A DISSERTATION ON

RECENT ADVANCES AND EXPERIMENTAL INVESTIGATIONS OF PROFENOFOS: AN ORGANOPHOSPHOROUS INSECTIDE

SUBMITTED TO THE

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BY

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DECLARATION

I hereby declare that the project entitled "**Recent Advances andExperimental Investigations of Profenofos: An Organophosphorous Insectide**" submitted in partial fulfilment for the award of thedegree of Bachelor of Technology in Food Technology iscompleted under the supervisionof**Dr. Meenakshi Tripathi**&**Mr. Kunal Prakash** is an authentic work.

Further, I declare that I have not submitted this work for the award of any other degree, elsewhere.

Signature and Name of the Student with date

It is certified that the above statement made by the students is correct to the best of myknowledge.

(Dr. Abdul Rehman)

Dean

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This is to certify that **Mr. Vikas Saini** student of M.Sc. Biotechnology (IV Semester), Integral University has completed his four months dissertation work entitled "**Recent Advances and Experimental Investigations of Profenofos: An Organophosphorus Insecticide**" successfully. He has completed this work from 17th Jan – 02nd June 2022 under the guidance of **Dr. Meenakshi Tripathi.** The dissertation was a compulsory part of his M.Sc. degree.

I wish him good luck and bright future.

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This is to certify that **Vikas Saini,** S/o Ishwar Datt from, **Intergral University** MSc in Biotechnology has successfully completed his dissertation in **"Recent advances and experimental investigations of profenofos: An Organophorous Insectide."** from 17th Jan-2022 to 02 June 2022 at **FARE Labs Pvt. Ltd.** and has been awarded excellent grade on the basis of his performance.

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Vikas Saini

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Abstract:

Pesticide play an important role in protecting various crops. Among various sets of the pesticide used worldwide, organophosphate are the most wide spread group. Profenofos [O-(4-bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate] is one of most commonly used organophosphate insecticides on crops, vegetables and orchards. The world health organisation classifies the compound as moderately hazardous (Toxicity Class II), and their residues have been found in vegetable like okra[*Abelmoschus esculentus* (L.) Moench], gooseberries (*Ribes* sp.), green chilies [*Capsicum frutescens* (L.)], curry leaves [*Murraya koenigii* (L.) Spreng], mint leaves [*Mentha piperita* (L.)], and coriander leaves [*Coriandrum sativum* (L.)]. Ingestion of profenofos (PFF) is the main exposure way for man.When PFF applied to agricultural fields, its residue spread in every part of the environment; ambient air, surface water, and soil. In this report we discuss the global use of PFF pesticide, and evaluate the homogeneity of the market sample and analyse the results using GC-FID. And, check the literature of their toxic effect on humans and other living organism on the environment and the biodegradation of this chemical by various microbial strain. To date, no complete biodegradation pathway has been established for PFF pesticide, calling for a study of this nature.

1. Introduction

Pesticide are important components of modern agriculture.Organ phosphorus pesticides (OPPs) from the most widespread group of all currently used pesticides around the world.Organophosphates (OPs) are precursors of many insecticides, herbicides, and nerve agents and are esters of phosphoric acid, thiophosphoric acid, and other phosphoric acids.[16a]The chemicals introduce to replace unruly and hazardous chlorinated pesticide, [9b]gaining popularity first in the United States[7a]and then worldwide. This class of pesticide, Profenofos[O-(4-bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate] one of the most used organophosphate insecticide on fields, vegetables, and fruit crop.This pesticide is moderately hazardous (Toxicity Class II;)[60a]and its residue have been found in vegetable such as okra, gooseberries, green chili, curry leaves, mint leaves, and coriander leaves.Ingestion of profenofos (PFF) is the primary exposure way for humans.[23b]When PFF is applied to agricultural fields, its residues spread into every part of the environment: ambient air, surface water, and soil.[4b, 26]The extensive use of OPPs has created a multitude of environmental and health issues due to their toxicity.

A variety of traditional procedures for removing or degrading OPPs, including as chemical treatment, cremation, and landfills, were later shown to be hazardous due to the potential of secondary exposure. Furthermore, such procedures are prohibitively expensive.As a result, there is a growing demand for bioremediation (microbial degradation), bio-mineralization, immobilised OP-degrading enzymes, adsorption, biosorption, and biotechnological tools to develop safe, convenient, eco-friendly, and environmentally practicable ways for OPP degradation.The most promising bioremediation strategy has proven to be microbial decomposition. We have studied the global use of PFF and other OPPs through the existing literature, their toxicity to humans and other living species in the environment, and their biodegradation by diverse bacterial and fungal strains in this work.

2. Origin of Profenofos

The first OP cholinesterase inhibitor, tetraethyl pyrophosphate, was produced in 1854.[55b]During world-war II, German businesses began producing thousands of OPs, and American industries began manufacturing agricultural OP insecticides as a result.In the United States, Profenofos was first registered in 1982[58d]to combat pests that had developed resistance to chlorpyrifos and other OPs.[23a]

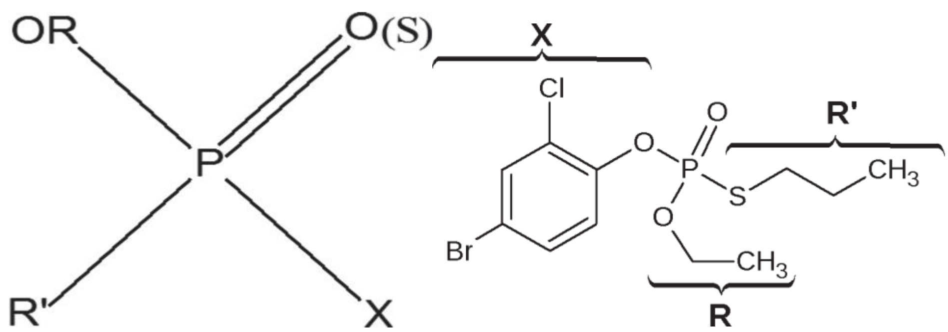

Fig. 1. (**a**)Basic structure of organophosphorus pesticide. (**b**) Structure of Profenofos.

3. Structure, Functions, and Usage of Profenofos

The basic structure of OPP, R is either a methyl or ethyl group, and R/ can be a methoxy, ethoxy, ethyl, phenyl, amino, substituted amino, or alkylthio group; X is a suitable leaving group; and double-bonded oxygen can be replaced by sulphur to form various structures.Because multiple functional groups may be incorporated into the OPP moiety, a variety of structures with diverse functions could be created.In the case of PFF, R is an ethyl group, R/ is a thio- propyl group, and X is an O-(4-bromo-2-chlorophenyl) (Fig. 1)

In 2001, OPPs were employed in the United States in an estimated 33 million kg (73 million lbs.) (70 percent of all insecticides).This is a stunning figure, although it should be recalled that between 1980 and 2001, the use of OPPs fell by 45 percent.[59b]OPPs are utilized as defoliants, fire retardants, solvents, and plasticizers in addition to being insecticides.[24]

Profenofos is a pesticide that is used to control cotton bollworms (*Helicoverpa zea*), tobacco budworms (*Heliothis virescens F.*), spider mites (*Tetranychus urticae*), nut-infesting eriophyid mites (Aceria guerreronis), armyworms (Mythimna unipuncta), whiteflies (Trialeurodesvaporariorum), cotton aphid (Aphis gossypii), plant bugs (Lygus lineolaris), and fleahoppers (Halticus bractatus).[37a58e53a]Every year, around 352,000 kg (775,000 lbs.) of active ingredient is applied to cotton, with about 85% of it being utilised to control Lepidopteran species.

Profenofos is widely used in many countries, including the United States, China, Thailand, Vietnam, Pakistan, Australia, Japan, Egypt, Brazil, Australia, and India[37a, 51a, 56b]to control pests in cotton [Gossypium hirsutum (L.)],coconut [Cocos nucifera (L.)], green chili, fruit, gooseberries, tomato [Solanum lycopersicum (L.)], spring onion, okra, curry leaves, mint leaves, coriander leaves, and in paddy and vegetable cultivation.[37a,57a, 60a, 27b,46b, 53a, 16b, 32b] Africa is a major user of OPPs, including (which has used these PFF; Egypt pesticides for more than 35 yr), Mali, Benin, Senegal, Ghana, Ethiopia, and South Africa (Fig. 3) (Dogheim et al.,fruits, and cotton [14abc40b38 48] use PFF as foliar spray on various vegetables,, fruits, and cotton. [14c 13a 18a3854c]

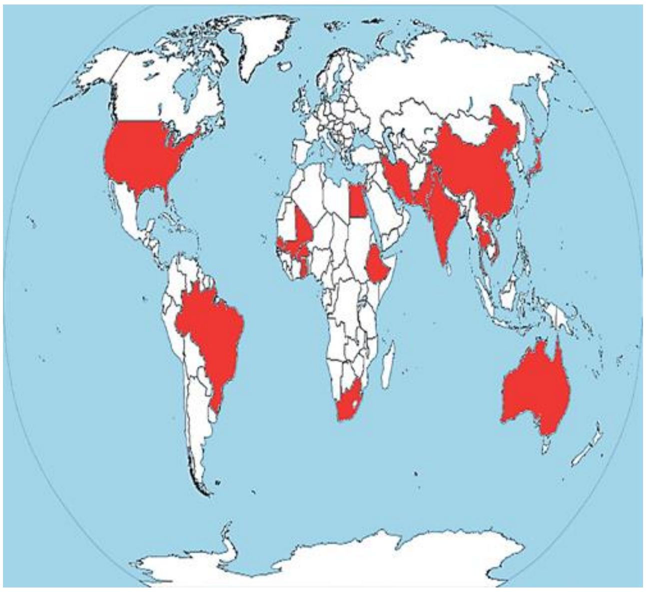

Fig. 2. Usage of profenofos in different parts of the world (marked in red).

Approximately 40% of Egypt's workforce is working in agriculture, the country's major industry, and has been exposed to OP insecticides such as chlorpyrifos and PFF, which are commonly used in agricultural fields, notably cotton fields.[2a]Farmers in numerous African nations (such as Benin, Senegal, Ghana, and Ethiopia) employ Fenom C (Syngenta Crop Protection AG), a combination of PFF and cypermethrin, to cultivate cotton, vegetables, pineapple [Ananas comosus (L.) Merr.], cowpea [Vigna unguiculata(L.) Walp.], and mixed cereals.[48]PFF is available in India in about 50 percent EC (emulsifiable concentrate) formulation. PFF residues are rarely discovered in market samples of crops and vegetables due to its widespread use in both open field and poly house environments.[4b, 26, 32b]

PFF is selectively harmful to insects over mammals due to the distinct metabolism of the propylthiol group.[35a]Even at low concentrations, it is highly persistent and poisonous.[62c]Unlike most OP insecticides, which need bioactivation of the oxon metabolite, the PFF parent form is a potent acetylcholine esterase (AChE) inhibitor.[50b, 45b]

4. Distribution of Profenofos in the Environment

Pesticides are widely used, resulting in their spread into the surrounding environment (water, soil, and/or air), particularly in areas near agricultural land. Parent chemicals and their residues provide a significant concern that must be addressed.Monitoring the quantities of pesticides in these medium will aid us in determining ambient pesticide levels and assessing potential concerns. Various investigations on the distribution and toxicity of OPP in various environmental compartments have been carried out all over the world.Pesticide residues were found in soil and water collected from four cotton-growing sites in.[13a]They discovered that *p,p*-DDT and its breakdown products, endosulfan I and II, endosulfan sulphate, and PFF were present in 77 percent of soil samples.According to L. Charerntanyarak and co-workers[26],pesticide dispersion in diverse environmental media in Thailand varies depending on the season, and the levels of several OPPs, including PFF, are higher in soil than in water and air. They discovered that the average value of PFF in soil was 41.8080 mg kg-1 in summer and 16.5956 mg kg-1 in winter[26], whereas the average value of PFF in water and air was 0.9520 mg L-1 and 0.0347 mg m-3 in summer and 0.3186 mg L-1 and 0.0020 mg m-3 in winter.[26] Pesticide residues were found in water, sediments, and muscle tissues of Cyprinus carpio taken from multiple sampling sites on the river Ravi at Balloki Headworks by.[39a]The most prevalent pesticides discovered in the fish tissue were endosulfan (13.62 1.50 mg kg-1) and PFF (13.72 0.86 mg kg-1), whereas PFF (1.40 0.15 ng L-1) and cypermethrin (1.41 0.05 ng L-1) were the most abundant pesticides found in the river water samples.Del Prado-Lu (2015) reported that different pesticide residues (triazophos, chlorpyrifos, cypermethrin, and malathion), including PFF, were found in about 42 percent of total soil samples collected from eggplant (Solanum melongena) farms in Santa Maria, Pangasinan, but the pesticide concentration was negligible in water samples collected from this farm.Cypermethrin, Acetamiprid, and endosulfan, coupled with PFF, were used to cultivate cotton in Burkina Faso, West Africa. In Burkina Faso, West Africa. In a traditional field, they ranged from 10 to 30 mg kg^{-1}, but in a new cotton zone, they ranged from 10 to 80 mg kg^{-1}. In soil samples collected from the new cotton zone, the level of PFF residue was somewhat higher (0.01–0.08 mg kg-1) than that of other pesticide residues.[46a]

5. Mechanism of Action

The enzyme AChE is found in all vertebrates and invertebrates, including mammals, in their central and peripheral nervous systems. It catalyzes the hydrolysis of the neurotransmitter acetylcholine (ACh) into acetic acid and choline, which modulates neuronal transmission.[31b, 53b,41b,46c,22a,11a]This step is critical for preventing tetany or hyper-regulation of the neuron caused by continuous stimulation of the post-synaptic neuron. ACh moves from vesicles in the pre-synaptic neuron to the synapse junction when a nerve impulse is passed from one neuron to another.It binds to post-synaptic neuron receptors and stimulates the next nerve fibre from there.

As shown in figure 3, the electrostatic attraction between an anionic site (the negative charge of the anionic site is due to the carboxylate anion of aspartic or glutamic acid) on the enzyme AChE and the positively charged nitrogen atom of the ACh results in the formation of an enzyme–substrate complex. The basic imidazole moiety of histidine then catalyzes acetylation of a serine hydroxyl (OH) in the ester site of the enzyme AChE, which interacts with the carboxyl group of glutamate (serine, histidine, and glutamate are part of a catalytic triad of the ester site of the enzyme AChE), resulting in an acetylated enzyme (Fig. 4B). Deacetylation occurs next, and the free enzyme, together with choline and acetic acid, is released as an end product (Fig. 4B). PFF inhibits AChE in a manner similar to the acetylation process described previously.

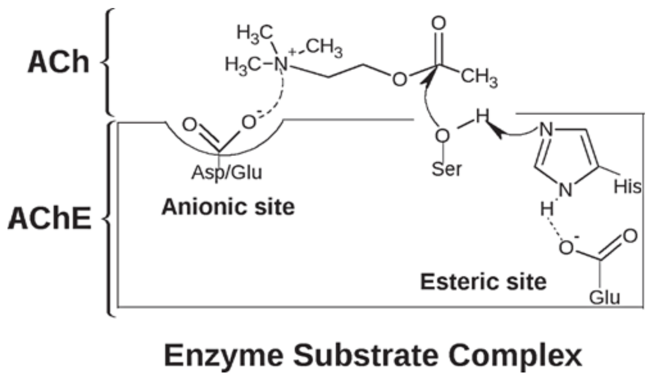

Fig. 3. Formation of enzyme substrate complex by acetylcholine and acetylcholinesterase enzyme. ACh, acetylcholine; AChE, acetylcholin- esterase; Asp, aspartate; Glu, glutamate; His, histidine; Ser, serine.

The phosphorylated serine hydroxyl moiety at the enzyme active site is more persistent than the acetylated one, and so does not get dephosphorylated (Fig. 5).As a result of the phosphorylation, the enzyme becomes non-functional, causing ACh to accumulate at the synapse junction, resulting in neuronal dysfunction.[19b, 8]

6. Toxicity and Health Effects

The main worry with these OPPs is that, with the right conditions, they can survive in the environment for a long time.[51b]As a result, widespread usage of this family of pesticides raises the potential of harmful effects on domestic animals, wildlife, and humans.[49d]Since the 1930s, when the pesticides were first developed for use as chemical warfare agents, OPP toxicity has been recognized.[42b]

Increased use of OPPs has had a long-term impact on society in agrarian countries such as India.Many OPPs (methyl parathion, chlorpyriphos, malathion, and others) are used to treat vegetables grown in different seasons (summer, rainy season, and winter), the residues of which can be found in many vegetables.[54a] Pesticide residues in soil pose a variety of direct and indirect health risks[5a] and are a major cause of morbidity and mortality in many developing countries.[49b]OP insecticides such as diazinon and malathion have a negative impact on the early in vitro development of mammalian oocytes by altering the expression of gene-encoding proteins involved in various cell processes.[6]Both of these OPPs are found in commercial formulations and have been shown to interfere with in vitro fertilisation and embryo development.[14d]Pesticide residues were found in various wildlife species in South Africa, including birds such as Cape vultures (*Gyps coprotheres*), African white-backed vultures (*Gyps africanus*), and bateleur eagles (*Terathopius ecaudatus*), and their populations were declining.[7b]

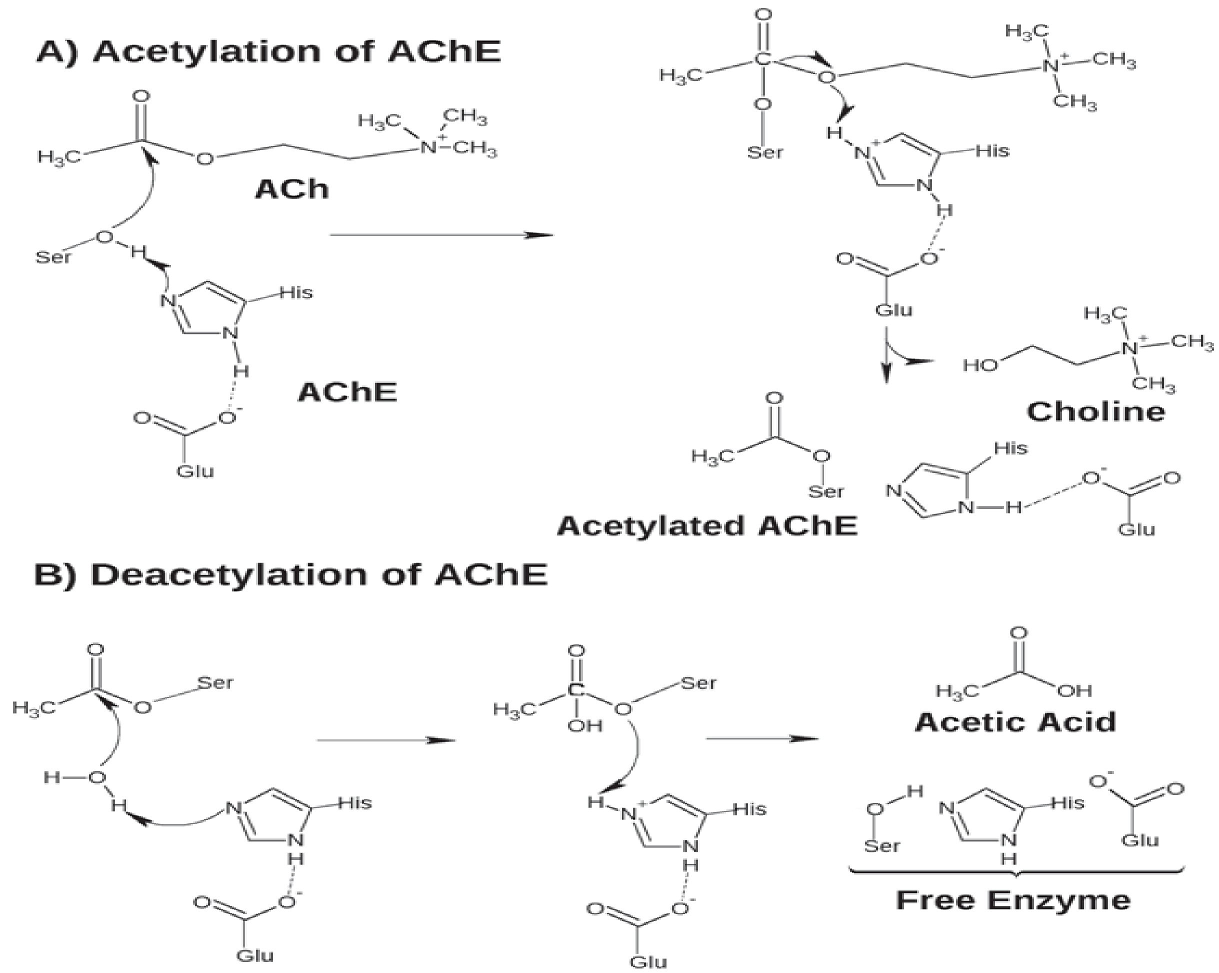

Fig. 4. (**a**) Acetylation of acetylcholinesterase enzyme. (**b**) Deacetylation of acetylcholinesterase enzyme. ACh, acetylcholine; AChE, acetylcholines- terase; Asp, aspartate; Glu, glutamate; His, histidine; Ser, serine.

Exposure to organophosphorus pesticides is also harmful to the human reproductive system, resulting in low sperm concentration. Furthermore, D.S. Rohlman and workers[8]discovered lower neurobehavioral performance, as well as learning deficits, in children of agricultural and non-agricultural parents in the United States. This has recently become a major concern because OPPs are being used in conjunction with pyrethroid (PYR) to increase the latter's toxicity to target insects, particularly those that have developed resistance to PYR.[49b]Many Class Ia (Extremely Hazardous, lethal doses (LD50) for the rat: oral = 5 and dermal = 50 mg kg-1 body weight) and Class Ib (Highly Hazardous, LD50 for the rat: oral = 5–50 and dermal = 50–200 mg kg-1 body weight) and Class Ic (Highly Hazardous, LD50 for the rat: oral = 5–50 and dermal = 50–200 mg kg-1 body weight) and Class OPPs (e.g., methyl parathion, parathion, triazophos, methamidofos, monocrotofos, demeton-S-methyl, terbufos, phorate, and dichlorvos) are widely used in agriculture and in the home.[60a]As a result, they induce a wide range of toxicity in all biosphere constituents. The use of these Class I insecticides has been phased out in many parts of the United States and the world due to their high level of toxicity (e.g., methamidophos in 2009, all products containing methyl parathion and their domestic use in 2010, and in 2013, respectively).[59d]

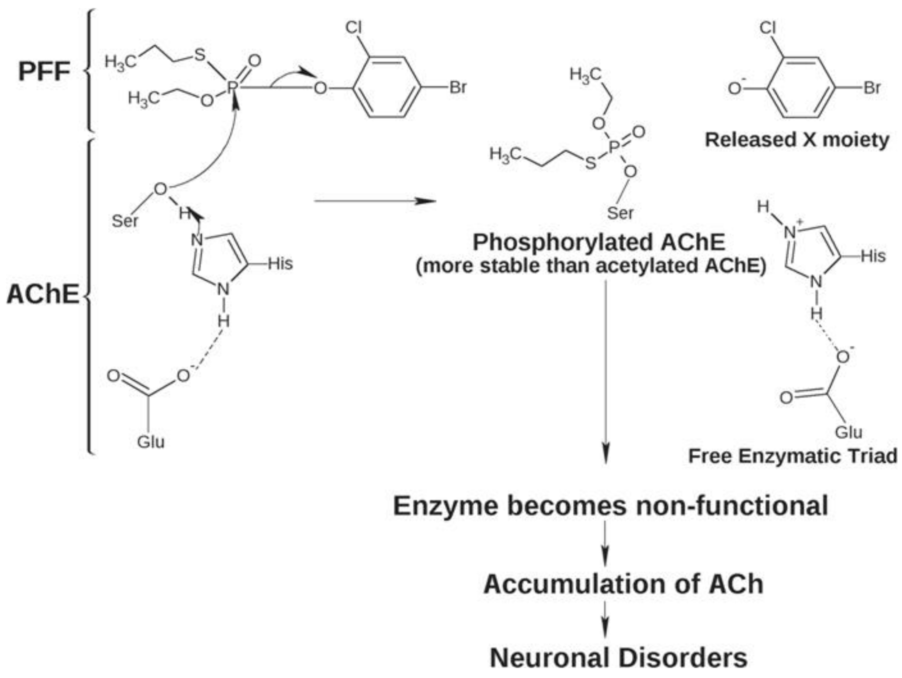

Fig. 5. Inhibition of acetylcholinesterase by profenofos (PFF). ACh, acetylcholine; AChE, acetylcholinesterase; X denotes the leaving group of PFF; Glu, glutamate; His, histidine; Ser, serine

Pesticides, in addition to their environmental toxicity, provide a significant societal concern because they account for one-third of all suicides worldwide.[23c, 15b]In Sri Lanka, the suicide rate due to pesticide self-poisoning was cut in half with the prohibition of Class I pesticides and endosulfan.[15a]

In tandem to Class I pesticides, another class of pesticides (Class II) with mild toxicity was introduced to the market due to increasing demand in the agricultural sector.[60a]Since then, dimethoate, diazinon, acephate, chlorpyrifos, fenthion, fenitrothion, phosmet, PFF, and quinalphos have all become commonplace. Following that, in June 2000, the USEPA issued a revised risk assessment and announced an agreement to phase out and eliminate specific chlorpyrifos applications.[58b]It also put limitations on diazinon in the US for a variety of purposes, including indoors and on all lawns, gardens, and turfs.[58c, 59a] In 2012, Acephate was outlawed.[59d]OPPs make up the majority of Class I and Class II pesticides, followed by carbamates, pyrethroids, organochlorines, and a coumarin derivative[60a] , demonstrating their high toxicity when compared to other pesticide classes.

Pesticide toxicity is determined not only by the pesticide's use, but also by its half-life in the environment. The half-life of a pesticide indicates whether or not it will accumulate in nature. Pesticides with shorter half-lives tend to accumulate less and remain in the environment for a shorter period of time. Pesticides with a longer half-life are more likely to accumulate after multiple applications, increasing the risk of contamination of nearby surface water, groundwater, plants, and animals.[25b]The half-life value varies greatly depending on environmental factors such as soil quality, aqueous medium, medium pH, and other environmental factors (TOXNET, 2014).For pH values of 5, 7, and 9, half-life values for PFF were 104 to 108 days, 24 to 62 days, and 0.33 days, respectively (TOXNET, 2002).

Many insects and pests acquire resistance to pesticides as a result of widespread use.[3,42b, 1b, 1c]OPP resistance was first detected 14 years after they were introduced.After 5 years of application, carbamate pesticide resistance developed, possibly due to conditioning from prior OPP exposure.[20a]The first European case of sheep scab mite (Psoroptesovis) resistance to an OPP (propetamphos) was reported in November 1996.[18b, 58a]found that the dusky cotton bug (Oxycarenus hyalinipennis) was resistant to a variety of OPPs (PFF, triazophos), as well as new chemistries (emamectin benzoate, spinosad, chlorfenapyr, imidacloprid, nitenpyram) and PYR insecticides (bifenthrin, deltamethrin, and lambda-cyhalothrin).Profenofos resistance in tobacco budworm (Heliothis virescens F.) and onion thrips (Thrips tabaci) has also been reported in Iran.[2, 70a, 44b]Farmers are forced to increase pesticide use depending on the insect target and insect population resistance to many OPPs. For example, when resistance to PFF at a lower dose developed, the LD50 value of PFF was increased from 50.49 to 176.03 mg mL^{-1}, and the resistance ratio increased from 29.70 to 103.55 for Musca domestica (Diptera: Muscidae) in dairy farms in Pakistan.[33a] Figure 7 depicts the overall toxic and health effects of PFF.

Profenofos residues in soil pose a number of environmental concerns, including negative effects on the following crop and migration to groundwater.[27b, 4c]As a result, humans are unavoidably exposed to pesticides and their residues.PFF and its metabolite 4-bromo-2-chlorophenol (BCP) were found in human plasma and urine in a study conducted by used lymphocytes from healthy human donors' peripheral blood samples to determine an in vitro toxicity profile of PFF.[23a, 50c]Chromosomal analyses of metaphase plates from samples treated with sub-lethal doses of PFF revealed satellite associations as well as chromatid breaks and gaps, indicating that PFF had an effect on chromosomes. The findings were supported further by a comet assay, which revealed that comet tail lengths had been reduced due to the breakdown of one of the DNA strands.The researchers also used the DNA diffusion assay to examine the intensity of apoptosis and necrosis caused by PFF and other OPs in cultured human peripheral blood lymphocytes under in vitro conditions.[50a]

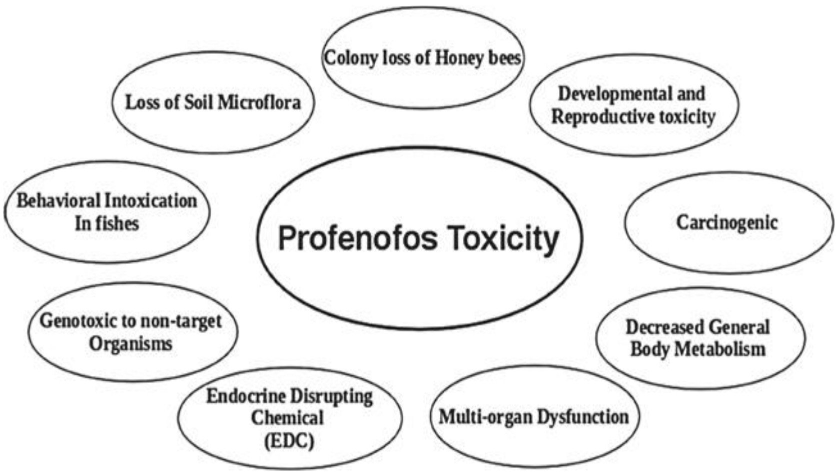

Fig. 6. Toxicity of Profenofos in different living organisms.

Profenofos has been shown to inhibit blood cholinesterase activity in rats.[40c]Profenofos is oxidatively bioactivated by both house fly (Musca domestica) and mouse liver abdomen microsomes to produce more potent AChE inhibitors (i.e., desthiopropylprofenofos and hydroxyprofenofos).[60b, 1a]In another study, the histopathological effects of PFF on the liver of Wistar albino rats were determined, and the results indicated that PFF was harmful even at a sublethal concentration of 1/10th of LD50 (330 mg kg body weight).Blood congestion in the central vein, damaged blood sinusoids, degenerate hepatocytes, periportal inflammation, pyknotic nuclei, and cell binucleation were discovered.[51c]

Fish and macroinvertebrates are acutely toxic to profenofos.[3a, 59c, 54b]In one of India's and Pakistan's indigenous fish species (Catla catla), the acute effects of a commercial formulation of PFF and a few additional OPPs and carbamates were studied.Behavioral intoxication, irregular swimming, laying on the bottom, asphyxia, lethargy and downward movements, and gulping before death were all observed in aquatic organisms. Ghazala et al[20b, 21a]found that when several organs (brain, gills, kidneys, meat, liver, and blood) of fingerlings were subjected to three different sublethal concentrations of PFF (0.038, 0.019, and 0.012 mg L-1) for 60 days, the pesticide inhibited cholinesterases (AChE and butyrylcholinesterase).Live video microscopy of zebrafish (Danio rerio) embryos exposed to PFF at various concentrations revealed aberrant development, skeletal abnormalities, and changed heart architecture, all of which led to changes in hatchling swimming behaviour after 6 days.[47]The widespread use of PFF and other OPPs has been suggested as a role in the demise of honey bee colonies (*Apis Mellifera* and *A. Cerana*). Pollen, honey, and bees have all been shown to have residues.[3b, 56a]Apis mellifera, bees that collect nectar from cotton blooms in cotton-growing areas of Burkina Faso, West Africa, have been observed to have developmental instability (increased tarsus skeleton length).[46a]In two agricultural soils in China, the toxicity of PFF to the soil springtail (Folsomia candida) was also demonstrated.[37c]

Profenofos is harmful to the developing and reproductive systems. Non-target aquatic and terrestrial organisms, such as mice[17], fish (e.g., Channa punctatus; rainbowfish [Melanotaeniidae duboulayi, family Melanotaeniidae]; cladoceran; euryhaline fish; and carp), insects (mosquitoes [Culex quinquefasciatus]), and earthworms (Eis) [35b, 60c, 9a, 32a, 49a, 5b] The herbicide causes chromosomal abnormality in both somatic and germ cells, indicating its potential mutagenicity.[17]Under controlled conditions,[37b]investigated the acute mortality and genotoxicity of PFF in frogs.PFF was very toxic to Rana spinosa tadpoles, with LC50 values of 1.59, 1.14, 0.77, and 0.58 mg L^{-1} at 24, 48, 72, and 96 hours, respectively.PFF at moderate to high sublethal doses may be genotoxic to the tadpole after 96 hours of exposure, according to the study's micronucleus test. Damage to the DNA of the tadpoles' erythrocytes caused the genotoxic impact.PFF had an immediate neurotoxic effect on Eisenia fetida, as well as morphological and histological changes.[9a]The herbicide inhibits metabolising enzymes in multiple tissues, resulting in decreased protein metabolism in the freshwater fish Labeo rohita.[44a] Profenofos is a powerful endocrine disrupting compound (EDC) that has been identified as one of the Wistar rats' male reproductive toxicants.[43a]It also caused testicular tissue disruption in rabbits that were exposed to it.[42a]

Profenofos also has an impact on the soil microflora, particularly the bacterial population, dinitrogen fixers, fungi, denitrifying bacteria, nitrifying bacteria, and their nitrogenase activity.[41a]In another study, the effect of PFF on Azospirillum brasilense cells cultured in a chemically specified media was studied.[22b]discovered that PFF inhibited N2 fixation, intracellular ATP levels, pantothenic acid, thiamine, and niacin synthesis, as well as cell proliferation.

7. Bioremediation

The negative consequences of the PFF chemical have prompted a surge in research into techniques to eliminate this xenobiotic from the environment. Bio-transformations have been extensively investigated for their ability to eliminate chemical contaminants at contaminated areas.[11b] Because it is environmentally sustainable and cost-effective, this innovative technology has emerged as a potential clean-up option for a variety of pollutants over the previous two decades.Bioremediation is the most effective method for removing contaminants through microbial metabolism.[10b, 40a, 10e, 52a, 31a]The solubility, mobility, degradability, and bioavailability of pollutants determine the efficiency of bioremediation.Pseudomonas, Agrobacterium, Enterobacter, and Flavobacterium species are the most well-known natural isolates capable of degrading organophosphates in the context of OPP remediation.[43b, 28b, 33b]They have organophosphate hydrolase (OPH) enzymes that aid in the degradation process.Because of the diverse metabolic and enzymatic activities of the bacterial strains that comprise them, syntrophic bacterial consortia play an important role in this process in many cases.[10c-d, 52b]This diversity aids in inducing each other's enzyme systems for utilising different pollutants, allowing the consortium to quickly adapt to contaminated environments.

Another bioremediation approach is biosorption, which includes employing inert and nonliving biomass to remove chemo-pollutants (organic chemicals, pesticides, metal ions, and colour molecules) from contaminated water (materials of biological origin like fungi, algae, and agricultural wastes).It has shown to be a commercially viable, low-cost, and environmentally beneficial technology.[10a, 19a, 25a, 45a]

8. Remediation of Profenofos by Other Methods

It can be challenging to execute effective biological treatment of wastewater containing PFF and other herbicides.Because of the toxicity of pesticide contaminants, biodegradation may not be successful in many circumstances.[28a, 36] Pesticide elimination from the environment via non-biological and/or natural means has also been described (EXTOXNET, 1998).Mansouriieh, N*et al* that low-cost zerovalent iron (ZVI) might be utilized to degrade PFF in a recent study.[40c] Gillham, R.W.found that zerovalent iron is a potent reducing agent that is conveniently accessible; it not only successfully reduces pollutants but also produces very little waste and secondary contaminants.[21b]ZVI nanoparticles are being employed to remediate a variety of chemo-pollutants, including dyes[16c], heavy metals[62b], and halogenated organics.[40c, 61]

PFF hydrolysis in buffered aqueous environments at various pH values was demonstrated by.[4a].Ouh decomposes via dealkylation in acidic and neutral media, yielding a dealkylated molecule and alcohols (ethanol and *n*-propanol); in an alkaline medium, PFF decomposes via hydrolysis, yielding BCP and EPPA.

PFF photo-transformation in diluted aqueous solutions was described by Martinique and co-workers.[62a]The photolysis studies were carried out in Martinique, a French West Indian island, using filtered water and natural Capot River water (which contained organic debris).[62a]The aerobic soil metabolism of PFF was investigated using [U-14C-phenyl]-PFF applied to various soils incubated at 21°C or 25°C under aerobic conditions. The rate of degradation varied depending on the kind of soil, temperature, moisture content of the soil, and dose.Only modest levels (0.1–1.6 percent) of PFF remained as the parent after 28 to 30 days.After 28 to 30 days, only small amounts (<0.1–1.6%) of PFF remained as the parent. BCP, 4-bromo-2-chlorophenyl ethyl ether (BCPEE), 2-thioethylenecarboxy-4 hydroxyphenyl methyl ether (THPME), 2-mercapto-4-hydroxyphenyl methyl ether (MHPME), and 4-bromo-2-chlorophenyl methyl ether (BCPME) were generated as intermediates in this process and were then destroyed in the conclusion.[18b]Pesticide residues in stockpiled cotton gin trash (CGT) were examined after two years (2002–2004) in another investigation.Among the 14 pesticide residues discovered, profenofos was one of them.Due to the simultaneous disintegration of the CGT matrix, the data revealed a complicated pattern of pesticide residue decay over time.[11c]Surface photooxidation of PFF in the presence of ambient ultraviolet light (185 and 254 nm), various gases (N_2 and Air), humidity, and various oxidants (ozone and hydroxyl radicals) was also investigated.After 60 minutes of exposure to both 254- and 185-nm UV light in humidified air and strong ozone conditions, profenofos film with a surface density of 0.38 g m^{-2} was entirely destroyed.[49c]

9. Profenofos Degradation Pathway

There hasn't been a whole PFF biodegradation route published yet.PFF hydrolyzes enzymatically to produce BCP and EPPA at first (Fig. 7).4-Bromo-2-chlorophenol is then converted to 3-methoxy phenol (3MP) and 3-chloro-4-methoxy phenol by biotic and abiotic mechanisms.[55a] PFF reacts with water during abiotic degradation to create BCP.[4a]There have been no reports on the destiny of 3MP and 3-chloro-4-methoxy phenol, and no verification of PFF's final degradation route has been published.

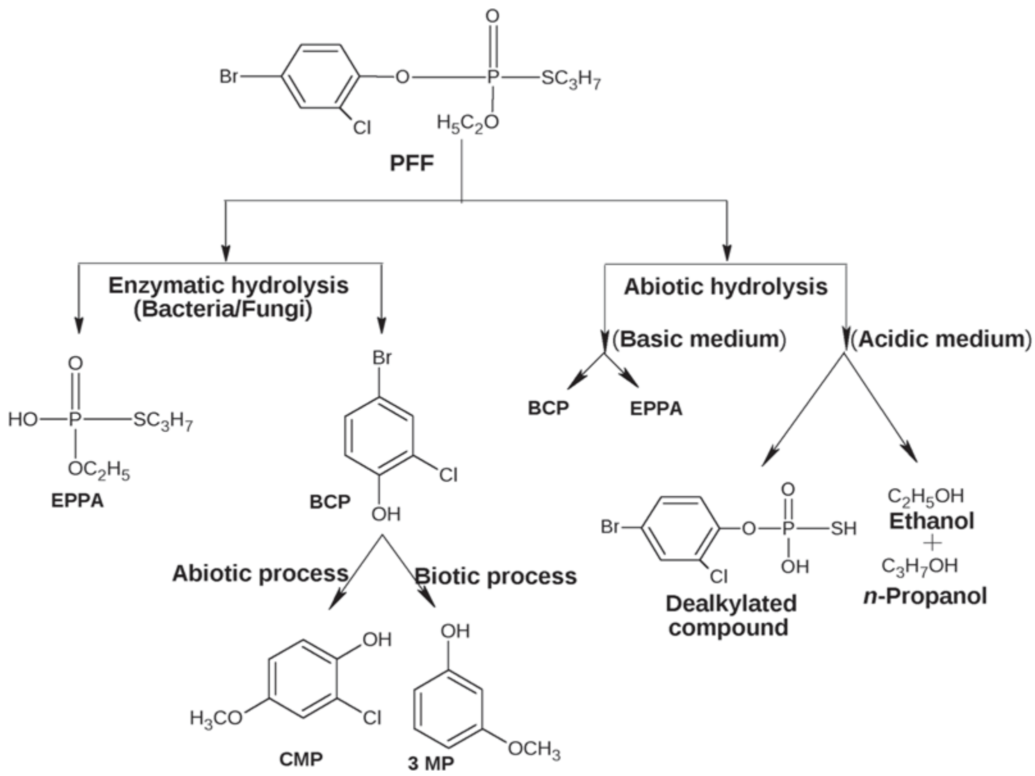

Fig. 7. Biodegradation pathway of profenofos (PFF). BCP,0-(4-Bromo-2-chlorophenyl); 3MP, 3-methoxy phenol; EPPA, O-ethyl-S-propyl-O- phosphorothioic acid; CMP,3-chloro-4-methoxy phenol.

10. Experimental Methods

10.1 Procedure:

In the beginning, we have purchase the market sample of Profenofos 50EC. To start the homogeneity test over the Test Item. We have mixed the sample in 2 Litre Beaker and stir it for 15 minutes. Then, transfer 50mL of volume each to the 25 HDPE Bottle. Label the code serially. Then, we have picked the random sample and start the analysis of Profenofos EC and other specifications as per the requirement of Indian Standard (IS 15238:2002 RA 2018).

It is recommended that the Active Ingredient of Profenofos has to be quantified with the help of GC-FID (Gas Chromatography- Flame Ionisation Detector) with the help of Internal Standard (Isopropyl 4-4` Dibromobenzilate AR Grade or equivalent).

Besides this, in the instrument, we have to set the Chromatography Conditions such as Injector Temperature, Detector Temperature and Oven Programming and other set of Chemicals are also required as mentioned in the IS. After preparing a set of solutions, we run the Standard Solution and Sample Solution over GC and analyze the results as per the given formula:

Purity, % =

Wt. of Standard/Wt. of Sample * Purity of Ref. Standard * Sample Area/ Standard Area* Dilution Factor

The table of the results of homogeneity studies are as follows:

In order to support the homogeneity results, the GC Chromatograph are attached herewith.

HOMOGENEITY CALCULATIONS RAW DATA SHEET											
PARAMETER: ACTIVE INGREDIENT											
S.No.	PT Item Code	Weight of Standard (In g)	Weight of Sample (in g)		Purity of Ref. Std.	Standard Area	Sample Area	Standard Area 1	Sample Area 1	Results, % by weight	Results, % by weight
			w1	w2						Test Portion, t1	Test Portion, t2
1	Profenofos Standard					678.66		665.81			
2	PFCH-2201-PR-02	0.1333	0.2000	0.2000	95.1	678.66	523.73	665.81	540.15	48.91	51.42
3	PFCH-2201-PR-03	0.1333	0.2020	0.2020	95.1	678.66	523.16	665.81	529.43	48.38	49.90
4	PFCH-2201-PR-06	0.1333	0.2058	0.2058	95.1	678.66	550.42	665.81	545.29	49.96	50.45
5	PFCH-2201-PR-07	0.1333	0.2049	0.2049	95.1	678.66	553.94	665.81	576.51	50.50	53.57
6	PFCH-2201-PR-14	0.1333	0.2020	0.2020	95.1	678.66	548.57	665.81	554.12	50.73	52.23
7	PFCH-2201-PR-17	0.1333	0.2006	0.2006	95.1	678.66	563.88	665.81	568.68	52.51	53.98
8	PFCH-2201-PR-20	0.1333	0.2157	0.2157	95.1	678.66	591.87	665.81	578.78	51.25	51.09
9	PFCH-2201-PR-24	0.1333	0.2157	0.2157	95.1	678.66	585.52	665.81	593.18	50.70	52.36
10	PFCH-2201-PR-27	0.1333	0.2094	0.2094	95.1	678.66	573.23	665.81	548.63	51.13	49.88
11	PFCH-2201-PR-30	0.1333	0.2033	0.2033	95.1	678.66	554.22	665.81	542.76	50.92	50.83

HOMOGENEITY CALCULATIONS RAW DATA SHEET						
PARAMETER: DENSITY, pH, PERSISTENT FOAM, EMULSION STABILITY						
S.No.	PT Item Code	Density	pH	Persistent Foam, mL	Emulsion Stability	Moisture, % by weight
					Qualitative	
1	PFCH-2201-PR-02	1.0887	6.72	6.2	OK	0.59
2	PFCH-2201-PR-03	1.0707	6.16	5.8	OK	0.62
3	PFCH-2201-PR-06	1.0784	5.96	6.0	OK	0.63
4	PFCH-2201-PR-07	1.0461	6.09	6.2	OK	0.65
5	PFCH-2201-PR-14	1.0678	6.36	5.6	OK	0.66
6	PFCH-2201-PR-17	1.0707	5.97	6	OK	0.58
7	PFCH-2201-PR-20	1.0662	6.18	5.8	OK	0.57
8	PFCH-2201-PR-24	1.0777	6.18	6.2	OK	0.56
9	PFCH-2201-PR-27	1.0998	6.37	6.4	OK	0.57
10	PFCH-2201-PR-30	1.1010	6.18	6	OK	0.56

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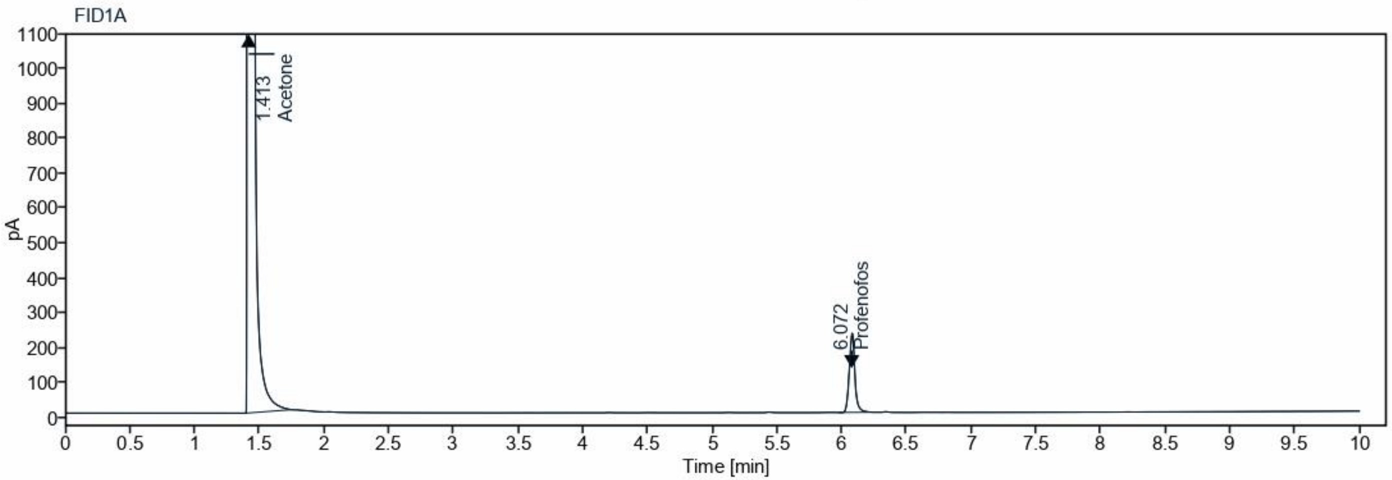
L-17/3, DLF Phase-II, IIFCO Chowk, M.G. Road
Gurgaon-122002, Haryana, INDIA

Sample Name: PROFENOFOS STANDARD

Acq. Method: Profenofos.amx **Operator:** GC Section

Data File: 2022-05-16 12-25-32+05-30-02.dx **Injection Date:** 2022-05-16 12:27:56+05:30



Signal: FID1A

S.No.	RT [min]	Area	Area%	Name
1	1.41	274898.69	99.754	Acetone
2	6.07	678.66	0.246	Profenofos
	Sum	275577.36	100.00	

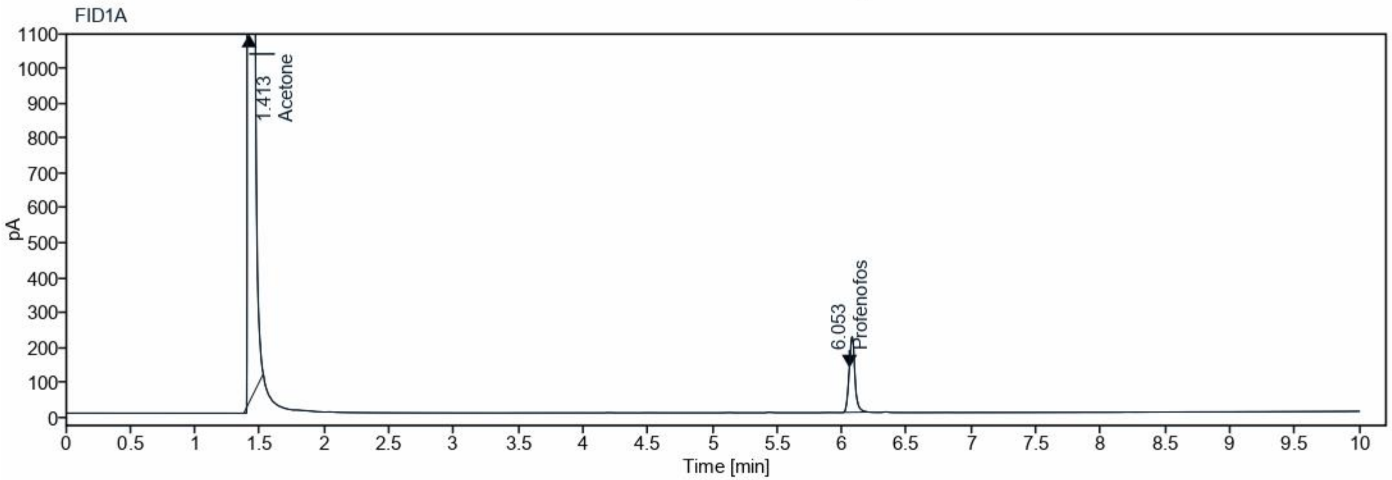
FARE Labs Pvt. Ltd.

L-17/3, DLF Phase-II, IIFCO Chowk, M.G. Road
Gurgaon-122002, Haryana, INDIA

Sample Name: PROFENOFOS STANDARD

Acq. Method: Profenofos.amx **Operator:** GC Section

Data File: 2022-05-16 12-37-59+05-30-03.dx **Injection Date:** 2022-05-16 12:40:11+05:30



Signal: FID1A

S.No.	RT [min]	Area	Area%	Name
1	1.41	271608.44	99.755	Acetone
2	6.05	665.81	0.245	Profenofos
	Sum	272274.24	100.00	

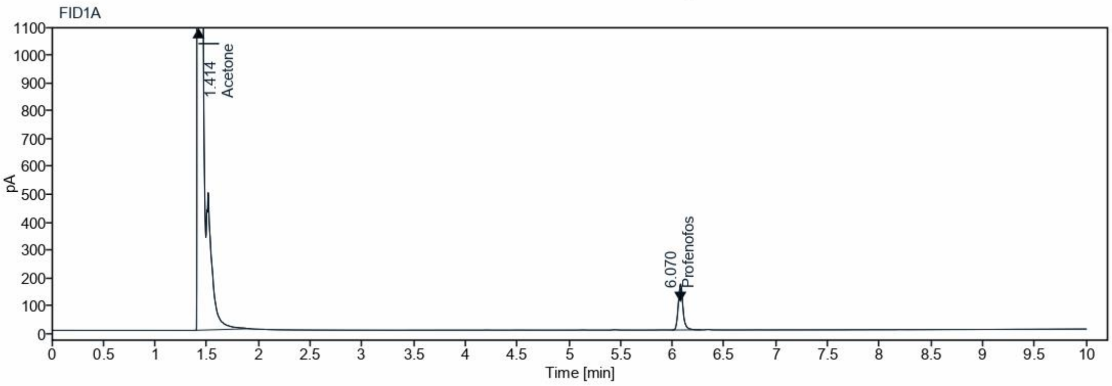
FARE Labs Pvt. Ltd.

L-17/3, DLF Phase-II, IIFCO Chowk, M.G. Road
Gurgaon-122002, Haryana, INDIA

Sample Name: PFCH-2201-PR-02 PT SAMPLE

Acq. Method: Profenofos.amx **Operator:** GC Section

Data File: 2022-05-16 12-50-12+05-30-04.dx **Injection Date:** 2022-05-16 12:52:49+05:30



Signal: FID1A

S.No.	RT [min]	Area	Area%	Name
1	1.41	273146.34	99.809	Acetone
2	6.07	523.73	0.191	Profenofos
	Sum	273670.07	100.00	

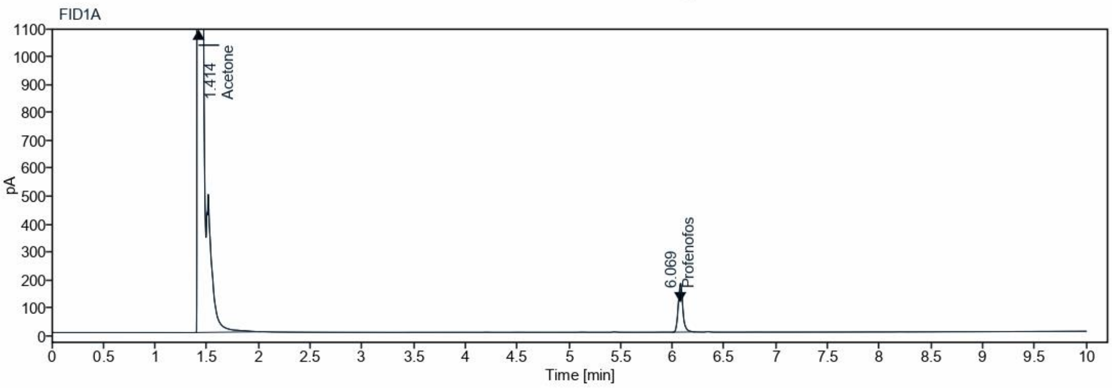
FARE Labs Pvt. Ltd.

L-17/3, DLF Phase-II, IIFCO Chowk, M.G. Road
Gurgaon-122002, Haryana, INDIA

Sample Name: PFCH-2201-PR-02 PT SAMPLE

Acq. Method: Profenofos.amx **Operator:** GC Section

Data File: 2022-05-16 13-02-50+05-30-05.dx **Injection Date:** 2022-05-16 13:05:25+05:30



Signal: FID1A

S.No.	RT [min]	Area	Area%	Name
1	1.41	277828.74	99.806	Acetone
2	6.07	540.15	0.194	Profenofos
	Sum	278368.89	100.00	

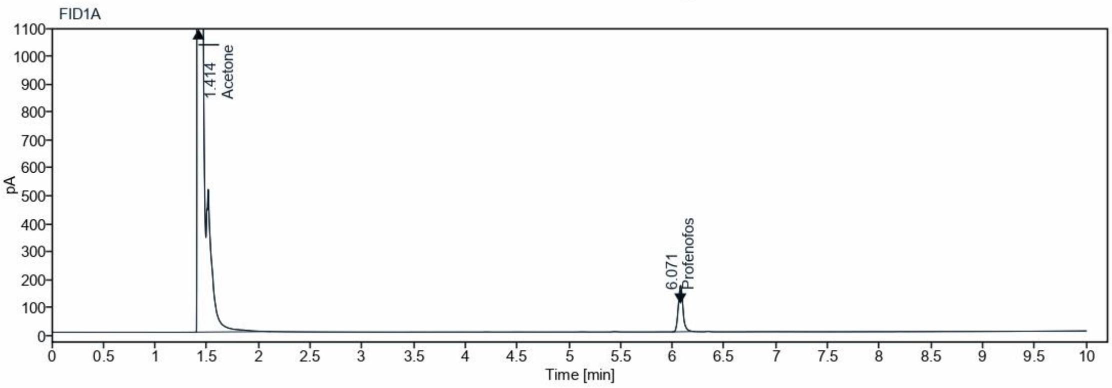
FARE Labs Pvt. Ltd.

L-17/3, DLF Phase-II, IIFCO Chowk, M.G. Road
Gurgaon-122002, Haryana, INDIA

Sample Name: PFCH-2201-PR-03 PT SAMPLE

Acq. Method: Profenofos.amx **Operator:** GC Section

Data File: 2022-05-16 13-15-27+05-30-06.dx **Injection Date:** 2022-05-16 13:18:07+05:30



Signal: FID1A

S.No.	RT [min]	Area	Area%	Name
1	1.41	278272.37	99.812	Acetone
2	6.07	523.16	0.188	Profenofos
	Sum	278795.53	100.00	

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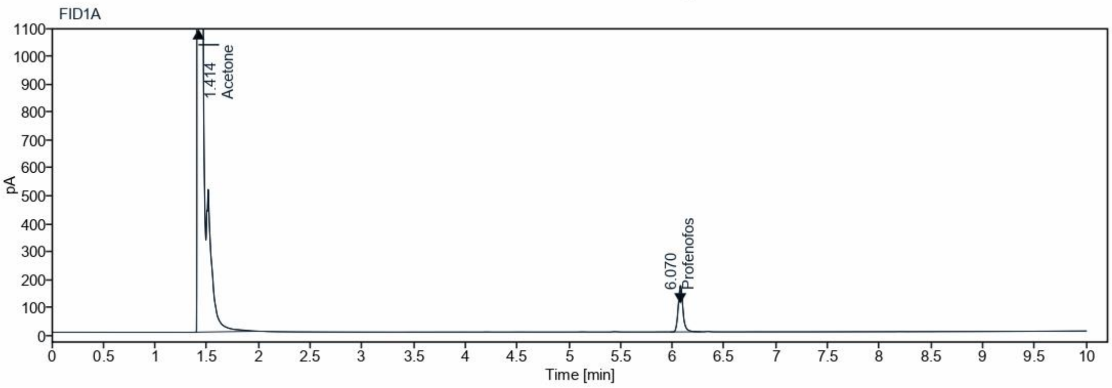
L-17/3, DLF Phase-II, IIFCO Chowk, M.G. Road
Gurgaon-122002, Haryana, INDIA

Sample Name: PFCH-2201-PR-03 PT SAMPLE

Acq. Method: Profenofos.amx **Operator:** GC Section

Data File: 2022-05-16 13-28-09+05-30-07.dx **Injection Date:** 2022-05-16 13:30:45+05:30



Signal: FID1A

S.No.	RT [min]	Area	Area%	Name
1	1.41	275623.58	99.808	Acetone
2	6.07	529.43	0.192	Profenofos
	Sum	276153.01	100.00	

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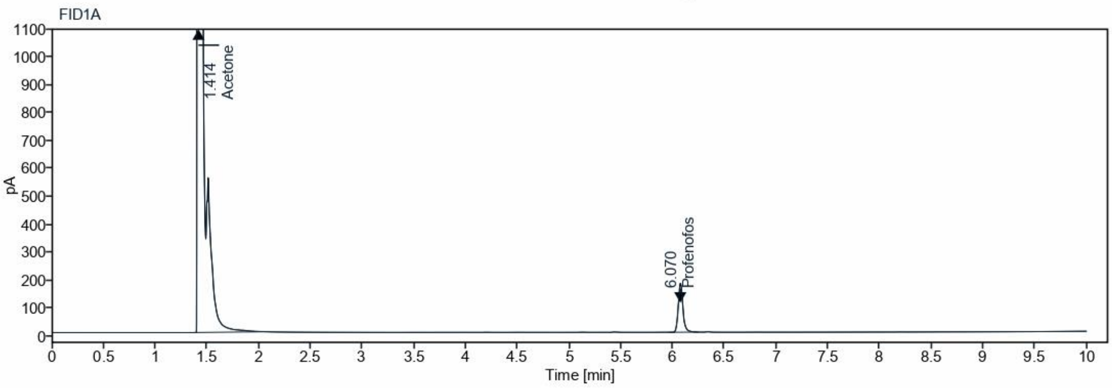
L-17/3, DLF Phase-II, IIFCO Chowk, M.G. Road
Gurgaon-122002, Haryana, INDIA

Sample Name: PFCH-2201-PR-06 PT SAMPLE

Acq. Method: Profenofos.amx **Operator:** GC Section

Data File: 2022-05-16 13-40-48+05-30-08.dx **Injection Date:** 2022-05-16 13:43:24+05:30



Signal: FID1A

S.No.	RT [min]	Area	Area%	Name
1	1.41	276754.20	99.802	Acetone
2	6.07	550.42	0.198	Profenofos
	Sum	277304.61	100.00	

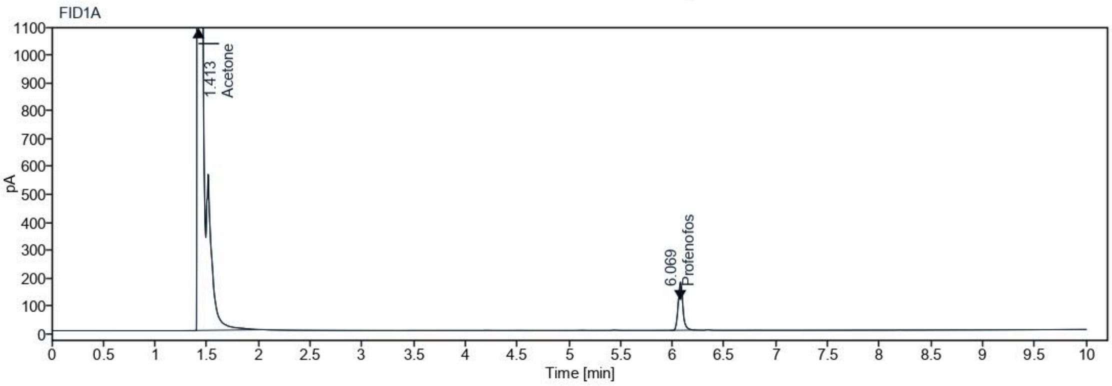
FARE Labs Pvt. Ltd.

L-17/3, DLF Phase-II, IIFCO Chowk, M.G. Road
Gurgaon-122002, Haryana, INDIA

Sample Name: PFCH-2201-PR-06 PT SAMPLE

Acq. Method: Profenofos.amx **Operator:** GC Section

Data File: 2022-05-16 13-53-28+05-30-09.dx **Injection Date:** 2022-05-16 13:56:03+05:30



Signal: FID1A

S.No.	RT [min]	Area	Area%	Name
1	1.41	275219.04	99.802	Acetone
2	6.07	545.29	0.198	Profenofos
	Sum	275764.33	100.00	

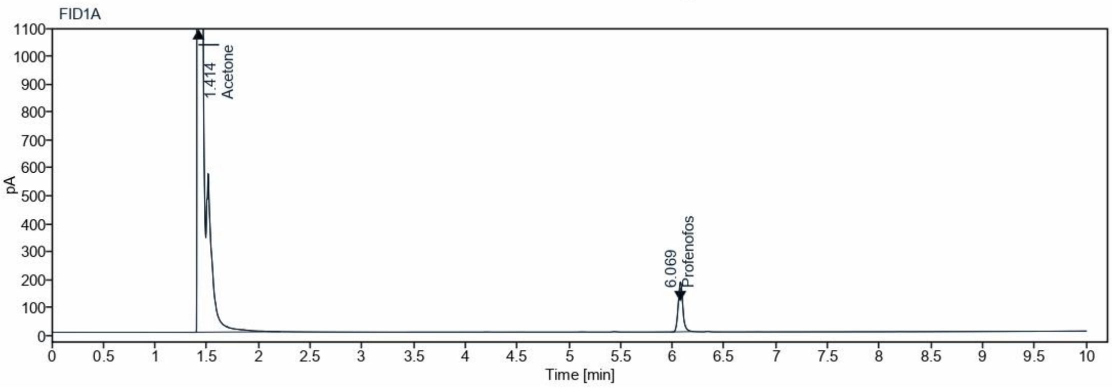
FARE Labs Pvt. Ltd.

L-17/3, DLF Phase-II, IIFCO Chowk, M.G. Road
Gurgaon-122002, Haryana, INDIA

Sample Name: PFCH-2201-PR-07 PT SAMPLE

Acq. Method: Profenofos.amx **Operator:** GC Section

Data File: 2022-05-16 14-06-08+05-30-10.dx **Injection Date:** 2022-05-16 14:08:23+05:30



Signal: FID1A

S.No.	RT [min]	Area	Area%	Name
1	1.41	275816.12	99.800	Acetone
2	6.07	553.94	0.200	Profenofos
	Sum	276370.05	100.00	

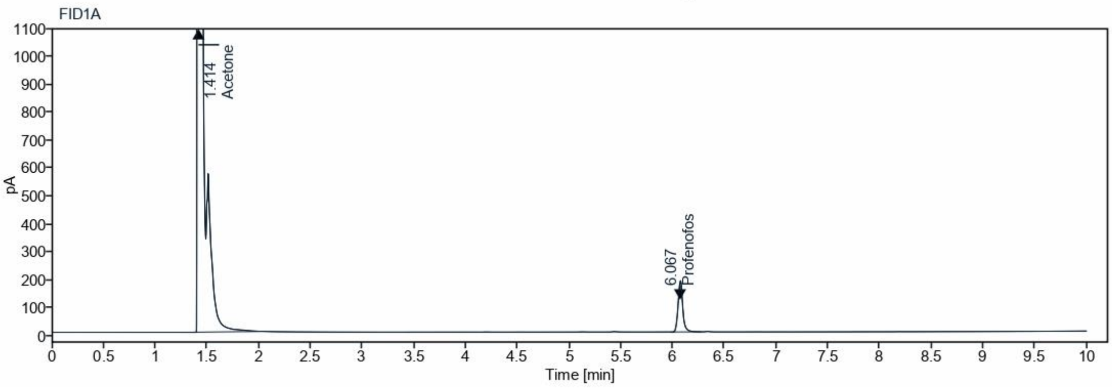
FARE Labs Pvt. Ltd.

L-17/3, DLF Phase-II, IIFCO Chowk, M.G. Road
Gurgaon-122002, Haryana, INDIA

Sample Name: PFCH-2201-PR-07 PT SAMPLE

Acq. Method: Profenofos.amx **Operator:** GC Section

Data File: 2022-05-16 14-18-27+05-30-11.dx **Injection Date:** 2022-05-16 14:21:07+05:30



Signal: FID1A

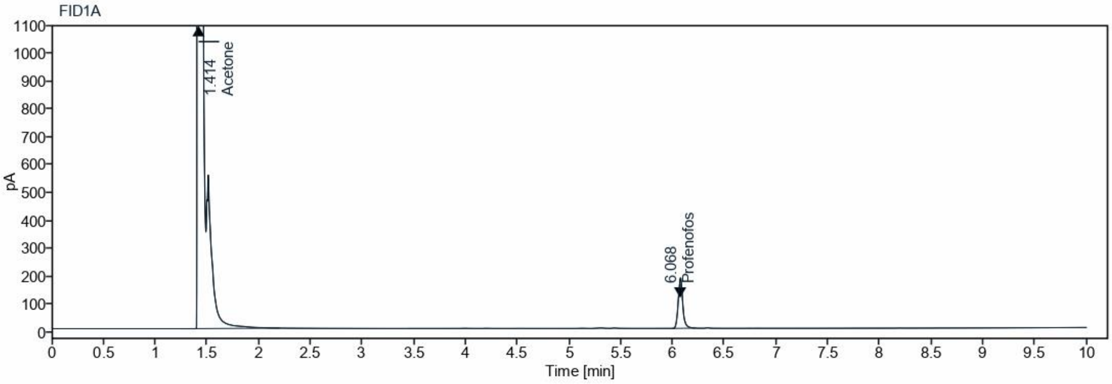
S.No.	RT [min]	Area	Area%	Name
1	1.41	277490.51	99.793	Acetone
2	6.07	576.51	0.207	Profenofos
	Sum	278067.02	100.00	

FARE Labs Pvt. Ltd.
L-17/3, DLF Phase-II, IIFCO Chowk, M.G. Road
Gurgaon-122002, Haryana, INDIA

Sample Name: PFCH-2201-PR-14 PT SAMPLE

Acq. Method: Profenofos.amx **Operator:** GC Section

Data File: 2022-05-16 14-31-10+05-30-12.dx **Injection Date:** 2022-05-16 14:33:32+05:30



Signal: FID1A

S.No.	RT [min]	Area	Area%	Name
1	1.41	275631.84	99.801	Acetone
2	6.07	548.57	0.199	Profenofos
	Sum	276180.41	100.00	

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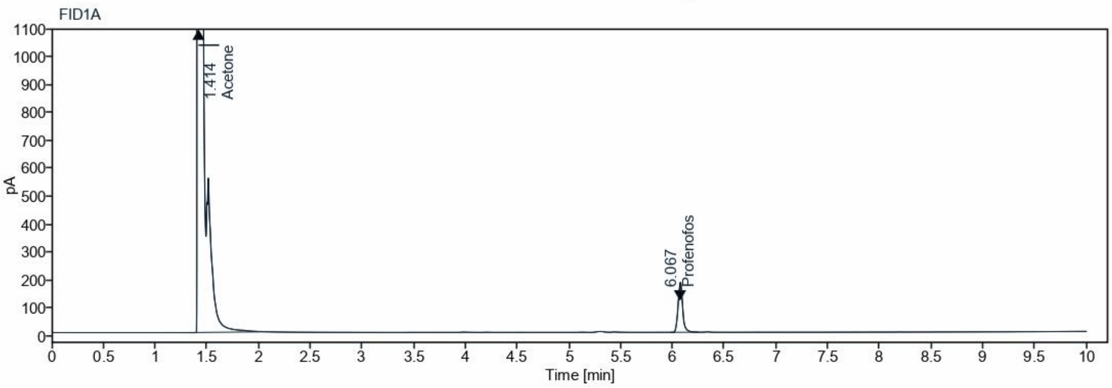
L-17/3, DLF Phase-II, IIFCO Chowk, M.G. Road
Gurgaon-122002, Haryana, INDIA

Sample Name: PFCH-2201-PR-14 PT SAMPLE

Acq. Method: Profenofos.amx **Operator:** GC Section

Data File: 2022-05-16 14-43-35+05-30-13.dx **Injection Date:** 2022-05-16 14:45:48+05:30



Signal: FID1A

S.No.	RT [min]	Area	Area%	Name
1	1.41	275786.20	99.799	Acetone
2	6.07	554.12	0.201	Profenofos
	Sum	276340.32	100.00	

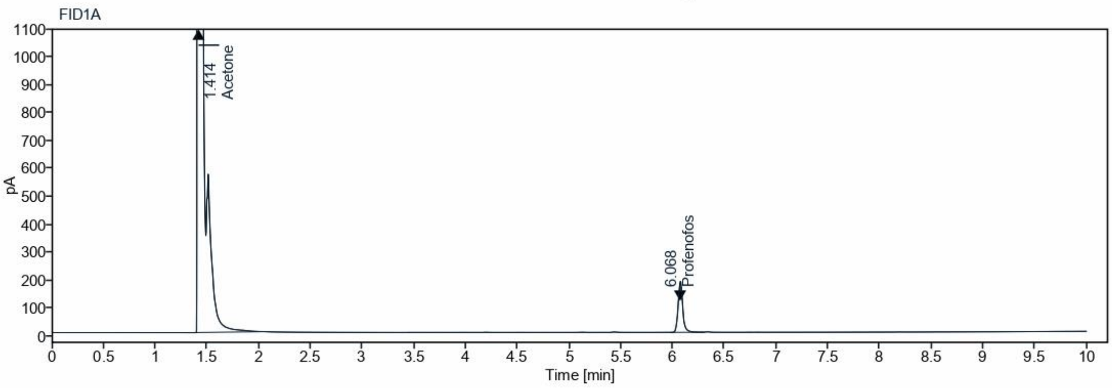
FARE Labs Pvt. Ltd.

L-17/3, DLF Phase-II, IIFCO Chowk, M.G. Road
Gurgaon-122002, Haryana, INDIA

Sample Name: PFCH-2201-PR-17 PT SAMPLE

Acq. Method: Profenofos.amx **Operator:** GC Section

Data File: 2022-05-16 14-55-50+05-30-14.dx **Injection Date:** 2022-05-16 14:58:05+05:30



Signal: FID1A

S.No.	RT [min]	Area	Area%	Name
1	1.41	274188.25	99.795	Acetone
2	6.07	563.88	0.205	Profenofos
	Sum	274752.12	100.00	

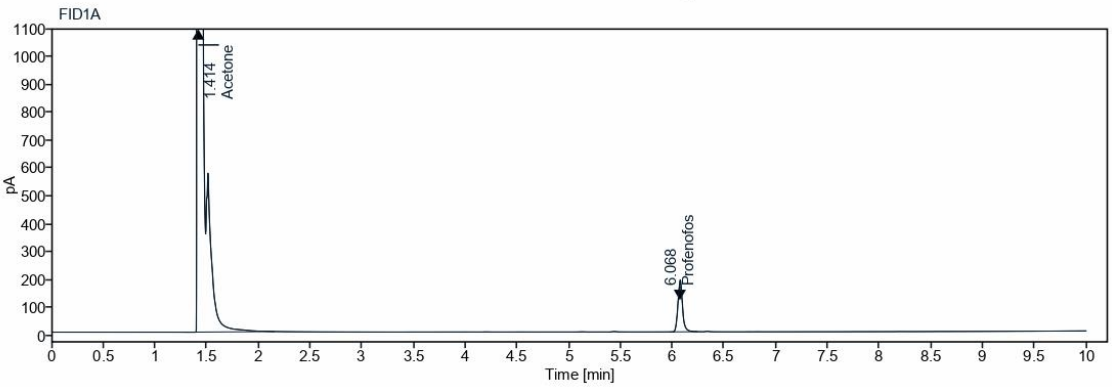
FARE Labs Pvt. Ltd.

L-17/3, DLF Phase-II, IIFCO Chowk, M.G. Road
Gurgaon-122002, Haryana, INDIA

Sample Name: PFCH-2201-PR-17 PT SAMPLE

Acq. Method: Profenofos.amx **Operator:** GC Section

Data File: 2022-05-16 15-08-05+05-30-15.dx **Injection Date:** 2022-05-16 15:10:41+05:30



Signal: FID1A

S.No.	RT [min]	Area	Area%	Name
1	1.41	277622.56	99.796	Acetone
2	6.07	568.68	0.204	Profenofos
	Sum	278191.24	100.00	

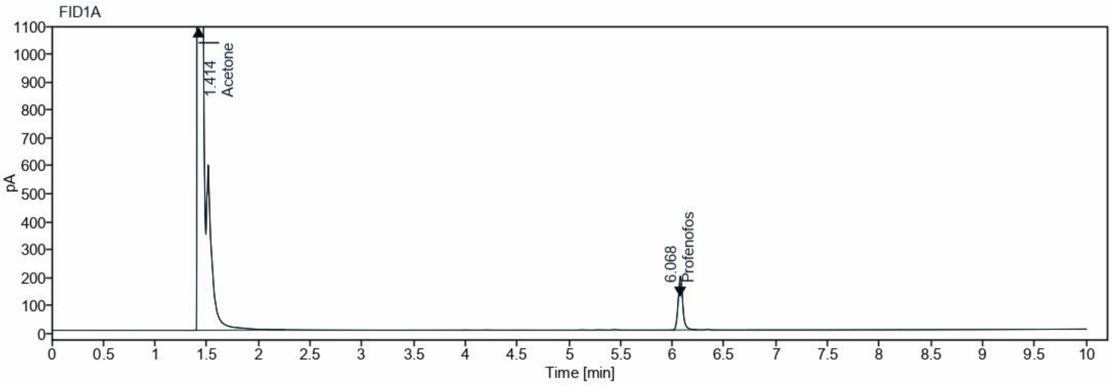
FARE Labs Pvt. Ltd.

L-17/3, DLF Phase-II, IIFCO Chowk, M.G. Road
Gurgaon-122002, Haryana, INDIA

Sample Name: PFCH-2201-PR-20 PT SAMPLE

Acq. Method: Profenofos.amx **Operator:** GC Section

Data File: 2022-05-16 15-20-44+05-30-16.dx **Injection Date:** 2022-05-16 15:23:30+05:30



Signal: FID1A

S.No.	RT [min]	Area	Area%	Name
1	1.41	277929.46	99.787	Acetone
2	6.07	591.87	0.213	Profenofos
	Sum	278521.32	100.00	

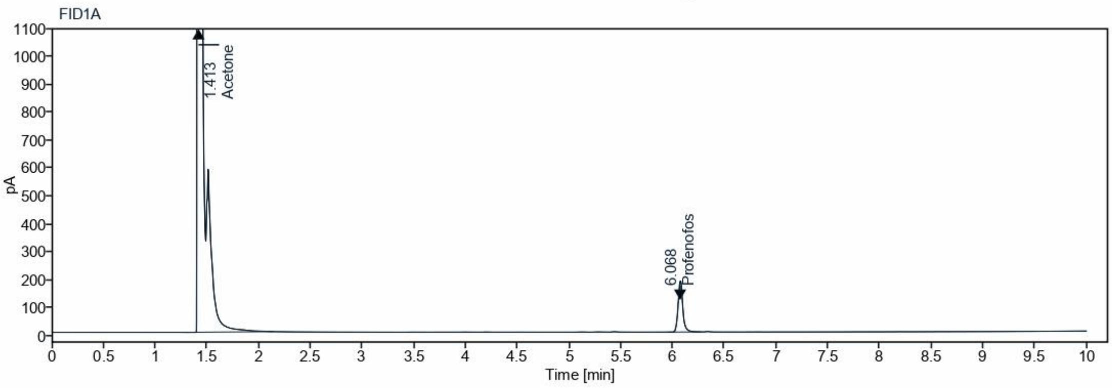
FARE Labs Pvt. Ltd.

L-17/3, DLF Phase-II, IIFCO Chowk, M.G. Road
Gurgaon-122002, Haryana, INDIA

Sample Name: PFCH-2201-PR-20 PT SAMPLE

Acq. Method: Profenofos.amx **Operator:** GC Section

Data File: 2022-05-16 15-33-33+05-30-17.dx **Injection Date:** 2022-05-16 15:36:09+05:30



Signal: FID1A

S.No.	RT [min]	Area	Area%	Name
1	1.41	276753.35	99.791	Acetone
2	6.07	578.78	0.209	Profenofos
	Sum	277332.13	100.00	

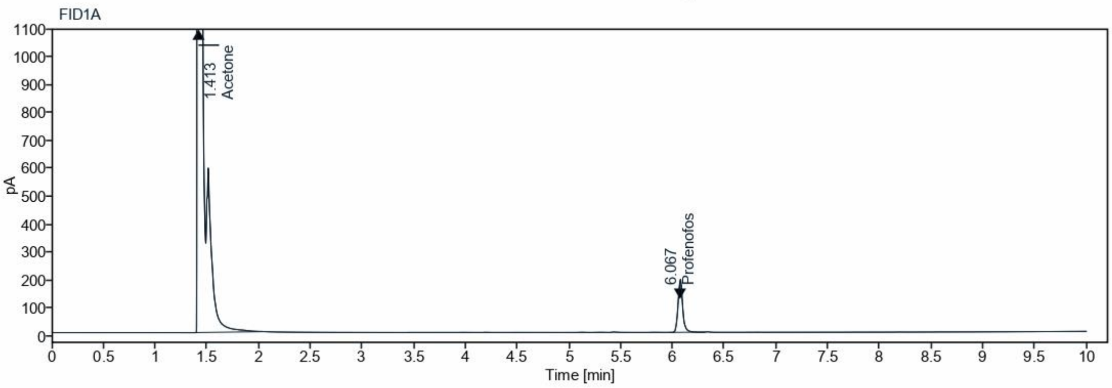
FARE Labs Pvt. Ltd.

L-17/3, DLF Phase-II, IIFCO Chowk, M.G. Road
Gurgaon-122002, Haryana, INDIA

Sample Name: PFCH-2201-PR-24 PT SAMPLE

Acq. Method: Profenofos.amx **Operator:** GC Section

Data File: 2022-05-16 15-46-12+05-30-18.dx **Injection Date:** 2022-05-16 15:48:47+05:30



Signal: FID1A

S.No.	RT [min]	Area	Area%	Name
1	1.41	273873.32	99.787	Acetone
2	6.07	585.52	0.213	Profenofos
	Sum	274458.84	100.00	

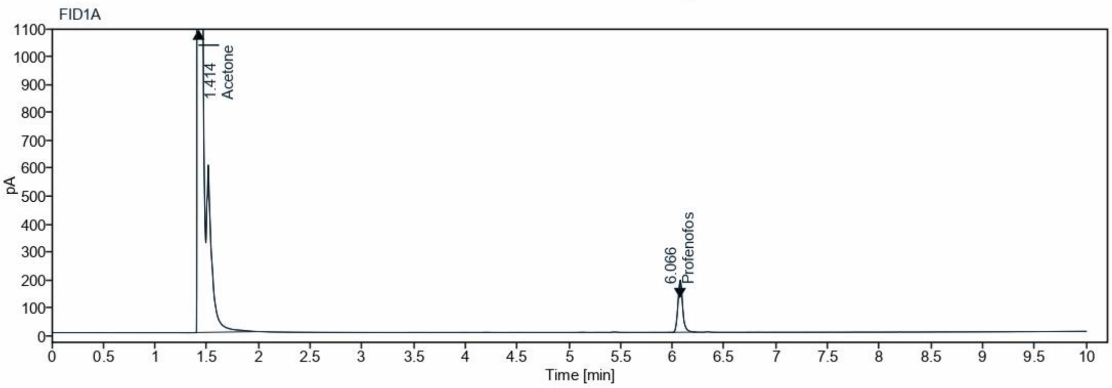
FARE Labs Pvt. Ltd.

L-17/3, DLF Phase-II, IIFCO Chowk, M.G. Road
Gurgaon-122002, Haryana, INDIA

Sample Name: PFCH-2201-PR-24 PT SAMPLE

Acq. Method: Profenofos.amx **Operator:** GC Section

Data File: 2022-05-16 15-58-50+05-30-19.dx **Injection Date:** 2022-05-16 16:01:28+05:30



Signal: FID1A

S.No.	RT [min]	Area	Area%	Name
1	1.41	271835.63	99.782	Acetone
2	6.07	593.18	0.218	Profenofos
	Sum	272428.81	100.00	

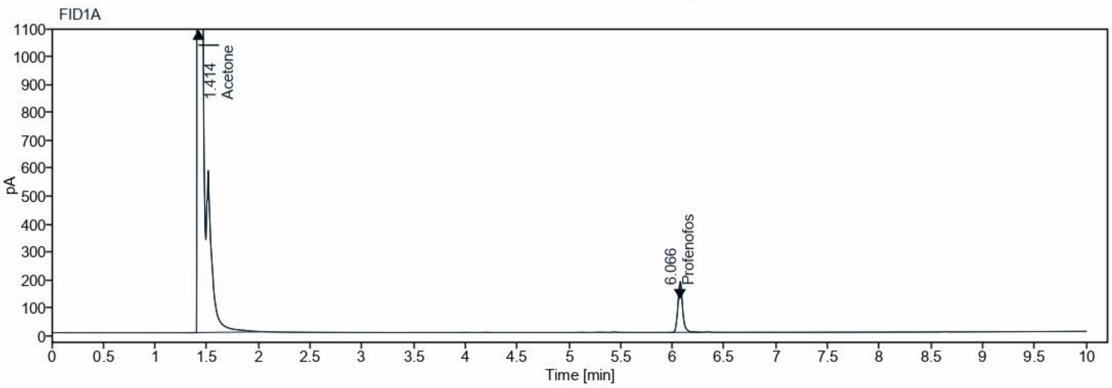
FARE Labs Pvt. Ltd.

L-17/3, DLF Phase-II, IIFCO Chowk, M.G. Road
Gurgaon-122002, Haryana, INDIA

Sample Name: PFCH-2201-PR-27 PT SAMPLE

Acq. Method: Profenofos.amx **Operator:** GC Section

Data File: 2022-05-16 16-11-31+05-30-20.dx **Injection Date:** 2022-05-16 16:14:07+05:30



Signal: FID1A

S.No.	RT [min]	Area	Area%	Name
1	1.41	274263.30	99.791	Acetone
2	6.07	573.23	0.209	Profenofos
	Sum	274836.53	100.00	

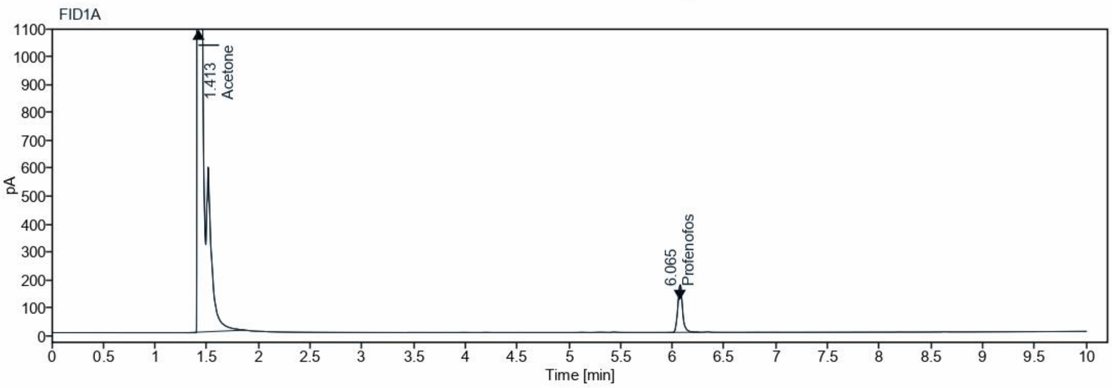
FARE Labs Pvt. Ltd.

L-17/3, DLF Phase-II, IIFCO Chowk, M.G. Road
Gurgaon-122002, Haryana, INDIA

Sample Name: PFCH-2201-PR-27 PT SAMPLE

Acq. Method: Profenofos.amx **Operator:** GC Section

Data File: 2022-05-16 16-24-09+05-30-21.dx **Injection Date:** 2022-05-16 16:26:26+05:30



Signal: FID1A

S.No.	RT [min]	Area	Area%	Name
1	1.41	275461.55	99.801	Acetone
2	6.06	548.63	0.199	Profenofos
	Sum	276010.18	100.00	

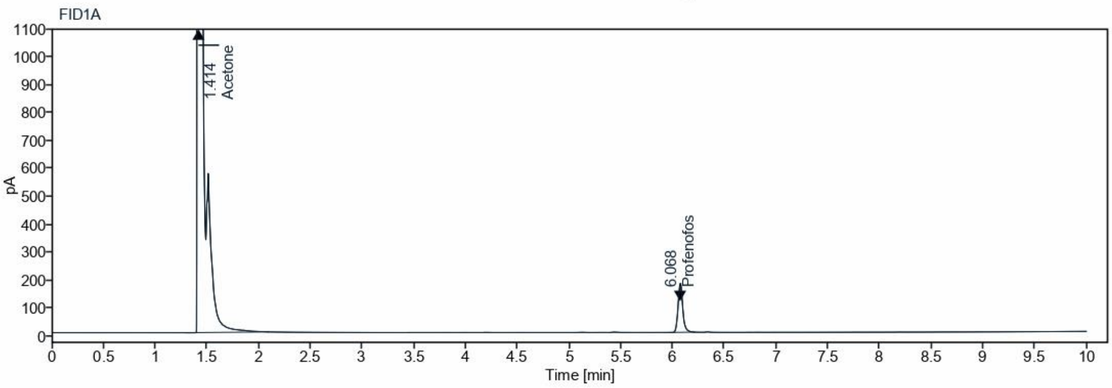
FARE Labs Pvt. Ltd.

L-17/3, DLF Phase-II, IIFCO Chowk, M.G. Road
Gurgaon-122002, Haryana, INDIA

Sample Name: PFCH-2201-PR-30 PT SAMPLE

Acq. Method: Profenofos.amx **Operator:** GC Section

Data File: 2022-05-16 16-36-27+05-30-22.dx **Injection Date:** 2022-05-16 16:39:03+05:30



Signal: FID1A

S.No.	RT [min]	Area	Area%	Name
1	1.41	274126.43	99.798	Acetone
2	6.07	554.22	0.202	Profenofos
	Sum	274680.64	100.00	

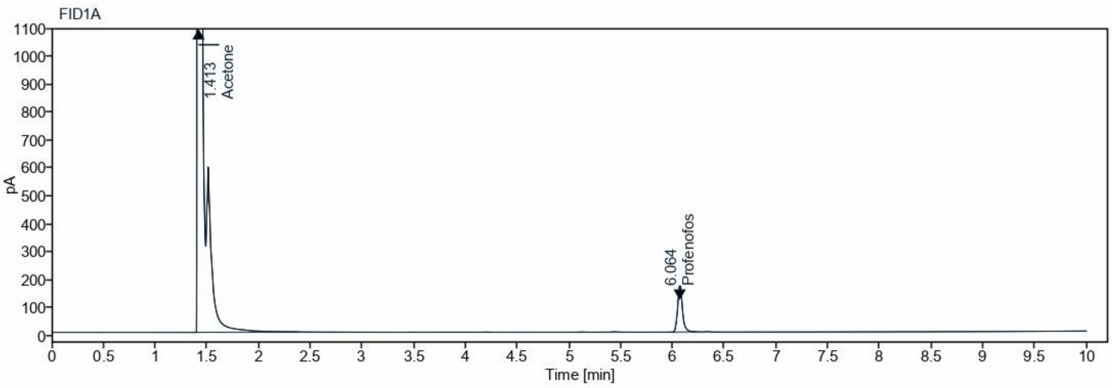
FARE Labs Pvt. Ltd.

L-17/3, DLF Phase-II, IIFCO Chowk, M.G. Road
Gurgaon-122002, Haryana, INDIA

Sample Name: PFCH-2201-PR-30 PT SAMPLE

Acq. Method: Profenofos.amx **Operator:** GC Section

Data File: 2022-05-16 16-49-07+05-30-23.dx **Injection Date:** 2022-05-16 16:51:17+05:30



Signal: FID1A

S.No.	RT [min]	Area	Area%	Name
1	1.41	273141.75	99.802	Acetone
2	6.06	542.76	0.198	Profenofos
	Sum	273684.50	100.00	

11. Future Considerations and Opportunities

Apart from the limited studies indicated above and experimental investigations over the market sample of Profenofos, no comprehensive investigation of PFF for homogeneity studies and biodegradation has been studiedso far, and, more importantly, no entire study of homogeneity, stability and biodegradation pathway has been established.Biodegradation by bacterial consortia has been documented a few times, however the roles of various partners and the degradation process for these cases have not been well studied. It is already reported that each member of the consortium does so that these bacteria can be employed to breakdown other contaminants from the same category.Furthermore, unlike biodegradation, the adsorption process has been depend over the homogeneity studies has yet to be investigated; unlike biodegradation, this approach can be utilised to remove PFF from potable water or water bodies near agricultural regions.Because PFF and other OPPs are widely used as agricultural pesticides and insecticides in many countries, contamination of soil and other environmental media by this harmful chemical is prevalent.Decontamination and removal of these pesticides from various environmental compartments is thus a pressing requirement that will necessitate additional research or studies in the near future.

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