion	10.42	0.36 ng mL^{-1}	0.30–3.0 ng mL^{-1}		
	Chlorpyrifos	10.97	0.18 ng mL^{-1}	1.5–15 ng mL^{-1}		
	Pirimiphos-ethyl	12.85	0.24 ng mL^{-1}	0.80–8.0 ng mL^{-1}		
	Prothiophos	12.98	0.47 ng mL^{-1}	0.40–4.0 ng mL^{-1}		
	Profenofos	14.28	0.51 ng mL^{-1}	2.0–20 ng mL^{-1}		
	Ethion	14.70	0.27 ng mL^{-1}	0.50–5.0 ng mL^{-1}		
	Triazophos	15.83	0.33 ng mL^{-1}	2.5–25 ng mL^{-1}		
GC-MS	Phosmet	—	0.50 $\mu\text{g kg}^{-1}$	0.05–0.2 mg kg^{-1}	Confirmatory and	84
	Phorate	—	0.70 $\mu\text{g kg}^{-1}$		residue analysis	
GC-MS	Chlorpyrifos	28.9	0.13 $\mu\text{g kg}^{-1}$	0.4–2500 ng g^{-1}	Confirmatory and	85
					residue analysis	
GC-ECD		5.79	0.014 $\mu\text{g g}^{-1}$	0.033–1.7 $\mu\text{g g}^{-1}$	Sensitive detection	86
					in environmental	
					samples	
HPLC-DAD	Parathion	3	0.10 $\mu\text{g L}^{-1}$	1–200 ng mL^{-1}	Liquid phase	87
					analysis of water	
					samples	
GC/FPD	Dimethoate	—	0.01 ng mL^{-1}	1 ppb–2 ppm	Food and	88 and
	Parathion-methyl		0.03 $\mu\text{g mL}^{-1}$		environmental	89
	Malathion		0.03 ng mL^{-1}		residue monitoring	
	Terbufos		0.04 ng mL^{-1}			
	Parathion		0.02 ng mL^{-1}			
SPF-GC-MS	Dichlorvos	4.82	4.0 ng L^{-1}	0.1–1.0 mg L^{-1}	Trace analysis in	2 and
	Methyl parathion	8.85	10 ng L^{-1}	0.1–2.0 mg L^{-1}	water samples	90
	Malathion	9.21	4.0 ng L^{-1}	0.1–2.0 mg L^{-1}		
	Parathion	9.46	5.5 ng L^{-1}	0.055–1.1 mg L^{-1}		

electrodes. Enzymatic biosensors measure the inhibition of AChE by OPPs, offering indirect quantification with good sensitivity and applicability to real samples like water and juice. Biomimetic sensors modified with amino acid-conjugated

polymers catalyse OPP hydrolysis, producing electroactive species detectable with low limits of detection. Advances include nanomaterial modifications (*e.g.*, copper nanowires, graphene oxide) enhancing sensitivity and selectivity.¹²⁵





Table 6 A summary of the most common electrochemical/optical sensors reported for the analysis of OPPs in complex samples

Sensing method	Analytes/OPP	Turnaround time	LOD	Linear range	Recommended application	Ref.
Electrochemical sensors						
Bimetallic nanowires network/ amperometry	Malathion	>15 min	0.037 pM	0.1 pM–100 nM	Ultra-sensitive detection in environmental monitoring	91
AChE/AuNRs/GCE/amperometry	Paraoxon dimethoate	5 min	0.7 nM 3.9 nM	1 nM–5 μ M 5 nM–1 μ M	Rapid screening in agricultural and environmental samples	90
SPE/amperometry	Chlorpyrifos oxon	10 min	0.3 nM	Not mentioned	Quick field monitoring of chlorpyrifos residues	92
<i>E. coli</i> /nafion-OMCs/amperometry	Paraoxon	—	(0.009 μ M) (0.010 μ M)	0.05–25 μ M 0.05–25 μ M	Biosensing in complex environmental and food matrices	93
AChE/GCE/graphene-nafion/ amperometry	Methyl parathion	—	(0.015 μ M) 70 nM	0.08–30 μ M Not mentioned	Sensitive detection of methyl parathion residues	94
AChE/Au electrode-Fe ₂ O ₄ / MWNTs/amperometry	Chlorpyrifos	4 s	0.1 nM	0.2–15 nM	Rapid, ultrasensitive detection in water and food	95
HRP/GCE/mercuric chloride	Malathion	10 min	24 μ M	25–500 μ M	Enzymatic sensor for pesticide residues in agricultural samples	96
SWNTs/MWNTs amperometry	Paraoxon	—	0.01 μ M for SWNTs 6.4 μ M for MWNTs	0.5–8.5 μ M 0.5–6.0 μ M	Electrochemical sensing in complex matrices	97
<i>E. coli</i> /carbon paste/nylon net/ silicon oil	Parathion	—	0.015 μ M	0.02–0.18 μ M	Electrochemical sensing in complex matrices	98
Cystamine coated Au on insulated p-type silicon/amperometry	Methyl parathion	<40 s	0.1 μ M	1–10 μ M	Fast detection for field applications	99
<i>E. coli</i> /mesoporous carbon and carbon-black/amperometry	Parathion	<10 s	0.12 μ M	0.2–8.0 μ M	Fast detection for field applications	99
CNTs-FIA/amperometry	Methyl parathion	<10 s	0.8 μ M	Up to 2 μ M	Flow injection analysis for real-time monitoring	99
GCE/CV	Methyl parathion	—	0.25 ng mL ^{−1}	1.0–100.0 ng mL ^{−1}	Electrochemical sensing in complex matrices	100
Graphite SPE/DPV	Profenofos	10 min	0.27 M	Not mentioned	Electrochemical sensing in complex matrices	101
MIP/MWCNTs/DPV	Parathion-methyl	3–5 min	60 nM	0.2–10 μ M	Selective electrochemical sensing in complex matrices	102
MIP/GCE/DPV	Isocarbophos	5 min	20 nM	75 nM to 50 μ M	Selective detection in environmental samples	103
GCE/CV	Fenitrothion	175 s	1.5 nM	5 nM–8.6 mM	Electrochemical sensing in complex matrices	104
SPE/CV	Chlorpyrifos	At least 14 min	0.14 nM	0.14 nM–0.29 mM	Sensitive pesticide residue detection in vegetables	105
MIP/Au modified with nitrogen-doped graphene/CV	Parathion methyl	—	0.01 μ g mL ^{−1}	0.1–10 μ g mL ^{−1}	Selective electrochemical sensing in complex matrices	106
CS MIP/GCE/CV	Parathion	50 min	3 μ g kg ^{−1}	0.015–15 mg kg ^{−1}		107



Table 6 (Contd.)

Sensing method	Analytes/OPPs	Turnaround time	LOD	Linear range	Recommended application	Ref.
Wearable tattoo sensor OPH/nafion/PVA/PANI/SPE/ potentiometry	Di-isopropyl fluorophosphates	<20 s	10 mM	10–120 mM	Selective electrochemical sensing in complex matrices On-body monitoring of exposure	108
Microfluidic arrays/fluorescence	Optical sensors Parathion	Longer than 15 min	0.38 pM	1 pM–10 M	Multiplexed screening in food safety	109
CQDs/fluorescence	Dichlorvos Chlorpyrifos	Longer than 30 min	8.6 nM 3.0 ng mL ⁻¹ 5 × 10 ⁻⁵ μM	0.01–1.0 μg mL ⁻¹ 0–1050 nM	Sensitive environmental water analysis Enzymatic fluorescent detection in water	110 111
CS/AuNPs/organophosphorus hydrolase/fluorescence	Paraoxon	<30 min	5 × 10 ⁻⁶ μM	10–250 nM	Ultrasensitive detection	112
OPH-conjugated nanomagnetic- silica/fluorescence	Paraoxon	—	50 nM	0.3–60 μM	Selective ultrasensitive detection	113
MIP film on QDs/fluorescence	Chlorpyrifos	40 min	5 × 10 ⁻⁵ μM	50–1050 nM	Ultrasensitive detection	114
OPH-conjugated AuNPs/ glutaraldehydecystamine/ fluorescence	Paraoxon	—	—	—	—	—
OPH6His/pyranine/silica-coated Ag NPs/fluorescence	Paraoxon	<3 min	2 ppb	5–100 ppb	Ultrasensitive detection	47 and 115
<i>E. coli</i> whole cells harboring plasmid pTinaPb-N/OPH/UV spectrophotometry	Methyl parathion Paraoxon	≤10 min	10 ppb 0.2 μM	20–100 ppb 0.5–150 μM	Selective detection	116
<i>E. coli</i> /MAP-based adhesion/UV spectrophotometry	Paraoxon	5 min	0.4 μM 1 μM 5 μM	1–200 μM 2.5–200 μM 5–320 μM	Selective detection	117
<i>Sphingomonas</i> sp. JK1/inner epidermis of onion bulb scale and glutaraldehyde/UV spectrophotometry	Methyl parathion	5 min	4 μM	4–80 μM	Selective detection	118
<i>Sphingomonas</i> sp. JK1/ glutaraldehyde as linker/UV spectrophotometry	Methyl parathion	5 min	4 μM	4–80 μM	Selective detection	119
Au NPs/colorimetry	Parathion	At least 35 min	0.7 ppb	Not mentioned	Colorimetric detection for rapid onsite analysis	120
Au nanopopcorn/SERS	Chlorpyrifos	Several minutes	1 μM	1.5–6.25 M	Surface-enhanced Raman for sensitive specific detection	121
<i>P. diminuta</i> /OPH immobilized on CNTs paste electrode/conductivity	Methyl parathion	≥ 10 min	0.1 μM	0.1–200 μM	Sensitive and selective detection in environmental and water samples	122
Competitive chemiluminescent enzyme immunoassay (CLEIA)	Triazophos	5 min	0.19	0.04–5 ng mL ⁻¹	Immunoassay for rapid high- throughput pesticide quantification	123

Colorimetric methods

Colorimetric assays frequently use gold and silver nanoparticles (AuNPs, AgNPs) aggregation and enzymatic reactions for rapid OPP detection. These methods provide visible color changes correlating with pesticide concentration, suitable for onsite screening and simpler instrumentation. Enzymatic colorimetric assays target AChE inhibition by OPPs, reflecting pesticide presence through intensity changes in chromogenic substrates. Such approaches are valued for their ease, specificity, and potential for miniaturization.¹²⁵

The variation in analytical LODs among the electrochemical and optical sensors for OPPs in complex samples stems from differences in sensing methods, sensor design, sample matrices, and sample preparation strategies.

Electrochemical sensors, such as amperometry using bimetallic nanowires or enzyme-based electrodes (*e.g.*, AChE immobilized on nanostructured materials), achieve ultra-low LODs (down to femtomolar or sub-nanomolar levels) due to the catalytic amplification of electrochemical signals and high specificity of enzyme-substrate interactions. These methods often require relatively short turnaround times (seconds to minutes), making them suitable for rapid environmental and food screening. The performance of these sensors is influenced by the electrode material, nanomaterial enhancements (gold nanoparticles, graphene, carbon nanotubes), and enzyme immobilization techniques which affect sensitivity and stability. In complex matrices, sample pretreatment or dilution may be necessary to reduce interference which can affect the LOD.

Optical sensors, including fluorescence-based and surface-enhanced Raman scattering (SERS), offer high sensitivity with LODs down to picomolar levels but generally have longer turnaround times (up to tens of minutes). The sensitivity depends on the fluorophore brightness, quenching mechanisms, and nanoparticle functionalization affecting sensor response. Optical systems often require more elaborate sample preparation to reduce background fluorescence or light scattering, especially from complex food or environmental samples.

Matrix complexity also plays a key role. Environmental water samples typically permit lower LODs due to fewer interfering substances compared to food or soil matrices, which require robust sample cleanup to prevent signal suppression/enhancement. Sample preparation approaches such as filtration, solid-phase extraction, or enzymatic digestion can concentrate analytes and remove interferents, directly impacting achievable detection limits.

Conclusions

Organophosphate pesticides are extensively utilised in agriculture, industry, livestock management, and home settings, posing a considerable health risk to humans and animals. These chemicals are the primary constituents of chemical nerve agents, including tabun, sarin, soman, and cyclosarin. Organophosphates are synthesized through the esterification of phosphoric acid, the oxidation of phosphite esters, and the alcoholysis of POCl_3 . Organophosphate pesticides continue to

be implicated in numerous severe human poisonings due to their fast distribution and accumulation in the liver, kidney, and adipose tissues; thus, inhibiting the function of acetylcholinesterase. The inhibition of this enzyme leads to the accumulation of ACh in the synaptic cleft causing overstimulation of the muscarinic and nicotinic ACh receptors, hence impeding neurotransmission. The contamination of water by pesticides is a significant ecological concern, particularly in areas of intense agriculture where leakage of these highly toxic compounds into water sources can adversely affect human and animal health. Groundwater and surface water contamination is a matter of concern, as the contaminants, particularly pesticides, can infiltrate drinking water supplies. Various techniques have been established to eliminate organophosphate pesticides from contaminated environmental samples, in order to minimize the potential health hazards. In this review, we provided an overview of organophosphate pesticides, including their classifications, potential health impacts, environmental risks, synthesis and analytical techniques, and prevalent remediation strategies. Despite the growing interest in organophosphate pesticide research, opportunities exist for scientists to formulate organophosphate pesticides with reduced toxicity to humans and cattle, as well as to devise novel ways for their effective removal and precise analysis. The advancement of such safe pesticides will be realised in the near future by targeting the insects' biomolecules and/or biochemical reactions that are not found in the human body. Computational biologists can significantly contribute to reaching this objective.

Future perspectives of organophosphorus pesticides

Market growth and trends

The global market for organophosphorus insecticides is anticipated to develop, with projections indicating a compound annual growth rate (CAGR) of approximately 4.5–5.2% from 2025 to 2034. This increase is propelled by rising agricultural demand, the necessity for efficient pest control measures, and the proliferation of contemporary agricultural methods, particularly in developing countries.

Technological breakthroughs are facilitating the creation of safer, environmentally friendly formulations with diminished toxicity, hence promoting regulatory compliance and environmental safety.

Precision agriculture and advanced application technologies, including drones and controlled-release formulations, are anticipated to improve the efficiency and sustainability of OPPs utilization.

Regulatory and environmental factors

Stricter rules and restrictions on dangerous substances, such as chlorpyrifos and malathion, are influencing the market, compelling manufacturers to innovate and create fewer toxic alternatives.



There is an increasing focus on integrated pest management (IPM) and sustainable agriculture methods, seeking to harmonize effective pest control with minimal environmental repercussions.

Environmental concerns, such as soil and water persistence, bioaccumulation, and toxicity to non-target organisms, are driving research towards improved degradation technologies and bioremediation strategies.

Health safety and research

Current investigations examine the mechanisms of organophosphorus pesticide toxicity, with specific emphasis on environmental concerns, such as soil and water persistence, bioaccumulation, and toxicity to non-target organisms, are driving research towards improved degradation technologies and bioremediation strategies. Neurotoxicity and long-term health impacts in humans and wildlife.

Future study is anticipated to investigate molecular pathways, encompassing RNA and microbe interactions, to formulate targeted therapeutics and safer pesticide designs.

Advancements in biosensors and analytical methodologies are enhancing the detection and monitoring of pesticide residues in the environment, hence facilitating improved risk assessment and management.

Transition to alternatives

Increasing consumer and regulatory demand for organic and sustainable agriculture is prompting a transition to biopesticides and natural pest management techniques.

Investment in research and development is expected to expedite the emergence of novel, less harmful pest management solutions, potentially diminishing dependence on conventional organophosphorus substances in the long term.

Conflicts of interest

There are no conflicts to declare.

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

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