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Synthesis of Mono and Di -2,6-Dichloro-3- Methyl aniline Organophosphate esters and Characterization by FTIR Spectroscopy and Biological Activity – Acute Oral Toxicity Study.

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Abstract- In this study, spectroscopic characterization, and assessment of acute oral toxicity of mono and di-2, 6-dichloro-3-metylaniline organophosphate esters derived from 2,6-dichloro-3-methylaniline are the main objective. We created the Mono-2, 6-dichloro-3-methylaniline organophosphate ester and di-2, 6-dichloro-3-methylaniline organophosphate ester and acute toxicity evaluations were carried out in vivo in accordance with OECD guidelines as per graded doses viz. 5, 50, 300, and 2000 mg/kg and the structural elucidation was done by using FTIR and in a 1:1 molar ratio, the mono 2, 6-dichloro-3-methylaniline organophosphate ester was created using Auger and Dupis' method. The Mono-2,6-dichloro-3-methylaniline organophosphate ester was identified as a white, odorless solid powder that melts on 330°C and gives 75% yield, a 2:1 molar ratio of 2, 6-dichloro-3-methylaniline to phosphoric acid, the Auger and Dupis method was also used to create the Di-2, 6-dichloro-3-methylaniline organophosphate ester. The synthesized Di-2,6-dichloro-3-methylaniline organophosphate ester, was isolated as a white powder possessing a sweet and aromatic odour, with 72% yield and a melts on 336°C. The compound exhibited a solid state and uniform crystalline morphology, reflecting good thermal stability.

1. INTRODUCTION

Organ-phosphorus compounds:-

Phosphate esters or organophosphates or OPEs are a class of organophosphorus compounds. It comprises a structure of the type $O=P(OR)_3$ having a phosphate molecule in centre with alkyl or aromatic substituents are esters of phosphoric acid. These esters are used as pesticides. DNA, RNA and ATP are also contain these types of esters linkage. Such ester linkage are also reported in a wide range of herbicides, insecticides, flame retardants and nerve agents.

P-C linked compounds having ages are identified as organo-phosphorus compounds that contains phosphorous and carbon. P-O-C linkages based phosphate esters are the most important organo-phosphorus compounds. Phosphorus-oxygen linkages containing Oxyphosphorus compounds are the most dominated in Phosphorus chemistry and generally known as phosphates. Mostly phosphorus esters that have phosphorus-oxygen linkages are organic phosphate esters found naturally. Phosphorous-carbon linkages organophosphorus are second most significant group whereas phosphorous-nitrogen linkages organophosphorus are perhaps the third group. Extensive phosphorous compounds and phosphoric acid on the earth are a vital source of industrial commodity based phosphorous. Deoxyribonucleic acid (DNA) are present in all living things that are also an organic phosphate ester. This is widely used in biochemistry and genetics. Organic esters are the most studied and crucial phosphorus compound that defends the persistence and advancement of the human race.

Although, inorganic phosphorus compounds are the utmost significant commercially. This organo-phosphorus compound has evolved rapidly. Following are the main four phosphorus compounds:

- Oxy-phosphorus compounds, contain covalent P-O linkages.
- Organo-phosphorus (carbon-phosphorus) compounds contain P-C linkages.
- Aza-phosphorus compounds contain P-N linkages.
- Metallophosphorus compounds contain P-metal linkages.

Sometimes two characteristics bonds are simplified the classification of phosphorous compound as following:

C-P-O : Organo-oxyphosphorus compound
N-P-O : Aza-oxyphosphorus compound
M-P-O : Metallo-oxyphosphorus compound
N-P-C : Aza-organophosphorus compound
M-P-C : Metallo-organophosphorus compound
M-P-N : Metallo-azaphosphorus compound

Organophosphates have been generally used in plasticizers and flame retardants. This is also used as performance additives to engine oil. Broad use of organophosphates in textile, furniture, electronics as plasticizers and flame retardants industries are due the low production cost and compatibility to numerous polymers. This is used as chemical bond neither add in final product physically. Heavy use of this in industries are leading to leak into the environment through volatilization, leaching, and abrasion increases the concentration in higher frequency in air, dust, water, sediment, soil and biota samples.[1]

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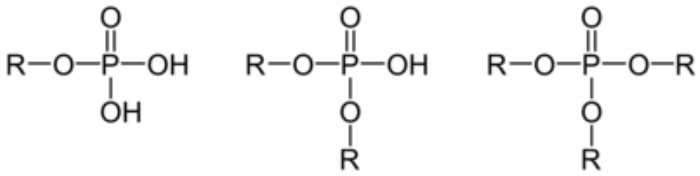
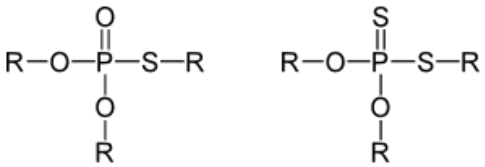
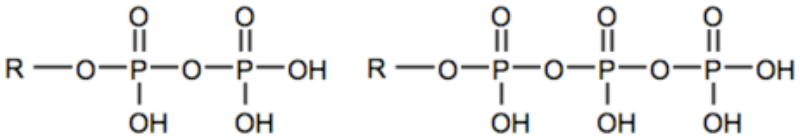
Organophosphates are primarily the esters of phosphoric acid. This can be mono-esters, di-esters or tri-esters depending on the number of attached organic groups (abbreviated as 'R' in the image below). Triesters are generally manmade, mono- or di-esters are usually biological organophosphates. The hydolysis of triesters can form diesters and monoesters.[2]



Organothiophosphates (P=S) or phosphorodiamidates (P-N) are a derivatives of organophosphates. This is included in pesticides as being organophosphates that are converted biologically into organophosphates.



Organophosphates included the esters of diphosphoric acid and triphosphoric acid due to biological cellular processes involve the mono- di and tri- phosphates of the same compound. AMP, ADP, ATP are the phosphates of adenosine playing a vital role in numerous metabolic processes.



Object and Scope of the proposed work-
The Objects of present work are;

- This synthesis is based on Auger and Dupis method by Allen's test.
- To synthesize organophosphate esters compounds (2, 6-dichloro-3-methylaniline).
- To synthesize three derivatives (Mono-ester, Di-ester) of 2, 6-dichloro-3-methylaniline organophosphate esters compound.
- Characterization of organophosphate esters compound on the basis of IR spectroscopy After synthesizing the organophosphate compound analyze their acute toxicity and comparative study.

[2]Materials and Methods:-

2.1 Synthesis of Mono 2, 6-dichloro-3-methylaniline organophosphate ester:-

The Mono-ester were synthesized by Auger and Dupis method in a ratio of 1:1. 3.4514 g of pure 2, 6-dichloro-3-methylaniline (parent compound) was dissolved in 15 ml of dry benzene in conical flask. 10.0 ml of pyridine in a RBF (round bottom flask) of 250 ml capacity and kept on a magnetic stirrer. Then a very small amount of the aniline (parent compound) was added slowly to RBF. Then 1.866 ml of H_3PO_4 is added drop by drop. The whole reaction mixture was refluxed for 06 hours at 60 to 65°C. After the stirring is completed, the stirred material was kept open so as to evaporate the solvent. The reaction mixture was kept at room temperature over-night. Then the oily residue left in the flask transferred into separating funnel and it was treated with water. Two layers were separated. Aqueous layer contained mono-2, 6-dichloro-3-methylaniline phosphate and benzene layer was rejected. The milky solution was treated with diluted HCl to remove unreacted pyridine as pyridine hydrochloride, the clear filtrate Barium hydroxide was then added to it became alkaline and then white precipitate began to separate and the obtained precipitate was filtered and washed several times with distilled water (containing few drops of acetic acid) to remove the inorganic impurities. It was then dried to obtain Ba-salt of mono-2,6-dichloro-3-metylaniline organo phosphate ester.[3-4]

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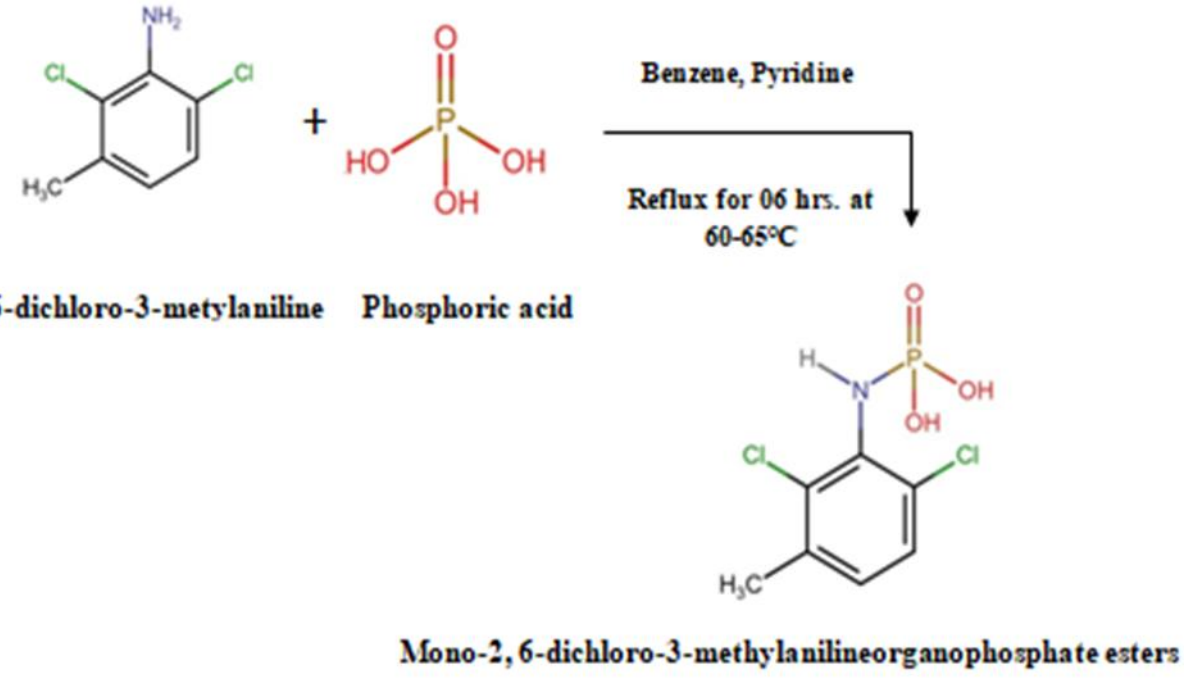
Synthesis of Mono-2, 6-dichloro-3-methylaniline organophosphate ester

[2.2] Chemical Reaction–



2, 6-dichloro-3-metylaniline **Phosphoric acid**

Mono-2, 6-dichloro-3-methylanilineorganophosphate esters

[2.3]FTIR Spectra of Mono-2,6-Di chloro-3- methyl aniline organophosphate ester

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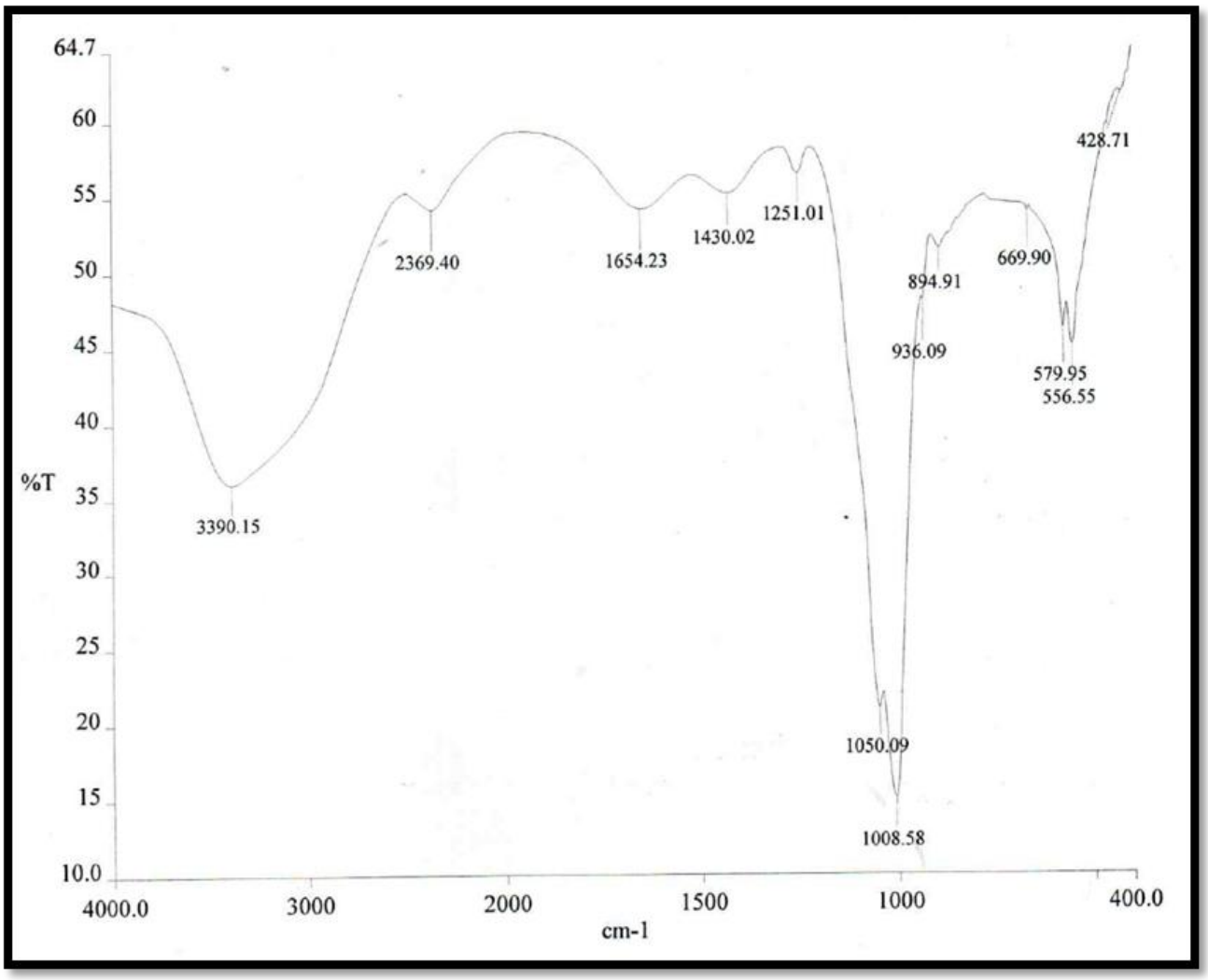

FTIR spectra of Mono-2, 6-dichloro-3-methylaniline organophosphate ester

[2.4] FTIR- Spectrum Frequency Range of Mono-2, 6-dichloro-3-methylaniline organophosphate ester

Sr. No.	Derivative	Frequency Range	Group Absorption (cm^{-1})	Appearance	Group	Compound Class
1	Mono-2, 6-dichloro-3-methylaniline organophosphate ester	3550-3200 (cm^{-1})	3390.15	Strong, Broad	O-H stretching	Hydroxyl Group
		2400- 2000 (cm^{-1})	2369.40	Strong	C-H stretching	Alkane
		2000-1650 (cm^{-1})	1654.23	Weak	C-H bending	Aromatic compound
		1500 -800 (cm^{-1})	1430.02	Weak	C-C stretching	Alkane
		1350-1200 (cm^{-1})	1251.01	Strong	C-N stretching	Aromatic amine
		1200-1000 (cm^{-1})	1050.09	Strong	P-N stretching	Compound containing Phosphate and Nitrogen group
		1020-930	1008.58	Strong	P-O	Phosphate group

		(cm^{-1})			stretching	
		1020-930 (cm^{-1})	936.09	Strong	P-O stretching	Phosphate group
		895-885 (cm^{-1})	894.91	Strong	C=C bending	Alkene
		730-665 (cm^{-1})	669.90	Strong	C=C bending	Alkene
		850-550 (cm^{-1})	579.95	Strong	C-Cl stretching	Halo compound
		850-550 (cm^{-1})	556.55	Strong	C-Cl stretching	Halo compound

In the FTIR spectrum of the mono-2, 6-dichloro-3-methylaniline organophosphate ester, several characteristic absorption peaks were observed. A strong, broad O–H stretching peak of the hydroxyl group appeared at 3390.15 cm^{-1}. Medium-intensity C–H stretching peaks corresponding to alkanes were observed at 2369.40 cm^{-1}. A C–H bending peak associated with an aromatic compound appeared at 1654.23 cm^{-1}, while a C–C stretching peak of an alkane was recorded at 1430.02 cm^{-1}. The C–N stretching peak of an aromatic amine was observed at 1251.01 cm^{-1}. A P–N stretching peak, indicative of the presence of both phosphate and nitrogen groups, appeared at 1050.09 cm^{-1}. P–O stretching peaks of the phosphate group were found at 1008.58 cm^{-1} and 936.09 cm^{-1}. Additionally, C=C bending peaks of an alkene were observed at 894.91 cm^{-1} and 669.90 cm^{-1}, while C–Cl stretching peaks of a halogenated compound appeared at 579.95 cm^{-1} and 556.55 cm^{-1}.

[2.5] Physico-Chemical Characteristics of Mono 2, 6-dichloro-3-methylaniline organophosphate ester

Compound –Chemical Name : Mono-2, 6-dichloro-3-methylaniline organophosphate ester
% Yield : 75 %
Melting Point : 330º C
Colour : White
Odour : Odorless
Appearance : Powder
State : Solid

[2.6] Solubility Studies of Mono 2, 6-dichloro-3-methylaniline organophosphate ester

Sr. no.	Solvents	Solubility
1	Petroleum Ether	Insoluble
2	Benzene	Insoluble
3	Ethyl acetate	Sparingly soluble
4	Acetone	Partially soluble
5	Chloroform	Sparingly soluble
6	Methanol	Soluble
7	Ethanol	Partially soluble
8	Water	Soluble
9	Dimethyl sulfoxide (DMSO)	Soluble
10	Dimethylformamide (DMF)	Soluble

[3.1] Synthesis of Di-2, 6-dichloro-3-metylaniline organophosphate esters-

Following the method of Auger and Dupis, this was prepared with some modification. A 2:1 ration of 2, 6-dichloro-3-metylaniline and H_3PO_4 were taken. 7.0 ml of pyridine was then added slowly. Stirred the solution by adding of 2-

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chloro-5-nitro aniline (3.45g) and H_3PO_4 (0.933 ml) in dry benzene (25.0 ml). Pyridine hydrochloride were then started to separate immediately as heat increases. The mixture of solution was placed on a magnetic stirrer at 60 to 65°C for a 10 hrs. After treatment of solution by water with (5%) NaOH that left the yellowish oily residue. Obtained filtrate was acidified with (5%) dilute HCl to precipitate the chloride. That was then washed with distilled water convers into di-ester. CCl_4 was dissolved to obtained impurities free di-ester**.[4]**

Figure 1 Synthesis of Di-2, 6-dichloro-3-methylaniline organophosphate esters

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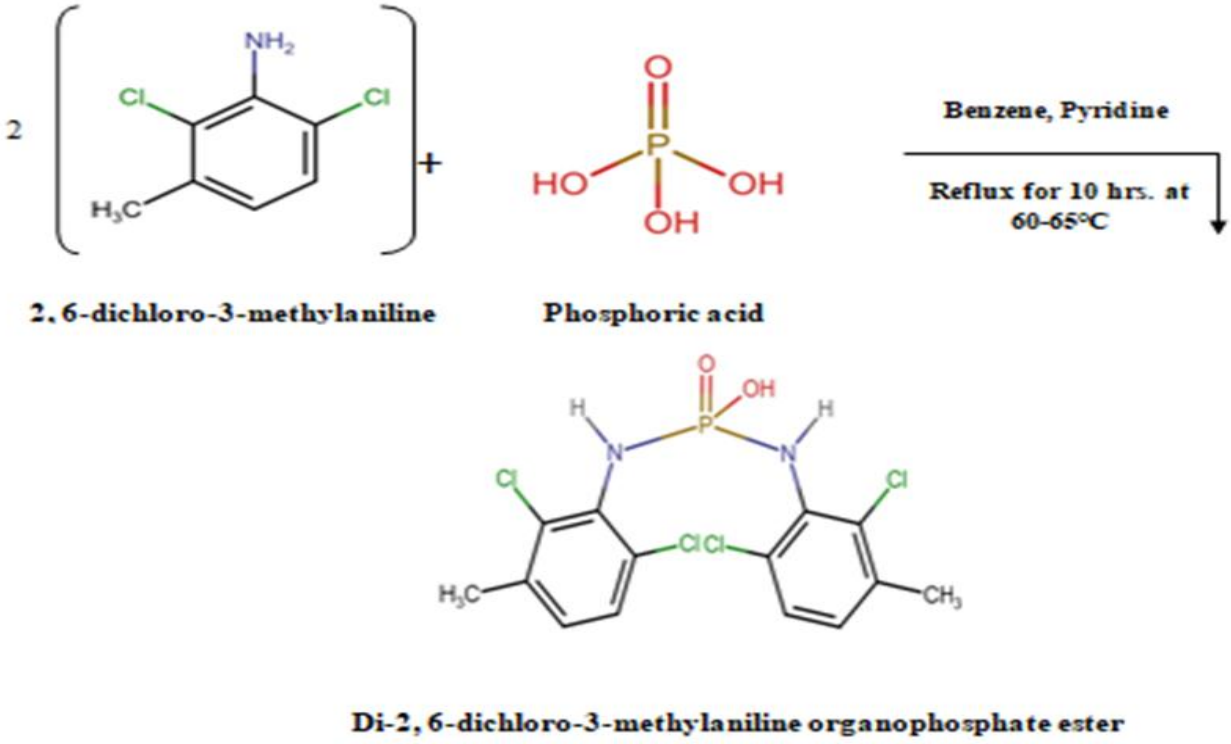
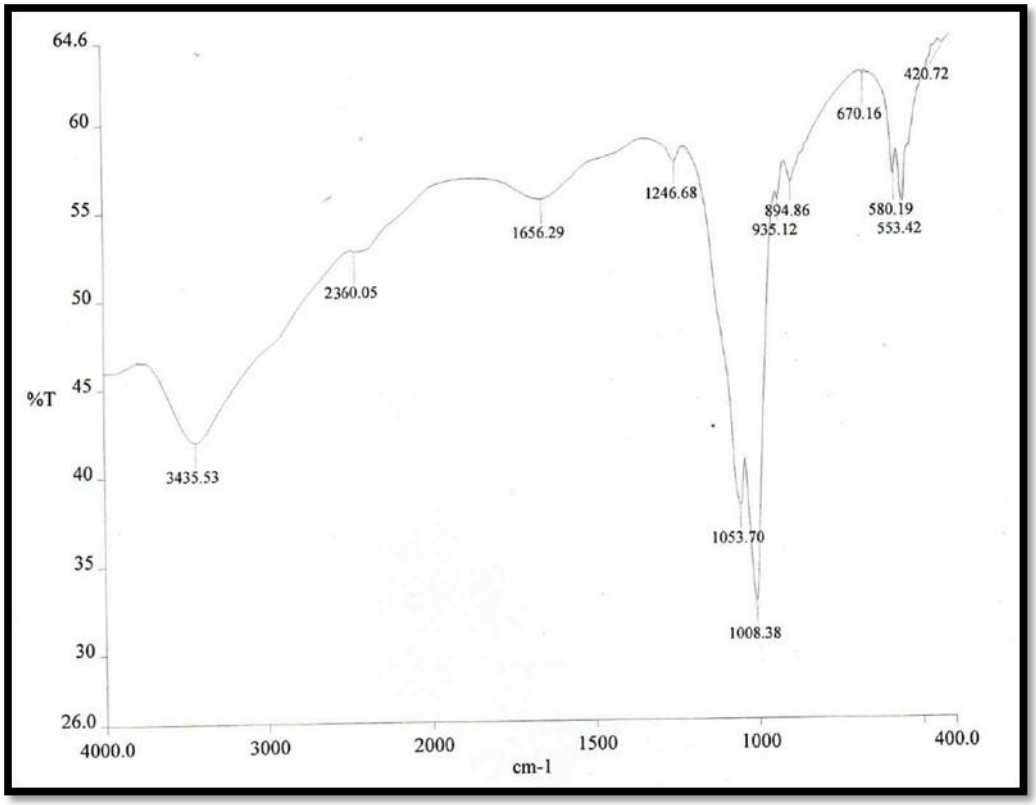
[3.2]ChemicalReaction:-



[3.3] FTIR spectra of Di-2, 6-dichloro-3-methylaniline organophosphate ester



FTIR spectra of Di-2, 6-dichloro-3-methylaniline organophosphate ester

[3.4] FTIR- Spectrum Frequency Range of Di-2, 6-dichloro-3-methylaniline organophosphate ester

Sr. No.	Derivative	Frequency Range	Group Absorption	Appearance	Group	Compound Class

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			(cm^{-1})			
2	Di-2, 6-dichloro-3-methylaniline organophosphate ester	3550-3200 (cm^{-1})	3435.53	Strong, Broad	O-H stretching	Hydroxyl Group
		2400- 2000 (cm^{-1})	2360.05	Strong	C-H stretching	Alkane
		2000-1650 (cm^{-1})	1656.29	Weak	C-H Bending	Aromatic compound
		1350-1200 (cm^{-1})	1246.68	Strong	C-N Stretching	Aromatic amine
		1200-1000 (cm^{-1})	1053.70	Strong	P-N Stretching	Compound containing Phosphate and Nitrogen group
		1020-930 (cm^{-1})	1008.38	Strong	P-O Stretching	Phosphate group
		1020-930 (cm^{-1})	935.12	Strong	P-O Stretching	Phosphate group
		895-885 (cm^{-1})	894.86	Strong	C=C Bending	Alkene
		730-665 (cm^{-1})	670.16	Strong	C=C Bending	Alkene
		850-550 (cm^{-1})	580.19	Strong	C-Cl Stretching	Halo compound
		850-550 (cm^{-1})	553.42	Strong	C-Cl Stretching	Halo compound

In the FTIR spectrum of the di-2, 6-dichloro-3-methylaniline organophosphate ester, several characteristic absorption peaks were observed. A strong, broad O–H stretching peak of the hydroxyl group appeared at 3435.53 cm⁻¹. Medium-intensity C–H stretching peaks of alkane were observed at 2360.05 cm⁻¹. A C–H bending peak associated with an aromatic compound appeared at 1656.29 cm⁻¹, while the C–N stretching peak of an aromatic amine was recorded at 1246.68 cm⁻¹. A P–N stretching peak, indicative of the presence of both phosphate and nitrogen groups, was observed at 1053.70 cm⁻¹. The P–O stretching peaks of the phosphate group appeared at 1008.38 cm⁻¹ and 935.12 cm⁻¹. Additionally, C=C bending peaks of an alkene were recorded at 894.86 cm⁻¹ and 670.16 cm⁻¹, while C–Cl stretching peaks of a halogenated compound were observed at 580.19 cm⁻¹ and 553.42 cm⁻¹.

[3.5]Physico-Chemical Characteristics of Di-2, 6-dichloro-3-methylaniline organophosphate ester –

Chemical Name : Di-2, 6-dichloro-3-methylaniline organophosphate ester
% Yield : 72 %
Melting Point : 336º C
Colour : White
Odour : Sweet and aromatic
Appearance : Powder
State : Solid

[3.6] Solubility Studies of Di- 2, 6-dichloro-3-methylaniline organophosphate ester

Sr. no.	Solvents	Solubility
1	Petroleum Ether	Partially soluble
2	Benzene	Sparingly soluble
3	Ethyl acetate	Slightly soluble
4	Acetone	Sparingly soluble

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5	Chloroform	Sparingly soluble
6	Methanol	Soluble
7	Ethanol	Soluble
8	Water	Partially soluble
9	Dimethyl sulfoxide (DMSO)	Soluble
10	Dimethylformamide (DMF)	Soluble

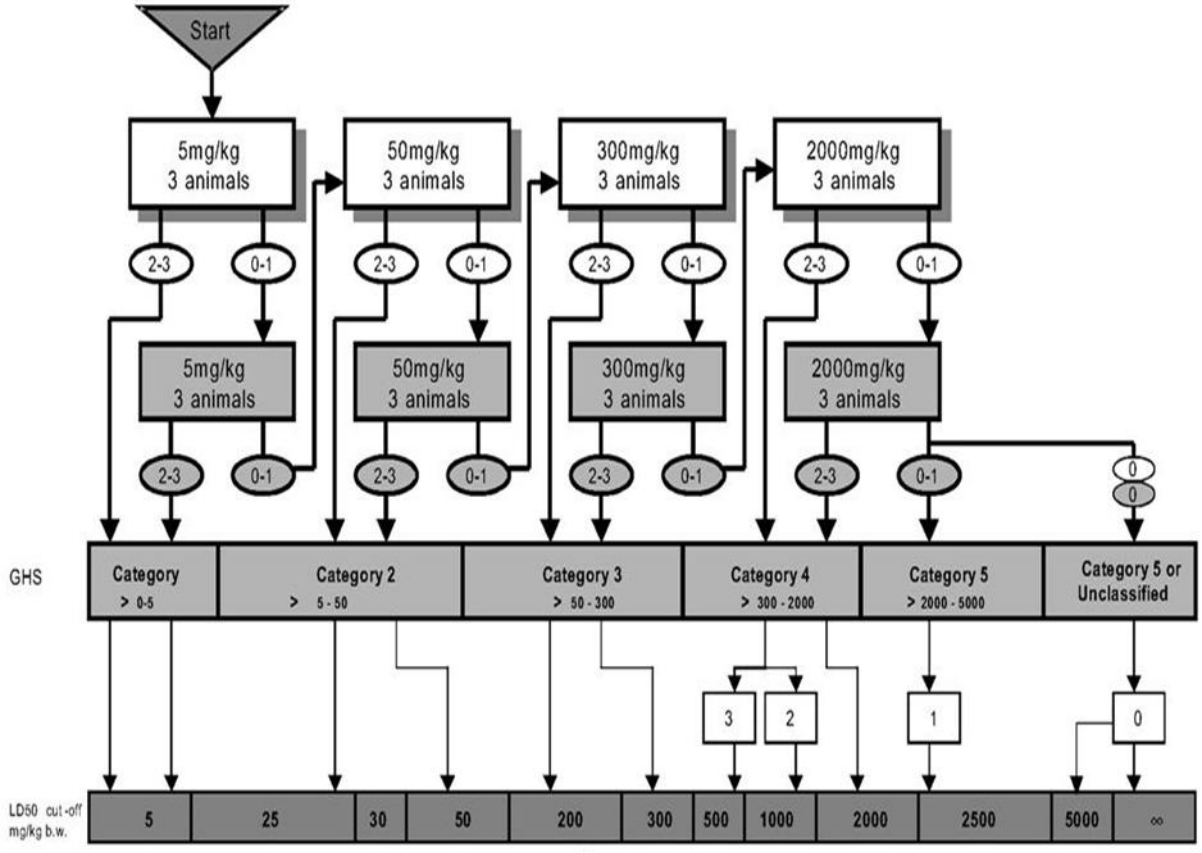
[4]Biological Activity – Acute Oral Toxicity Study of Mono-2, 6-Dichloro-3- Methyl aniline Organophosphate ester

[4.1]Acute oral toxicity study

Acute oral toxicity is a measure of the adverse biological effects that result from ingesting a chemical substance in a short period of time. It provides an estimate of how toxic a compound is when taken by mouth and helps determine its LD_{50} (lethal dose, 50%)—the dose.

[4.2]Acute oral toxicity study was checked as per the flow diagram of OECD 423 Guideline-

The acute toxic class method outlined in this guideline, is a stepwise procedure for use of three animals of single sex per step, which is depending on the mortality and/or moribund status of the animals, an average of 2–4 steps may be required to assess the acute toxicity of the test substance is administered orally into the group of experimental animals at one of the predefined dose levels. The testing procedure is illustrated in the explanatory diagrammatic representation below, with each step using three animals of a single sex .[5]



[4.3] Results

[4.3.1]Acute oral toxicity of Mono-2, 6-Dichloro-3- Methyl aniline Organophosphate ester -

The acute oral toxicity of the Mono-2, 6-dichloro-3-metylaniline phosphate esters was evaluated to assess their safety profiles. The study was conducted following standardized guidelines for acute toxicity testing (e.g., OECD Guideline 423), with observations made over a 14-day period post-administration. [5]

[4.3.2]Acute oral toxicity of Mono-2, 6-dichloro-3-metylaniline phosphate esters

Body weight changes and Mortality

Group	Dose (mg/kg)	Rat		Sex	Day of	Body Weight (gm)	No.

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		No.			Death	**0 Day**	**7 Day**	**14 Day**	dead/Tested
A	5 mg/kg	R1		M	--	163	167	172	0/3
	"	R2		M	--	169	174	175	
	"	R3		M	--	166	169	168	
B	50 mg/kg	R1		M	--	187	185	190	0/3
	"	R2		M	--	186	191	188	
	"	R3		M	--	190	200	202	
C	300 mg/kg	R1		M	--	179	169	185	0/3
	"	R2		M	--	188	180	195	
	"	R3		M	--	199	188	203	
D	2000 mg/kg	R1		M	--	178	166	162	0/3
	"	R2		M	--	182	170	167	
	"	R3		M	--	199	202	180	

[4.3.3]Acute toxicity behaviour changes of Mono-2, 6-dichloro-3-methylaniline phosphate ester

Sr. No.	**Toxicological parameters**	**Observations of isolated compound of Mono-2, 6-dichloro-3-metylaniline phosphate ester**			
		(5 mg/kg)	**(50 mg/kg)**	**(300 mg/kg)**	**(2000 mg/kg)**
1	Changes in skin and fur	Normal	Normal	Mild Hair loss	Hair Loss
2	Eyes	Normal	Normal	Normal	Normal
3	Mucous membranes	Normal	Normal	Runny nose	Runny nose
4	Salivation	Normal	Normal	Normal	Normal
5	Stool	Normal	Normal	Normal	Hard
6	Urine	Normal	Normal	Normal	Normal
7	Sleeping pattern	Normal	Normal	Normal	Normal
8	Behavior pattern	Normal	Normal	Normal	Normal
9	Somatomotor activity	Not seen	Not seen	Not seen	Not seen
10	Mortality (14 days)	No	No	No	No

The acute oral toxicity assessment of the isolated compound Mono-2, 6-dichloro-3-metylaniline phosphate esters was conducted at doses of 5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg, with observations recorded over a 14-day period.

At lower doses (5 and 50 mg/kg), animals exhibited no significant toxicological symptoms, and all physiological parameters remained normal. At 300 mg/kg, mild hair loss was noted, and at 2000 mg/kg, noticeable hair loss was observed, indicating a dose-related effect on skin and fur. At all tested doses, there were no indications of neurological or behavioral toxicity in the eyes, salivation, urine output, sleeping pattern, behavior pattern, and somatomotor activity.

However, animals at 300 mg/kg and 2000 mg/kg showed runny noses, suggesting some mucous membrane irritation at higher doses. Furthermore, only at 2000 mg/kg was hard stool observed, indicating a slight gastrointestinal impact at the maximum dose examined.

Crucially, during the 14-day observation period, there was no mortality in any dose group, suggesting that Mono-2, 6-dichloro-3-metylaniline phosphate esters have a high safety margin with regard to acute oral toxicity. Even at higher doses, mono-2, 6-dichloro-3-metylaniline phosphate esters showed good tolerability and minimal toxicity.

Mono-organophosphate ester derivatives have been shown to have no acute toxicity, as no deaths and no significant changes in behaviour have been recorded at the dose used. The minor signs including slight lethargy and reduced activity occurred for a short time only and passed within 24 hours; therefore, they indicate that mono-organophosphate ester derivatives are very tolerable.

[4.4.1] Acute toxicity of oral dose of the Di-2, 6-dichloro-3-methylaniline organophosphate ester

Body weight changes and Mortality

Group	**Dose (mg/kg)**	**Rat No.**	**Sex**	**Day of Death**	**Body Weight (gm)**			**No. dead/Tested**
					0 Day	**7 Day**	**14 Day**	
A	5 mg/kg	R1	M	--	171	175	173	0/3
	"	R2	M	--	170	173	179	
	"	R3	M	--	175	179	184	

B	50 mg/kg	R1	M	--	126	131	129	0/3
	"	R2	M	--	125	145	138	
	"	R3	M	--	130	146	141	
C	300 mg/kg	R1	M	--	147	-	-	2/3
	"	R2	M	--	157	129	-	
	"	R3	M	--	174	146	105	
D	2000 mg/kg	R1	M	--	161	-		3/3
	"	R2	M	--	160	-	-	
	"	R3	M	--	157	-	-	

[4.4.2]Acute toxicity behavior changes of Di-2, 6-dichloro-3-methylaniline organophosphate ester

Sr. No.	Toxicological parameters	Observations of synthesized compound of Di-2, 6-dichloro-3-methylaniline organophosphate ester			
		(5 mg/kg)	(50 mg/kg)	(300 mg/kg)	(2000 mg/kg)
1	Changes in skin and fur	Hair loss	Hair loss	Hair loss	Hair loss
2	Eyes	Normal	Normal	Normal	Flaky eyes
3	Mucous membranes	Normal	Runny nose	Bleeding Nose	Bleeding Nose
4	Salivation	Normal	Normal	Normal	Normal
5	Stool	Normal	Diarrhea	Hard stool	Constipation
6	Urine	Normal	Normal	Normal	frequent
7	Sleeping pattern	Normal	Normal	Normal	Sleepless
8	Behavior pattern	Normal	Aggressive	Lazy	Lazy
9	Somatomotor activity	Not seen	Not seen	Not seen	Not seen
10	Mortality (14 days)	No	No	Yes	Yes

The acute oral toxicity profile of the synthesized compound of Di-2, 6-dichloro-3-methylaniline organophosphate ester was evaluated at increasing dose levels of 5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg. A range of clinical signs was recorded over a 14-day observation period post-administration.

Hair loss was continuously observed at all doses and thus it was concluded that the effect on the skin and fur was not dependent on the dose. The ocular condition of the test animals was good at the lower doses while there was slight flakiness at the highest test dose (2000 mg/kg). The condition of the mucous membranes became more impacted as doses increased: it was normal at 5 mg/kg, runny nose at 50 mg/kg, and bleeding nose at both 300 and 2000 mg/kg. Salivation was normal in all groups of animals.

The gastrointestinal effects were dose dependent: 5 mg/kg caused no alteration in stool while at 50 mg/kg diarrhea, at 300 mg/kg hard stool, and at 2000 mg/kg constipation were observed. At low doses urine output was normal, but at the highest dose it became frequent. The sleeping pattern was not disturbed up to 300 mg/kg; however, sleeplessness was reported at 2000 mg/kg.

Among the noticeable changes in the behavior of the experimental animals were the aggressiveness observed at 50 mg/kg and the laziness observed at the higher doses. The somatomotor activity was completely absent in all groups. What is more, no deaths were registered in the groups administered with 5 and 50 mg/kg, while death was experienced in the groups receiving 300 and 2000 mg/kg, thus confirming dose-dependent toxicity. The compound Di-2, 6-dichloro-3-methylaniline organophosphate ester has a relatively low toxicity at lower doses and moderate toxicity at higher doses, which, however, coems to be the reason for its significant potential toxicity and lethality at the highest dose points. This situation draws the attention of a careful selection of doses for the next pharmacological or toxicological investigations.

The di-2, 6-dichloro-3-methylaniline organophosphate ester caused greater harm in an acute way, for instance, some test animals showed short-lived signs of discomfort like raised fur, less eating, and small loss of weight. Deaths occurred at high doses, which means that the di-2, 6-dichloro-3-methylaniline substituted organophosphate ester compound is much more toxic than the mono substituted analogs at least in terms of lethality.

[5] Overall Conclusion

The findings are unambiguously pointing that the increased substitution of the phosphate ester backbone affects not only the electronic and structural properties of the compounds but also their biological safety profiles. The three compounds were successfully synthesized and their structures were confirmed, but only the mono-2, 6-dichloro-3-methylaniline substituted organophosphate ester exhibited consistently low toxicity and offered favorable physicochemical

characteristics. The di-2, 6-dichloro-3-methylaniline substituted organophosphate ester, on the other hand, demand cautious dose consideration and further safety assessment before any possible use.

The data presented are a crucial starting point for the organophosphate derivatives' design, synthesis, and toxicity assessment, and are potentially useful for subsequent research on the discovery of safer and better derivatives for agrochemical, pharmaceutical, or chemical purposes.

The research distinctly shows that the incorporation level on the phosphate ester backbone is the one aspect that characterizes the organophosphate derivatives both in their physicochemical behavior and in their biological safety. The improved safety profile and stability of mono-2, 6-dichloro-3-methylaniline organophosphate ester indicate that a reduction in substitution may lessen the inherent reactivity of the phosphate group, thus decreasing the chances of cholinergic toxicity and making it more compatible with living organisms. On the other hand, the increased toxicity in the di-2, 6-dichloro-3-methylaniline substituted organophosphate ester analogues clearly demonstrates that higher substitution can lead to the alteration of the molecular polarity, steric configuration, and electronic distribution, which in turn could facilitate their being picked by biological targets like acetylcholinesterase. These results underline the necessity of structural optimization and careful alteration of substitution patterns during compound manufacture so as to secure the best possible compromise between activity and safety. Generally, this research presents a robust experimental and theoretical foundation for the logical design of next-generation organophosphate-based materials, thus redirecting the research effort towards less, more selective, and application-specific chemical entities in agriculture, industry, and pharmaceuticals that are safe and efficient.

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