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# *Molecular Toxicology of Organophosphate Poisoning*

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***Abstract***

*The use of organophosphates (pesticides and other compounds to eradicate pests), currently, to increase the fulfillment of the population's consumption needs has a double-edged sword effect, on the one hand it can increase the need for food to be consumed by the population. The negative effect that can arise is the safety of organophosphate drugs which can contaminate the soil and water sources around the place where organophosphate drugs are used. The negative effects of organophosphates are associated with the effects of xenobiotics on humans who consume them. Xenobiotics are associated with toxicdynamic effects where organophosphates cause irreversible inhibition of the enzyme acetylcholinesterase (ACh). ACh is one of the main enzymes in the nervous system that terminates impulse conduction through the hydrolysis process of acetylcholine enzymes. Acetylcholinesterase is a specific molecular target of organophosphate pesticides. The inhibition of the Ach enzyme causes the inhibition of the acetylcholine enzyme which is normally always hydrolyzed by the Ach enzyme and is a specific biological marker of pesticide poisoning. Inhibition of ACh will cause the accumulation of the enzyme acetylcholine, resulting in negative effects of organophosphate poisoning which can lead to death. In this paper, the authors collect from various sources related to the study of molecular toxicology toxidynamic effects of drug safety and organophosphate poisoning. The results of this review article show that organophosphate poisoning is associated with irreversible inhibition of the acetylcholinesterase enzyme which results in death in the individual concerned.*

***Keywords:*** *Acetylcholinesterase, Drug Safety, Organophosphate, Toxicology, Xenobiotic*

***Review Article***

## Toksikologi Molekuler Keracunan Organofosfat

**Abstrak**

Penggunaan organofosfat (pestisida dan senyawa lain untuk membasmi hama), saat ini, untuk meningkatkan pemenuhan kebutuhan konsumsi penduduk menimbulkan efek pedang bermata dua, di satu sisi dapat meningkatkan kebutuhan pangan untuk dikonsumsi oleh penduduk. Efek negatif yang dapat timbul adalah keamanan obat organofosfat yang dapat mencemari keadaan tanah dan sumber air di sekeliling tempat penggunaan obat organofosfat. Efek negatif organofosfat dikaitkan dengan efek xenobiotik terhadap manusia yang mengkonsumsi. Xenobiotik berhubungan dengan efek toksikodinamik dimana organofosfat menyebabkan inhibisi ireversibel terhadap enzim

asetilkolinesterase (Ach). Ach merupakan salah satu enzim utama di dalam sistem persarafan yang bersifat mengakhiri hantaran impuls melalui proses katalisis hidrolisis enzim asetilkolin. Asetilkolinesterase merupakan target molecular spesifik pestisida organofosfat. Penghambatan enzim Ach menyebabkan inhibisi enzim asetilkolin yang secara normal selalu dihidrolisis oleh enzim Ach dan merupakan marker spesifik biologi pada keracunan penggunaan pestisida. Inhibisi Ach akan menyebabkan akumulasi enzim asetilkolin sehingga mengakibatkan timbulnya efek negatif keracunan organofosfat yang berujung kematian. Dalam penulisan ini, penulis melakukan pengumpulan dari berbagai sumber yang berhubungan dengan kajian toksikologi molekuler efek toksidinamik keamanan obat dan keracunan organofosfat. Hasil review article ini bahwa keracunan akibat organofosfat berhubungan dengan inhibisi ireversibel pada enzim asetilkolkinesterase yang mengakibatkan kematian pada individu yang bersangkutan.

**Kata Kunci:** Asetilkolinesterase, Keamanan Obat, Organofosfat, Toksikologi, Xenobiotik.

## PENDAHULUAN

Indonesia as an agricultural country in the world and a developing country, of course, tries to maintain the food consumption needs for its citizens. The occupation that is often associated with people living in Indonesia is farming. However, there are risks for farmers working in agricultural areas. The problem is related to increasing food production and how to deal with pests which are often done by farmers by providing materials known as organophosphates (pesticides and other substances). Organophosphates have two effects with the first effect being to increase agricultural production by repelling pests, but on the other hand it can cause toxicdynamic processes in farmers and relationships associated with agricultural areas (Lionetto *et al*., 2013; Ghorab and Khalil, 2015). The World Health Organization (WHO) noted that every year there is an increase in cases due to pesticide poisoning to farmers as subjects who work in agricultural areas with the death rate reaching tens of thousands of people. This problem often occurs in Indonesia, which is one of the developing countries (Fiananda, 2014). The purpose of this study is to explain the dangers of organophosphate poisoning as a pesticide commonly used in agricultural countries such as Indonesia.

Organophosphates or commonly called pesticides are chemical compounds that are widely used throughout the world. This compound was first introduced in 1854 with toxicity effects that were not known until 1931. The first organophosphate compound developed was the TEPP tethraethyl pyrophosphate compound which was made in Germany with the aim of making it a nicotine substrate. Over time, organophosphate compounds are associated with problems that can be considered a dual effect, both beneficial and negative. The first is to improve agricultural and household processes and eliminate all forms of plant pests whose presence can cause crop failure. In addition, the use of organophosphate compounds at home can repel all forms of pests that can interfere with household life, such as: repelling mosquitoes, cockroaches, and other insects. What is then obtained is the presence of organophosphate substances given to plants or fruits that can leave residues, whether these residues can be left in fruit material, leaves, or fall to the ground which can cause pollution problems related to the use of pesticides. (Ghorab and Khalil, 2013; Sinha and Sharma, 2003; Kaushal *et al*., 2020).

## METODE

This study is based on the point of view of the molecular literature on the molecular mechanisms of organophosphate poisoning and its negative effects on the human body. The review method used is by searching some literature based on journal materials from search engines in Pubmed, Google Scholar using the keywords: Acetylcholinesterase AND Organophosphate AND Toxicology AND Xenobiotics. After obtaining some of the journal articles, inclusion and exclusion criteria were carried out to determine which articles could be used as sources of literature review and carried out an in-depth exploration of the studies discussed in the journal manuscript.

Data extraction using extraction based on PRISMA Flowchart which is presented in Figure 1.

**Searching Strategy:**

A literature search was conducted from January to May 2020 to identify published studies of organophosphate, acetylcholinesterase, and organophosphate molecular toxicology related organophosphates and acetylcholinesterase. Authors managing medical references develop individual search strategies and take citations from ScienceDirect, PubMed, and Google Scholar. Controlled mix of words and vocabulary (Organophosphate AND Acethylcholinesterase AND Molecular toxicology of Organophosphate) were used.

Criteria: This literature review includes all studies that examine the relationship between organophosphates, acetylcholinesterase, and the molecular toxicology of organophosphates.

The following inclusion and exclusion criteria were used for literature selection:

**Inclusion Criteria**

The article should include a discussion of the relationship between organophosphates, acetylcholinesterase, and the relationship between them in molecular toxicological processes.

**Exclusion Criteria**

Exclusion criteria were carried out by removing all journals that had nothing to do with the keywords mentioned, journals that only contained abstracts, and journals that could not be accessed or downloaded.

**Data Extraction**

This article produces data covering the perspective of organophosphates as pesticide materials used by farmers, the relationship between organophosphates and the inactivation of acetylcholinesterase enzymes, and the molecular toxicological mechanism of organophosphates in inactivating acetylcholinesterase enzymes.

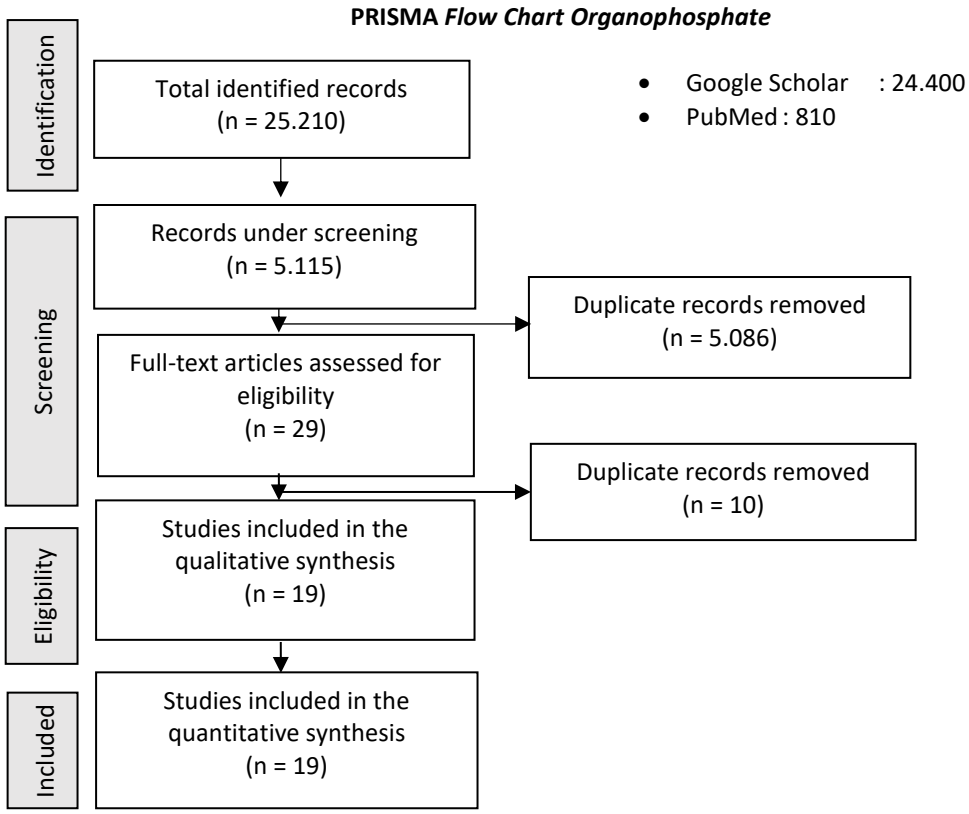


**Figure 1**. Flow diagram identification of organophosphate and acetylcholinesterase literature exploration.

The Optional Reporting Item Diagram for the four-phase Systematic Review and Meta-analysis (PRISMA) can specify the number of studies identified, screened, and included in the systematic review.

Based on the literature exploration of the point of view of the molecular literature on organophosphate poisoning, it is certain that organophosphates can cause two effects, either a positive effect or a negative effect. The first effect is to increase the cultivation of food demand, but the latter effect is dangerous if used incorrectly. The latter effect is related to the mechanism by which organophosphates can irreversibly inhibit the need for acetylcholinesterase to break down the enzyme acetylcholine.

## DISCUSSION

### Organophosphate

Before discussing the toxicological effects caused by organophosphates, we will first discuss the ingredients of organophosphates. The negative effects caused by organophosphates can be divided into two forms based on the intended target, namely: organophosphates that can be used to kill insects and organophosphates that can be used as potential materials for terrorist activities (neurotoxic substances made during the Second World War) due to side effects. The use of organophosphates is as an acetylcholine inhibitor that affects neuromuscular transmission. The use of organophosphates as a terrorist crime relates to the use of gases associated with the nervous system. Organophosphates used to eradicate insects based on structural characteristics are divided into 13 groups, namely: phosphate groups, phosphonates, phosphates, phosphorothiates, phosphonotiates, phosphorodithioates, phosphorotrithioates, phosphoroamidothioates. The components contained in organophosphates are esters, amides, or thiol phosphonic acid derivatives (Ghorab and Khalil, 2015; Kamanyire and Karalliedde, 2004; Paudyal, 2008; Mladenovic *et al*., 2020).

There are several groups of organophosphates, namely very toxic and moderately toxic groups. For highly toxic organophosphate groups, namely: azinphos-methyl, bomyl, carbophenothion, chlorfenvinphos, chlormephos, chlorthiophos, coumaphos, cyanofenphos, demetone, dialifor, dicrotophos, dimephos, diaphath, sulfur, endulfon, fenamiothions, phenthophosphos. , isofenphos, mephosfolan, methamidophos, methidathion, parathion methyl, mevinphos, and others. As for the organophosphate group with moderately toxic groups, namely acephate, bromophos-ethyl benzulidem, bromophos, chlorphoxim, chlorpyrifos, crotoxyphos, crufomate, cyanophos, cythioate, DEF, demteon-S-methyl, diazinon, dichlofenthion, dichlorvosethion, EPi ethoprop, etrimfos, and others.

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**Organophosphates as Xenobiotics and Toxicokinetic-Toxicodynamic Effects**

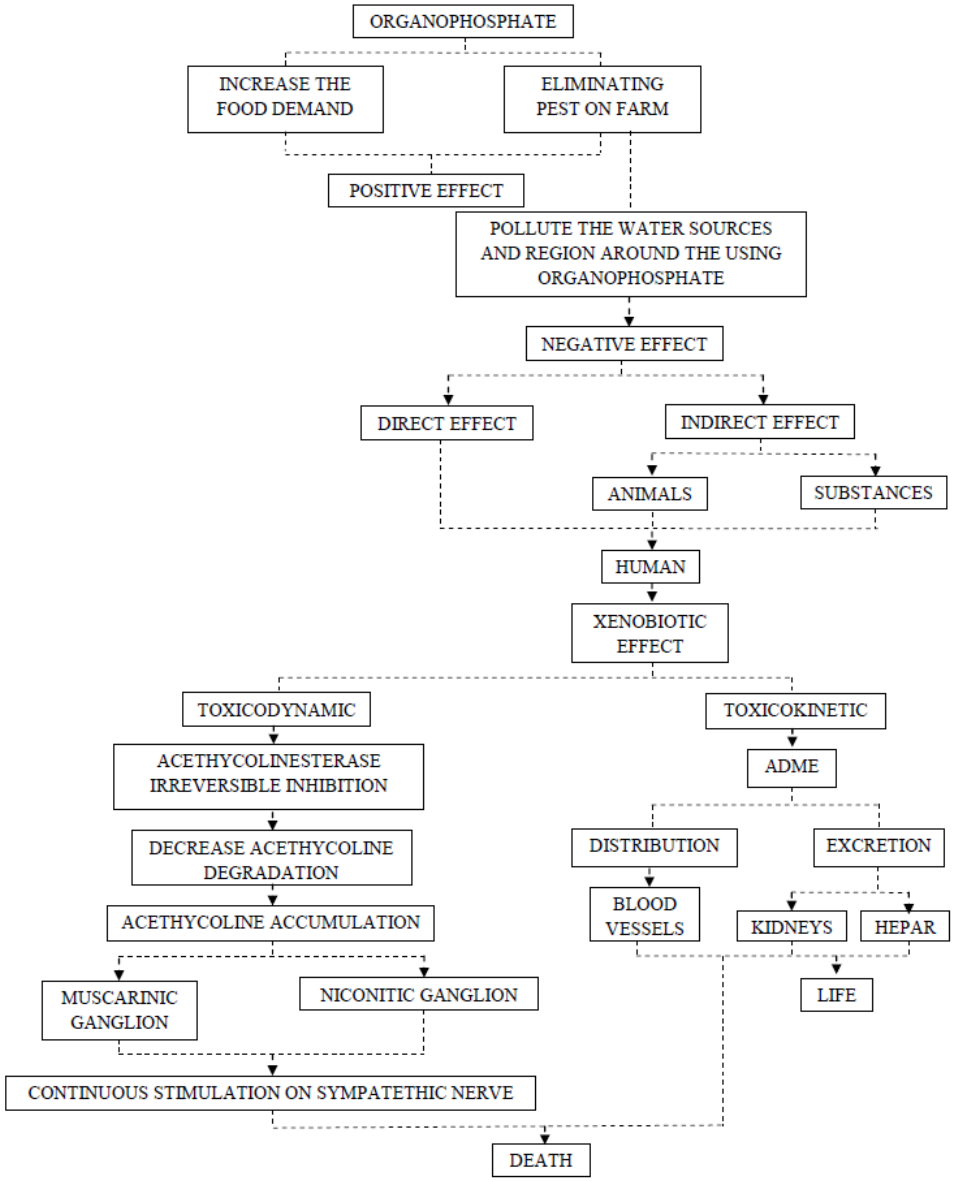


**Figure 2.** Conceptual framework of the process of organophosphate poisoning.

The conceptual framework for organophosphate poisoning is described in Figure 2. It explains the positive and negative effects of using organophosphates. The first is related to increasing the demand for food and eliminating pests in agriculture. There are models that explain how organophosphates can enter from spraying agricultural products (both fruits, vegetables, etc.) which then particles can enter the soil and about worms that live in the soil (because they fall from the surface of fruits, vegetables, etc.), eaten by herbivores, entering the water which then pollutes the water which then becomes a habitat for fish and is a source of drinking water, or about insects which are then eaten by birds, mammals and others, with the last one being humans who consume all the products mentioned previously so that there is a buildup of organophosphate materials, which are often called xenobiotics (Vermeire *et al*., 2010; Perwitasari *et al*., 2017). The latter is often associated with the effects of xenobiotics which are usually not found in the

human body so that when the body is exposed to xenobiotic substances, the body will try to excrete xenobiotic substances. The processes associated with the elimination process consist of: absorption, distribution, metabolism, and excretion (ADME) with the most important thing here is that xenobiotic materials can enter because of the transport process in the body which consists of two, namely: convection (transport of xenobiotics along with flow). blood) and diffusion (transport of xenobiotics across biological membranes). Systemic circulation still plays an important role in the xenobiotic transport process between organs and body tissues, so the rate of blood circulation in organs and tissues will determine the speed of xenobiotic distribution in the body. Xenobiotic organophosphates, in this case, are a group of xenobiotics that are easily soluble in fat, with fat being one of the largest components in composing cell membranes and walls so that xenobiotic organophosphates can be easily absorbed and entered into cells. In addition to the transport process, there are also metabolic and excretory processes associated with the xenobiotic elimination process. Elimination of xenobiotics occurs through a biotransformation process, with the biotransformation process occurring mostly in the liver and a small portion in the kidneys, skin, blood, and others. Biotransformation is divided into two parts, namely: phase I (functionalization reaction) and phase II (conjugation reaction) (Wirasuta, 2008; Hidayatullah *et al*., 2020).

**Organophosphate mechanism against acetylcholinesterase enzyme inactivation**

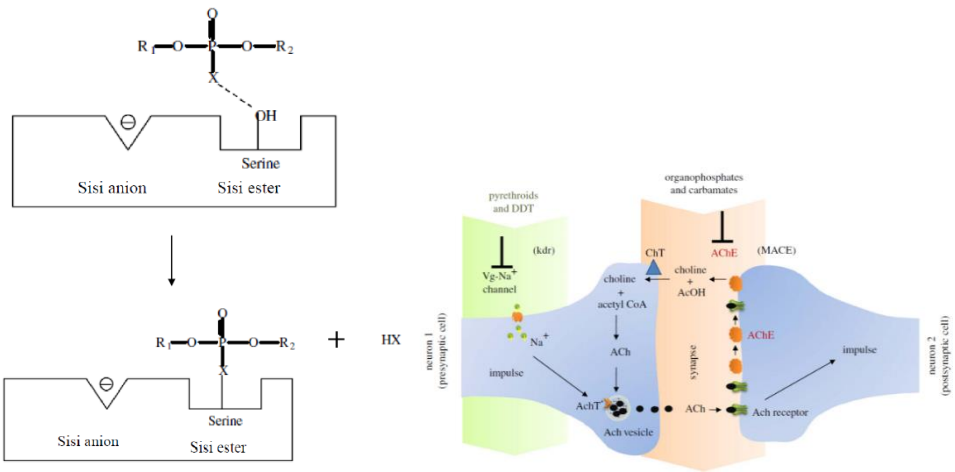


**Figure 3**. The process of inactivation and location of acetylcholinesterase by organophosphates in presynaptic and postsynaptic cells. (Fiananda, 2014; David et al., 2013: 368)

Figure 3 describes the organophosphate mechanism of acethycolinestherase inactivation by blocking the acetylcholine cleavage enzyme, which is an irreversible acetylcholinesterase block at pre and post-synaptic (free nerve endings) nerve endings and this mechanism of action is considered to be the main effect of the organophosphate component which is often associated with disease or side effects. which can be caused by organophosphates. Acetylcholinesterase is located in the central nervous system (CNS) in the gray matter, sympathetic ganglion, myoneural junction, and erythrocytes (Sinha and Sharma, 2003; Kamanyire and Karalliedde, 2004; David *et al*, 2013; Eddleston, 2019).

Organophosphates can also regulate nicotinic and muscarinic acetylcholine receptors, as well as adrenergic receptors caused by acetylcholinesterase inhibition which can also trigger damage to the central nervous system and peripheral nervous system and are associated with the somatic nervous system that innervates skeletal muscles and the sympathetic and parasympathetic nervous systems in the nervous system. autonomous. Muscarinic receptors for acetylcholine are found predominantly in the bronchi, smooth muscle, heart, exocrine glands, and pupils whereas nicotinic acetylcholine receptors are found in motor nerve endings of skeletal muscle and autonomic ganglia. Phosphorylation of the acetylcholinesterase

enzyme causes accumulation or accumulation of acetylcholine in cholinergic synapses and overstimulation of the cholinergic nervous system pathways with manifestations, namely hypersalivation, lacrimation, vomiting, urination, diarrhea, and miosis (the most easily observed observation) which is in accordance with the presence of muscarinic receptors and nicotinic acetylcholine. The pharmacodynamic effects of organophosphates on the central nervous system (CNS) are anxiety, dizziness, confusion, ataxia, and convulsions. The most important consequence of inhibition of the enzyme acetylcholinesterase is depression or suppression of the function of the cardio-pulmonary system. Death can occur due to inhibition of the enzyme acetylcholinesterase associated with respiratory system failure caused by a combination of central and peripheral effects, particularly those associated with broncho-alveoar constriction, increased bronchiolar secretion, bronchiolar paralysis, paralysis of the respiratory muscles, and shutdown of the respiratory control center. in the brain. The part of the brain that is the most significant influence is on the neo-cerebellum, thalamic nucleus, and cortex caused by the accumulation of esterase, resulting in a condition called Neurotoxic esterase (NTE) (Paudyal, 2008; Pittmann, 2007; Hung *et al*., 2015; Dhamayanti and Shaftarina; Lenina *et al*., 2020).

Structurally, the acetylcholinesterase enzyme consists of two active sites, namely: an esteratic site and an anionic site, with acetylcholinesterase using both active sites to capture and hydrolyze acetylcholine, thereby releasing choline. Antiacetylcholinesterase can be associated with and affect the hydrolysis of acetylcholine on the esteratic attachment and this applies to the overall action of the organophosphate, but also depends on the electrophilicity of the inhibitor (transesterification) of serine hydroxyl by the phosphate atom which depends on the electrophilic nature of the enzyme and inhibitor (Paudyal, 2008).

**Toxicology effect of organophosphate**

One of the things related to the toxicological effects of organophosphates is the ability to metabolize organophosphates, considering that organophosphates are easily absorbed through the skin, lungs, mucous membranes, conjunctiva, and gastrointestinal tract so that the severity of symptoms or symptoms of organophosphate effects depends on the components of the organophosphate material, dose, route, frequency, and length or duration of exposure. (Sinha & Sharma, 2003) on the components of organophosphate ingredients, doses, routes, routes, digestive tract, and digestive tract. frequency, and duration or duration of exposure. This is due to the very high solubility of organophosphates in fat, and even organophosphates were found to inhibit the blood brain barrier (Blood Brain Barrier) (Kamanyire and Karalliedde, 2004; Paudyal, 2008; Eddleston, 2019).

Effects that occur after inhibition of acetylcholinesterase can occur in two possible reactions, namely: 1. Spontaneous reactivation of acetylcholinesterase occurs which takes hours or days depending on the type of organophosphate used. Spontaneous reactivation occurs within a half-life of 0.7 hours for dimethyl substances and 31 hours for diethyl substances. Spontaneous reactivation can also be accelerated by administration of nucleophilic reagents as antidotes or antitoxins, for example, oximes. 2. Due to the effect of exposure to acetylcholinesterase with organophosphates, the acetylcholinesterase enzyme complex loses one alkyl group, resulting in a longer response to reactivation and is associated with the aging process. The reactivation process in the second possibility is influenced by: pH, temperature, type of OP component (dimethyl OP group has a half-life of 3.7 hours while diethyl OP has a half-life of 33 hours). The second possibility relates to the usefulness of oximes before 12 hours after poisoning by organophosphates (Paudyal, 2008; Lionetto *et al*., 2013).

In addition to some of the effects mentioned above, there are other organophosphate effects, namely: mutagenic and carcinogenic effects, effects on reproductive organs, effects on immune cells resulting in toxicity to the immune system, cytogenic effects, immune suppression in the immune system and the onset of cancer, cancer teratogenic effects, determinant effects, and effects on lipid metabolism (Ghorab and Khalil, 2015; Cedergreen *et al*., 2017; Vicou *et al*., 2009; Mladenovic *et al*., 2020).

## CONCLUSION

Organophosphate poisoning is related to irreversible inhibition of the enzyme acetylcholinesterase which results in death in the individual concerned.

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