CHAPTER - 1

INTRODUCTION
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Forensic Science Laboratories provides scientific aids to the Police Investigating Officers in crime investigation, pertaining to crimes registered under various acts of Indian Penal Code and criminal procedure code etc. Forensic Science Laboratories are multidisciplinary institution doing highly specialized and sophisticated analytical work.

The indispensable analytical reports issued by the forensic science laboratories help the police and the Judiciary in the detection of crime and administration of criminal justice by providing objective scientific evidence against the guilty or at times clearing the innocent.

Ordinarily, a forensic science laboratories has the following main divisions. (1) Chemistry including toxicology, (2) Ballistics, (3) Biology-Serology, (4) Physics and chemical instrumentation. The main laboratory examination is directed towards materials identification. The main and biggest division of forensic science laboratory is forensic chemistry and toxicology which pressed into service whenever a chemical or toxicological examination such as identification of drugs and/or insecticides, like poisonous materials is required in a suspected articles or in a biological materials like viscera, or postmortem blood, stomach wash etc. of a victim of poisoning. The present day requirement is not merely to identify the toxic material qualitatively by
general and non-specific tests of yester years but also to identify it specifically, using efficient extraction procedures and modern techniques like thin-layer chromatography, gas chromatography, spectrophotometry etc.

In the recent years, use of different types of insecticides and fungicides is increased in agriculture to protect the crops and commercial plants from insects, to get good yield and also they are often used in houses to kill the mosquitoes, cockroaches and bed bugs. Easy availability of these insecticides frequently misused in suicidal and homicidal poisoning. Consequently their detection and determination in biological materials such as viscera, stomach-wash, blood, urine, vomit sample etc. and in non-biological materials such as clothes of deceased, container of poisons material, earth mixed with vomit etc. is the problems of forensic toxicology.

Insecticides are mainly divided into four groups: These are (1) Organophosphorus, (2) Organochloro, (3) Carbamate, and (4) Pyrethroid insecticides.

Organophosphorus Insecticides

As such today, organophosphorus compounds form an important class of pesticides. More than 100,000 different organophosphorus compounds have been synthesised and evaluated as pesticides, out of these more than 80 are widely used in agriculture.
Inspite of the enormous structural diversity of organophosphorus insecticides, all the compounds can be represented by the classical hypothetical structure as

\[
\begin{align*}
 & R' \quad \text{O(S)} \\
 & \quad \text{P} \quad X \\
 & R
\end{align*}
\]

where \( R \) and \( R' \) are short chain alkyl, alkoxy, alkylthio or amide groups and \( X \) is a labile leaving group or groups that can be metabolised in vivo to a labile entity. The biological activity of the organophosphorus compounds is due to the capacity of the central 'P' atom to phosphorylate the active site of the enzyme cholinesterase (chE) which is an essential constituent of the nervous system, not only of insects but also of higher animals. The phosphorylated enzyme is irreversibly inhibited and is, therefore, no longer able to carry out its normal functions of rapid removal and destruction of neurochrome (Acetylcholine) from the nervous synapse. This results in the accumulation of acetylcholine and consequent disruption of the normal functioning of the nervous system giving rise to typical cholinergic symptoms associated in insects with organophosphorus poisoning, like hypersensitivity, hyperactivity, tremors, convulsions, paralysis and death.

The chemical structure of some of the organophosphorus and thiophosphorus insecticides are given in figure 1.
Various methods have been reported in the literature for the detection and determination of organophosphorus insecticides. These include Gas chromatography, Spectrophotometry, high performance liquid chromatography, Fluorometry etc. But all these methods are reported for pure compounds, or for formulated products, or for extract from water samples, grains etc. and are highly susceptible to the impurities co-extracted with insecticides from biological materials (such as viscera, blood, urine and stomach-wash) in poisoning cases. However, an elaborate clean-up procedure is essential for their detection in biological materials by above reported methods. Hence Thin-layer chromatography is the method of choice for the detection and determination of these insecticides in biological and non-biological materials in poisoning cases. TLC method can be successfully used for the separation, detection and determination of pesticide residues in biological and non-biological materials.

Most TLC analyses have been carried out by traditional one dimensional ascending development in a glass tank at ambient temperature. The analyses are performed on silica gel G particle size 15 μm or high performance silica gel layers including an organic binder, but chemically bonded or impregnated layers.
ORGANOCHELORINE INSECTICIDES

Organochlorine insecticides are widely used in agriculture for the protection of crops. The extreme toxicity of these insecticides towards insects particularly those affecting man and its low toxicity against mammals helped its rapid spread. DDT (Dichlorodiphenyl trichloro-ethane) is the first synthetic insecticide. This compound first synthesised by Zeidler in 1874 was tested as an insecticide by Swiss workers during 1939-1940, and was found to be very effective. However, DDT is being used much less at present for housefly control, since the fly has developed resistance to DDT.

Recently the use of BHC, endosulfan and endrin has been continually increasing in India and this is reflected in the increasing number of criminal cases referred to forensic science laboratories, concerning the misuse of these insecticides. Out of these, benzene hexachloride (BHC) was first synthesised by Michael Fersdrey in 1825. In 1942, the Imperial Chemical Industries Laboratories in England found this compound to have considerable insecticidal properties but did not give considerable encouragement since consistent results were not obtained. This inconsistency was later found to be due to the presence of isomers of different toxicity.
Endosulfan (thiodan) is the adduct of hexachlor cyclopentadiene and 1,4-dihydroxy-2-butane, subsequently chlorinated with $\text{SO}_2\text{Cl}_2$ to produce the endosulfan. The technical material is a brownish solid, M.P. 70 to 100°C, consisting of about 4 parts of $\alpha$-cis isomer and one part of $\beta$-trans isomer. The symptoms of poisoning in insects are hypersensitivity, hyperactivity with violent bursts of convulsions and finally complete prostration with convulsive movements. The site of these disturbances lies in the ganglia of the central nervous system.

Endrin is a stereoisomer of dieldrin. It is produced by the oxidation of isodrin with hydrogen peroxide in acetic acid at the lowest possible temperature. Endrin is a white crystalline solid melting at 200°C. It is insoluble in water but is soluble in most organic solvents. Endrin exceeds in toxicity for man, domestic animal and insects than many other compounds of this series and hence recently baned by the government of India.

Other organochlorine insecticides such as chlordane, Heptachlor, Aldrin, Dieldrin, Isodrin and Toxaphene are also available in India but has a limited use for the protection of crops in agriculture. The chemical structure of some of the organochlorine insecticides are given in Figure 2.

A gas-chromatographic methods have been described in the literature for the detection and determination of
organochlorin insecticides in food materials, milk, water samples, urine, meat and meat products and in formulated products. However, a tedious and time-consuming cleanup procedure is essential before GC analysis. Hence TLC is the suitable and appropriate method for the detection of chlorinated pesticides in biological and non-biological materials in forensic case work.

**CARBAMATE INSECTICIDES**

The N-methyl and N,N-dimethylcarbamic esters of phenols and heterocyclic enols possess useful insecticidal properties. Heterocyclic N,N-dimethyl carbamate compounds were discovered by Gysin. The two important insecticides that were isolated are Isolan and Dimetelan. Aromatic N-methylcarbamates are derivatives of phenyl-N-methyl-carbamates with a great variety of groups like chloride, alkyl, alkylthio, alkoxy- and dialkylamino- in the side chain. The carbamate insecticides are generally crystalline materials of low water solubility but soluble in organic solvents. Among the derivatives of carbamic acid, the aryl esters of N-methyl carbamic acid are used for control of insect pests. Maximum insecticidal effect is shown by the aryl esters of N-methyl carbamic acid.

The esters of N-alkylcarbamic acids cause inhibition of cholinesterase. The monosubstituted phenyl N-methyl carbamates depress the cholinesterase activity more strongly
as the resistance of the compound to hydrolysis increases. The greatest activity is shown by 1-naphthyl-N-methyl carbamate which is used in agriculture under the name sevin or carbaryl or Naphthylcarbamate. Similarly, 2-isopropoxy phenyl-N-methylcarbamate i.e. propoxur or Baygon is widely used as a house-hold insecticide against bed bugs, mosquitoes and house fly etc.

Other carbamate insecticides such as carbofuran (Furadan, 2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate), Temik (Aldicarb, 2-methyl-2-methylthiopropionyl-oxime-o,N-methyl carbamate) are also in use in India as an effective insecticide in agriculture. Figure 3 shows the structural formula of some of the carbamate insecticides.

Among the various analytical methods used for the detection and determination of carbamate insecticides, TLC is the most useful and most applicable method for the detection of these insecticides in biological material in forensic toxicology.

**PYRETHROID INSECTICIDES**

Naturally occurring pyrethrum has been used for the control of harmful insects for many centuries. The insecticidal principle in pyrethrum is found in the flower heads of certain plants of chrysanthemum genus, family compositae. Only a few species of this large family like Chrysanthemum
(Pyrethrum) roseum Web and Mohr., C. cinerariefolium Trev., C. marshalli Ach. and C. tamrutene have been found to be a valuable source of this insecticide.

The structure of the active principle in pyrethrum was established only in 1950. Staudinger and Ruzicka found two active principles Pyrethrin I and Pyrethrin II which were esters of a keto alcohol (Pyrethrolone) and two acids (Chrysanthemum monocarboxylic acid and C. dicarboxylic acid). Two more compounds were identified and are named as cinerins I and II. All these compounds are viscous liquids, soluble in a variety of solvents but not in water.

Pyrethrum affects the nervous system of insects such as cockroaches, resulting in muscular excitation, convulsion and paralysis.

With the structural requirements for the photostable pyrethroids, many new compounds which retained the chemical and stereochemical features of parent materials and their biological activity were synthesised during 1973-1977. In 1974 Okno and others described highly active compounds like cypermethrin, fenvalerate and deltamethrin. These compounds are viscous lipophylic liquids having high boiling point and low volatility. They are practically insoluble in water, highly photostable, biodegradable, have low mammalian toxicity, and do not leave residues in the biological
systems. Their movement from the site of application is limited in the environment due to their low volatility in soil and in plants as they are nonsystemic in nature because of low polarity. However, they are found to be effective even at 1/10 to 1/50th of the normally recommended rates of conventional pesticides.

TLC is found to be very useful for the detection of unabsorbed quantity of these pyrethroid insecticides, present in the stomach and intestine in homicidal and suicidal poisoning cases referred to forensic science laboratory.

The chemical structure of some of the pyrethroid insecticides are shown in Figure 4.

**DRUGS - BENZODIAZEPINES**

Various types of drugs are frequently encountered in forensic case work. Their characterization in biological and non-biological material is the problem in front of forensic scientists. According to the action on the body of living animals, these drugs are divided into different groups. These are sedatives, hypnotics, narcotics, tranquilizing, analgesic, antipyretic, anaesthetics and so on. These drugs, in a therapeutic dose, are used as a medicine to cure the diseases. But in over doses, they show poisonous effects on the body. Hence these drugs sometimes
are misused for homicide and suicide, sometimes due to negligence they are consumed in over doses, and ultimately show poisonous effect and death.

Now-a-days, the use of benzodiazepine drugs are increasingly being used for clinical aspects, because of its hypnotic, tranquilizing and anticonvulsing properties. Easy availability of these drugs sometimes misused for road robbery, and for narcotic action.

Due to effective hypnotic and tranquilizing properties of benzodiazepines, they are frequently encountered in clinical and forensic case work samples involving road traffic offences, road robbery and/or drug overdoses.

On September 1985 amendments were made to the misuse of Drugs Act such that 22 benzodiazepines become controlled as class C drugs. These changes were made so that the India complied with the requirements of the United Nations Convention on Psychotropic substances, 1971.

The chemical structure of certain 1,4-benzodiazepines which are generally used in India for clinical purpose are shown in Fig. 5.

For the detection and determination of benzodiazepines different methods such as chromatographic, spectroscopic, mass spectrometric have been described in the literature.
Many benzodiazepines are readily broken down into benzo-phenone derivatives under the conditions used to hydrolyse tissues prior to toxicological analysis. Thus several workers prepared the benzophenones from the parent benzodiazepines and their GC, TLC and MS properties were described. Among these techniques thin-layer chromatographic technique is found to be more suitable for separation, detection and determination of benzodiazepines in biological and non-biological materials in clinical and forensic case work.
CHEMICAL STRUCTURES OF SOME OF THE
ORGANOPHOSPHORUS AND THIOPHOSPHORUS INSECTICIDES

**Dichlorvos**: (DDVP, Nuvan, 0,0-dimethyl 0,2,2-dichlorovinyl phosphate)

\[
\begin{align*}
\text{H}_3\text{CO} & \quad \text{P} \quad \text{O} \quad \text{C} = \text{C} \quad \text{C} \quad \text{Cl} \\
\text{H}_3\text{CO} & \quad \text{Cl}
\end{align*}
\]

**Phosphamidon**: (Dimecron, 0,0-dimethyl-0(2-chloro-N,N-diethyl-carbamyl)-methylvinyl phosphate)

\[
\begin{align*}
\text{H}_3\text{CO} & \quad \text{P} \quad \text{O} \quad \text{C} = \text{C} \quad \text{C} \quad \text{N} \quad \text{C}_2\text{H}_5 \\
\text{H}_3\text{CO} & \quad \text{CH}_3 \quad \text{Cl} \quad \text{Cl} \quad \text{O} \quad \text{C}_2\text{H}_5
\end{align*}
\]

**Ethyl parathion**: (0,0-diethyl-0-4-nitrophenyl thiophosphate)

\[
\begin{align*}
\text{HCO} & \quad \text{S} \quad \text{P} \quad \text{O} \quad \text{N} \quad \text{O}_2 \\
\text{HCO} & \quad \text{C}_5\text{H}_2
\end{align*}
\]

Fig. 1
**Methyl parathion**: (0,0-dimethyl-0-4-nitrophenyl thiophosphate)

**Fenitrothion**: (Sumithion : 0,0-dimethyl-0-4-nitro-3-methyl phenyl thiophosphate)

**Malathion**: (0,0-dimethyl-3-1,2-dicarboethoxyethyl-dithiophosphate)

**Dimethoate**: (Rogor : 0,0-dimethyl-3-(N-methyl-carbamoylmethyl) dithiophosphate)

**Thimet**: (Phorate : 0,0-diethyl-3-(ethylthiomethyl) dithiophosphate)

*Fig. 1*
CHEMICAL STRUCTURES OF SOME OF THE ORGANOCHLORINE INSECTICIDES

**DDT** : (Dichlorodiphenyl trichloroethane)

![DDT structure]

**BHC** : (Gammexane : Benzene hexachloride; 1,2,3,4,5,6-hexachlorocyclohexane)

![BHC structure]

**Endosulfan** : (Thiodan; 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexa-hydro-6,9-methano 2,4,3-benzo-dioxathiepin-3-oxide)

![Endosulfan structure]

Fig.-2
Aldrin: (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endoexoxo-5,8-dimethano naphthalene)

Dieldrin: (1,2,3,4,10,10-hexachloro-6,7,epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4,5,8 endo,exo-dimethano-naphthalene)

Endrin: (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4, 5,8-endo endo dimethano naphthalene)

Fig-2
CHEMICAL STRUCTURES OF SOME OF THE CARBamate INSECTICIDES

Carbaryl: (Sevin; 1-Naphthyl-N-methyl carbamate)

\[
\text{OCONHCH}_3
\]

Propoxur: (Baygon; 2-Isopropoxy phenyl-N-methyl carbamate)

\[
\text{OCONHCH}_3
\text{OCH(CH}_3\text{)}_2
\]

Carbofuran: (Furadan; 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate)

\[
\text{OCONHCH}_3
\]

Temik: (Aldicarb; 2-methyl-2-methylthiopropional-oxime-0,N-methyl carbamate)

\[
\text{CH}_3
\text{CH}_3\text{S}\text{CH}--\text{NOCONHCH}_3
\text{CH}_3
\]

Fig-3
CHEMICAL STRUCTURES OF SOME OF THE PYRETHROID INSECTICIDES

Fenvalerate: (Cyano(3-phenoxyphenyl) methyl-4-chloro-alpha-(1-methyl ethyl) benzene-acetate

\[
\begin{align*}
\text{Cl} & \quad \text{O} \quad \text{C} \equiv \text{N} \\
\text{Cl} & \quad \text{H} \quad \text{H} \quad \text{C} \equiv \text{C} \quad \text{C} \equiv \text{O} \\
\text{CH}_3 & \quad \text{H} \quad \text{H} \quad \text{O} \quad \text{CN} \\
\end{align*}
\]

Cypermethrin: \(-\alpha\)-Cyano-3-phenoxy benzyl-cis trans-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate.

\[
\begin{align*}
\text{Cl} & \quad \text{H} \quad \text{H} \quad \text{C} \equiv \text{C} \quad \text{C} \equiv \text{O} \\
\text{CH}_3 & \quad \text{H} \quad \text{H} \quad \text{O} \quad \text{CN} \\
\end{align*}
\]

Deltamethrin: \(-\alpha\)-Cyano-3-phenoxy benzyl-cis-3-(2,2-dibromovinyl)-2,2-dimethyl cyclopropane carboxylate.

\[
\begin{align*}
\text{Br} & \quad \text{C} \equiv \text{CH} \quad \text{C} \equiv \text{C} \quad \text{C} \equiv \text{O} \quad \text{CN} \\
\text{Br} & \quad \text{H} \quad \text{H} \quad \text{O} \quad \text{CN} \\
\text{H}_3\text{C} & \quad \text{CH}_3 \quad \text{H} \quad \text{H} \\
\end{align*}
\]

Fig. - 4
CHAPTER 1, FIGURE 5: CHEMICAL STRUCTURES OF SOME OF THE BENZODIAZEPINES

Diazepam: 7-Chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one

Nitrazepam: 1,3-dihydro-7-nitro-5-phenyl-2H-1,4-benzodiazepin-2-one

Fig. 5
**Oxazepam**: 7-Chloro-1,3-dihydro-3-hydroxy-5-phenyl-2H-1,4-benzodiazepin-2-one

![Oxazepam Structure](image)

**Chlordiazepoxide**: 7-Chloro-N-methyl-5-phenyl-3H-1,4-benzodiazepin-2-one amine-4-oxide

![Chlordiazepoxide Structure](image)

**Fig. 5**