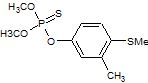
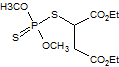
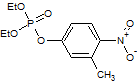
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Chemical Information Retrieval

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Organophosphate is the term used to define esters of phosphorous acids and is typically used to represent the phosphor-carbon bond in these molecules.1 Effective organophosphates contain a phosphoryl group, two lipophilic groups attached to the phosphorous atom, and a leaving group, commonly a halide, also bound to the phosphorous atom.1 The side-chains are diverse and lead to different reactivities which correspond to the different levels of poisoning caused by organophosphates.2 In 1932, a German chemist, Gerard Schrader, was inspired to study the effects of organophosphates on insects after Willy Lange discovered the toxic effects of organophosphates on the parts of the nervous system that are related to acetylcholinesterase.1 Organophosphates are effective inhibitors of acetylcholinesterase, which is essential to nerve function in insects, animals, and humans. Organophosphates toxic effects and increased hydrolytic rates when exposed to sunlight, soil and air, 1 made them an ideal replacement for the former active ingredient in pesticides, organochlorides. However, organophosphates are shown to be more toxic in humans than organochlorides. Pesticides are inherently toxic since they are composed of more than 200 organophosphates, with the active ingredient found on the label or MSDS.2 Structures of common organophosphates found in pesticides can be seen in Figure-1.3



(a.) Parathion Ethyl (b.) Malathion (c.) Fenthion

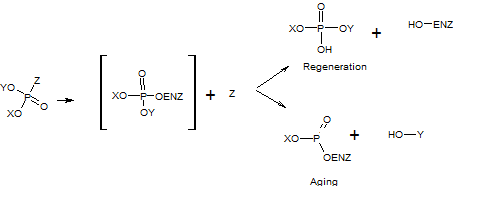
**Figure-1: Structures of common organophosphate pesticides, including parathion, malathion and fenthion.**

The utilization of organophosphates as components in pesticides has become increasingly common in the global agriculture sector. Globally, production and consumption of pesticides has increased 10-fold between 1955 and 1985.4 As the global population increases, there is a greater demand on the industrial and agricultural sectors for the production of food. This demand requires preservation of crops that can be destroyed by natural disasters or pests. To address the issue imposed by insects, pesticides are employed. To meet the higher demand for food, the use of pesticides is increased which in turn increases pollution on the environment.5 Organophosphate contaminants are generally found in freshwater reserves and soil.5 The presence of organophosphates in soil and water is a result of water run-off caused by rain or flooding. Another common form of organophosphate contamination is caused by inhalation which can result from the distribution of pesticides among the crops or by chemical fires that occur at warehouses where pesticides are stored.6 Agriculture workers are the most common victims of organophosphate exposure; however, the contamination of fresh-water by organophosphates endangers society, animal-life and the enivronment. Organophosphate poisonings are also found as the cause for many suicides due to the relative ease in obtaining organophosphate containing pesticides. Organophosphate poisonings more commonly occur in under-developed nations, such as Sri-Lanka, however, even the most regulated countries struggle to prevent the impairment or death of citizens that were exposed to organophosphates.7

Toxic exposure of organophosphates on agriculture workers leads to more than 3 million poisonings and 200,000 deaths per year, globally.2 Most of the poisonings occur in deprived nations, such as Taiwan 7 , however, in the United States, 13,348 toxic organophosphates exposures were reported.8 Organophosphate exposure occurs through respiratory, gastrointestinal, ocular and dermal absorption of pesticides. Symptoms occur more rapidly from the inhalation of the organophosphate odor.2The organophosphates from the pesticides accumulate in fat, liver and kidneys. Patients can begin to exhibit symptoms as quickly as 12 hours. The symptoms are a result of inhibition of the enzyme, acetylcholinesterase.

Acetylcholinesterase is a biological enzyme found in insects, animals and humans. It is an effective enzyme that is of great importance which is found in the central nervous system. Acetylcholinesterase is found at neuromuscular junctions and synapses in the central nervous system, as well as, the membranes of red blood cells. It is an enzyme that rapidly hydrolyzes the neurotransmitter, acetylcholine. Acetylcholine binds to the hydroxyl group on the serine residue 203 in acetylcholinesterase, which forms an intermediate that is broken down by hydrolysis of acetylcholine and regeneration of acetylcholinesterase.2 The choline that is produced from the hydrolysis of acetylcholine is recycled back into the nerve terminals where it is used to produce new acetylcholine molecules.9 Acetylcholinesterase breaks-down 5000 molecules of acetylcholine every second.9 The presence of cholinesterase inhibitors prevents the acetylcholinesterase from functioning. Without the activated acetylcholinesterase, the acetylcholine receptors are over stimulated and eventually breakdown due to the accumulation of acetylcholine.2 The accumulation of acetylcholine results in neuromuscular paralysis across the entire body which leads to death by asphyxiation.9

Organophosphates are one example of a common inhibitor of acetylcholinesterase. When organophosphate is present, phosphorylation occurs at the serine residue 203, forming an organophosphorous intermediate that is stabilized, slowing the rate of hydrolysis or even preventing regeneration of acetylcholinesterase, depending on the phosphorylated group bound to acetylcholineserase.2 Figure-2 is a general schematic of acetylcholinesterase inhibition caused by organophosphate pesticides.2



**Figure-2: General scheme of acetylcholinesterase inhibition caused by organophosphates.**

The side-chains bound to the organophosphate determine its affinity to acetylcholinesterase, rate of hydrolysis, regeneration of acetylcholinesterase, and toxicity.2 For example, acetylcholinesterase ages if the phosphorylated enzyme loses an alkyl group before it is regenerated. An aged enzyme is permanently phosphorylated and cannot be regenerated.2 These effects are enhanced in insects, ultimately leading to their death, which make the use of organophosphates in pesticides ideal. Research has been done on the effects of organophosphates present in humans. The studies have shown that organophosphates have neurological and detrimental effects on human, which has led to their use in chemical war-fare agents, such as the presence of s…. in the nerve gas, VX. (REF) Interestingly enough, organophosphates are still used as pesticides. Due to their high toxicity on humans, strict regulations must be employed on the use of organophosphates as pesticides to prevent over-exposure.

Despite regulations and instructions associated with the use of pesticides, misuse and environmental contamination leads to exposure of the skin and respiratory tract and ultimately acute toxic effects, especially in agricultural workers.10 Organophosphate poisoning is most common amongst agriculture workers in underdeveloped countries, but can also be found as the cause for many suicides.10 The following studies discussed further focus on undesired exposure to organophosphate pesticides. Pesticides lead to 1 million accidental poisonings and 2 million suicide attempts world-wide. With 13,000 poisoned patients and 1,000 deaths occurring in Sri Lanka and 100,000 cases occurring in China.10 The main ingredient in each pesticide can vary depending on the major organophosphate present. A study was done in California between 1982 and 1990 to determine the type of organophosphate that led to poisoning amongst non-agricultural workers and agricultural workers. People who were not agricultural workers were poisoned by organophosphates of moderate toxicity including malathion, chlorphyrifos, dichlorovos, dimethoate, and propetamphos. Poisonings amongst agricultural workers were associated with high-toxicity organophosphates including mevinphos, methomyl, methamidophos, oxydemeton, and parathion, as well as moderately toxic organophosphates including dimethoate and phosalone.10 The level of toxicity determines the systemic effects observed in victims of organophosphate poisoning.

Organophosphate poisoning can occur through consumption of contaminated water or inhalation of the odours. Pesticides found in the soil pose a threat to ground water, water reserves and wells due to surface runoff caused by rain.11 A case study involving contaminated waters was done in Somalia’s North-West Zone. A pesticide store was damaged after a bombing, which led to the theft of chemical drums in which some contained organophosphate pesticides. The looters dumped the chemical drums, contaminating 3700 sq. m. of soil with up to 3,728 ppm of pesticides. The high concentration of pesticides found in the soil and water led the death of lizards, frogs and humans, as well as many cases of organophosphate poisonings amongst the citizens of Solamiland. 11 Organophosphate poisoning does not need to only occur through consumption, but can also be a result of inhalation. The inhalation of organophosphates can result from the spraying of crops with pesticides. An example of exposure through inhalation involves an oil-company employee that noticed the odor of malathion, an organophosphate pesticide, coming from a helicopter. The employee reported symptoms including a sore-throat, headache, and burning skin. The cholinesterase activity was measured and was reported as normal. In this case, malathion is a less toxic pesticide. The odour is a result of mercaptans and sulphides present in organophosphates which can provoke symptoms such as a headache and nausea which are independent from acetylcholinesterase inhibition. Atypical symptoms of organophosphate poisoning that arise from inhalation include tightness of the chest, wheezing and bronchoconstriction result from poisoning and require careful attention.10 Inhalation of organophosphates can also result from the burning of pesticides. One of the most common hazards of plants that store pesticides is a chemical fire. The decomposition of pesticides and their by-product result in hazardous consequences on the environment.6 The level of toxicity is hard to measure due to the complex combinations of different chemical reactions that would ensue.

The acute systemic effects vary according to individual pesticides and are the determining factor of organophosphate poisoning.10 However, exposure to eye, skin or lungs causes topical reactions without systemic illness. In 1994, 1,332 possible pesticide poisoning cases reported had involved 48.9 % with only topical symptoms, 34.1% with systemic systems and topical systems, and 16.7% with only systemic symptoms.10 The initial symptoms that occur are a result of overstimulation of the receptors in the nervous system that interact with acetylcholine. The degree of the inhibition of the acetylcholinesterase is impossible to determine. Therefore, the rate of acetylcholinesterase inhibition is related to the onset of symptoms.10 The main symptoms of organophosphate poisoning include miosis, urination, diarrhoea, diaphoresis, lacrimation and excitation of the nervous system.10 Organophosphate poisoning cannot be treated by general physicians. Treatment from a specialist is required in which many patients are typically administered to hospitals for remediation.10 A patient exposed to organophophates is diagnosed with organophosphate poisoning once their blood test of red-cell and plasma cholinesterase is reported as being positive.10 The tests include observing the rate of regeneration of the acetylcholinesterase enzyme after treatment of the same blood sample with the cholinesterase antidote pralidoxime. The results are compared to a baseline value. Patients are treated with an antidote, typically atropine, which reverses the muscarinic symptoms caused by organophosphate poisoning. The prescription required depends on the amount of exposure. Pralidoxime is another antidote that remediates symptoms in exposed patients. Pralidoxime treats nicotinic and muscarinic symptoms. For patients with the most serious case of poisoning, oxygen ventilation, clearance of secretions and treatment with an antidote is required. For patients that don’t require hospitalization, washing of the skin is required and future exposure to pesticides is not recommended.10 Long-term neurological effects have been found in patients with high exposure to organophosphates over a long period of time. The fate of victims who were highly exposed to toxic organophosphates over a short period of time resulted in death.

Organophosphate poisonings that do not end in mortality have a likely chance of initiating long-term neurological and nervous system effects due to inhibition of the acetylcholinesterase enzyme resulting in over accumulation of acetylcholine. Multiple studies have been done on assessing the effects of organophosphate poisoning on neurological and nervous systems. One such report involved studying agricultural workers from Nicaragua who had high exposure to organophosphates between July 1, 1986 and July 1, 1988 and comparing the behaviour of their central nervous system to that of men who were not exposed to such organophosphates.4 The group that was deemed poisoned was exposed to pesticides two years prior to the study. The two groups participated in six subtests developed by the World Health Organization that is used as a neuropsychological test battery. The poisoned groups’ performance on five of the six tests was much worse. The two groups also completed additional tests that assessed verbal and visual attention, visual memory, visuomotor speed, problem solving, motor steadiness and dexterity. The poisoned group’s performance was unsatisfactory on three of the six tests. The study found that there was a decrease in neuropsychological performance amongst the group that was poisoned by organophosphates. From this study, it was observed that exposure to organophosphate pesticides resulted in a decline in neurological performance. Another neurological study was extended beyond underprivileged nations to that of a more regulated country. The results were strikingly similar.

The same results were found in a study that was done a few years later that involved determining the chronic neurological effects of organophosphate poisoning. The study was done on 128 men that were poisoned by organophosphate pesticides in California from 1982 to 1990. The men underwent neurological physical examination, five nerve conductions tests, two vibrotactile sensitivity test, ten neurobehavioral tests and one postural sway test. The study found that the poisoned men performed worst on two neurobehavioral tests. When the study focused on comparing men who were severely poisoned as opposed to the men who were less exposed, the more highly-exposed group showed worse vibrotactile sensitivity.12 Though the size of the study is limited, this study coupled with the previous study confirms the harmful effects organophosphate exposure has on human. They also reinforce that notion that exposure of organphosphate pesticides must be eliminated. Detection of organophosphate pesticides in the environment is critical in prevention against organophosphate poisoning.

In order to detect the presences of pesticides in the environment and then remediate areas that have been contaminated, analytical techniques are used to measure the concentration of the different types of organophosphates present.13 Environmental contamination caused by pesticides can be studied by applying analytical methods such as gas-chromatography used in conjuction with mass-spectrometry (GC/MS), high-pressure liquid chromatography (HPLC), liquid chromatography used in conjuction with mass spectrometry (LC/MS), ultraviolet-visibile absorption spectroscopy (UV-VIS), solid-phase extraction disks (SPE-disks) and solid-phase microextraction (SPME) followed by GC/MS, and optical sensor sol-gel techniques. 14 European Union regulations set a 0.5 microg/L limit for all pesticides present and 0.1 microg/L for individual compounds present. Therefore, detection limits of organophosphates in water samples must be below 0.1 microg/L.3 The presence of pesticides in food is monitored by multiresidue extraction methods (MRMs), with the most common method involving solvent extraction, using acetone or ethyl acetate, followed by gas chromatography (GC).15 GC-MS is successful in detecting the presence of pesticides because it allows for simultaneous confirmation and quantitation of a large number of pesticides. However, the limitation of using GC arises from the high limits of detection, which need to be decreased.15 One option in decreasing the limits of detection includes increasing the injection volume of the sample. Large column injection based on cold on-column and programmable temperature vaporizing injections that inject 1-2 microliters are typically used. One study included using a split/splitless injector that injects 10 microliters.15 This technique analyzed 110 pepper, cucumber and tomato samples and found organophosphate resides on 46% of the samples analyzed, with 23% of the samples exceeding the tolerance. GC-MS has also been used to determine the bi-products of organophosphate combustion. GC-MS has proven to more effective in confirming these products than the typical micro-scale experiments that are used for investigation.6

LC/MS is another technique used to determine trace levels of organophosphates.16 Direct-electron ionization is a relatively new interfacing device that combines a nano-HPLC column with a mass spectrometer that has electron ionization capability, which is expected to optimize the introduction of the liquid into the EI source and detect the presence of organophosphates at concentration levels of a few parts per billion (ppb).17 The direct liquid introduction previously used was limited in success due to clogging of the transport capillary, unstable source pressure and sample sputtering. It also allows micro- and nano-HPLC columns to perform well at very low flow rates. EI spectra give structural information that is easily interpreted and obtained. The coupling of HPLC with MS is a better alternative to GC/MS since organophosphates are thermally labile and chemically reactive which results in decomposition during analysis.18 Studies using this technique have identified several organophosphate pesticides in water samples below 0.5 ppm.17

Another technique used to determine organophosphates in environmental samples includes the use of sol-gel based mid-infrared sensors on aqueous solutions. This is a better alternative to gas or liquid chromatographic techniques. LC/GC techniques rely heavily on sensitivity. The concentration levels of organophosphates changes due to the changing environment. These techniques do not account for the changing conditions. A better detection technique is to use a monitoring device that measure the presence of the organophosphates while still in the environment, rather than in samples taken from contaminated sites. This type of technique would rely on developing biosensors to study the organophosphate interaction with surface-immobilized enzymes.19 One limitation in using biosensors is that they are difficult to purify and they have low stability. Optical sensors are better alternative such that they can have “intrinsic molecular specificity, robustness, remote sensing capability, and are absent of interferences such as electromagnetic fields or surface potential.”19 The sol-gel technique is a method used to develop ceramics by hydrolysis and polymerization of organic materials. This method would allow for preparation of analyte layers with large surfaces areas, as well as, advantageous optical, dielectric, thermal and acoustic properties that are stable under sever environmental conditions which have shown to detect organophosphates at low ppm concentrations.19 One study used this method to determine the presence of the following organophosphate pesticides: parathion, fenitrothion and paraoxon in aqueous solution. ZnSe ATR crystals were coated with a sol-gel film that which the oranophosphates interacted. The increased surface area and porosity of sol-gel optical sensors allowed for lower limits of detection of these organophosphate containing pesticides.5

The techniques described above are examples of different detection methods used to determine the presence of organophosphates in environmental samples. Organophosphate compounds have been the primary components in pesticides since the 1980’s and have shown to cause acute effects on the nervous system with high-levels of exposure.20 Corrective measures can then be taken to prevent harmful exposure to these pesticides. Currently, remedial solutions of organophosphates include avoiding use or limited use and chemical solutions. Current synthetic work involves developing catalytic mimics of the metalloenzyme, acetylcholinesterase; that can be used to increase the rate of hydrolysis of the organophosphate bond. However, extensive research on effects on the environment as well as human health needs to be considered when employing these methods. Considering the neurological and detrimental side-effects on exposure to organophosphate pesticides, it is clear that an alternative solution to the utilization of organophosphate pesticides must be found.

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