CHAPTER I

GENERAL INTRODUCTION AND LITERATURE SURVEY
1A.1 General Introduction

In the present era, the advances of synthetic chemistry and changing conditions of living standard have placed an ever-increasing number of highly poisonous but chemically unstable substances within the reach of modern man. Nowadays, so many people are interested in using large numbers of chemicals as a medicine, even though medical practitioners prescribe so many medicines. Such substances in prescribed dose are medicine, but in case of overdose it becomes poison. Such substances play a primary, secondary, direct or indirect role in bringing about unnatural death. These types of cases are received for chemical analysis in Forensic Science laboratories.

1A.2 Historical Approach of Forensic Science

The combination of a medical and legal approach is dealing with crime used by United States today were formulated by British people in the 12th century, when King Richard I established “Office of the Coroner”. The coroner's main duty was to keep a record of all criminal cases in county. The office of coroner was also responsible for investigating all deaths thought to be the result of suicide or homicide.

With time, the need for a more scientific investigation of unnatural deaths became apparent, and coroners began calling on physicians for help. Over the centuries, it became clear that medical schools needed to prepare doctors for this responsibility. As a result, in 1807 the University of Edinburgh in Scotland established a Department of Legal Medicine. There are as many as 1100 forensic science laboratories in 89 countries. Crime in some form or the other has existed since the beginning of human race. With the advancement in science and technology the concept of crime as well as the methods adopted by criminals in its commission has undergone a phenomenal change. On one hand the intelligent criminal has been quick to exploit science for his criminal acts; on the other hand the investigator is no longer able to rely on age old art of interrogation and methods to detect crime. In this context FORENSIC SCIENCE has found its existence.
1A.3 History of forensic Toxicology

Until the 1700s convictions associated with homicidal poisoning were based only on circumstantial evidence rather than the identification of the actual toxicant within the victim. In 1781, Joseph Plenic stated that the detection and identification of the poison in the organs of the deceased was the only true sign of poisoning. Years later, in 1813, Mathieu Orfila (considered the father of toxicology) published the first complete work on the subject of poisons and legal medicine. By 1836, James M. Marsh developed a test for the presence of arsenic in tissue. Then, in 1839, Orfila successfully used Marsh's test to identify arsenic extracted from human tissues. By 1918, the Medical Examiner's Office and Toxicology Laboratory was established in New York. The chief forensic toxicologist was Alexander O. Gettler who is considered the father of American toxicology.

The Forensic Toxicology is important branch in Forensic Science dealing with the detection of foreign compound, which responsible for the death or ill effect. The report or the analytical findings of Forensic Toxicologist is having significant role in crime detection particularly in homicidal cases. In most of the homicidal cases the ingested compound is in lesser amount, which is forcibly ingested or administered through vehicle. The widely used vehicles for administration of poison are tea, coffee, liquors, cold-drinks, biscuits and food materials. The accuse always tries to adulterate the toxic compounds in small quantity to ignore the reaction from the victim.

In most of the homicidal and suicidal poisoning cases medical officer preserves organs such as stomach, intestine, liver, spleen, kidney, heart, brain etc. and the biological fluids such as blood, urine, vomits. All these samples are submitted to Forensic Toxicology Department for the detection of foreign compound or the toxic compounds.

Analytical toxicology is an important branch of forensic Science. The number of fatal poisoning cases received for toxicological analysis is constantly increasing. There is tremendous rise in the use of insecticide; pesticide and other potentially poisonous substances in last five-year for crop protection. Former use these
insecticides, pesticides which were misused lead to suicidal, homicidal or accidental poisoning cases. In developed country sedative, tranquilizer and other synthetic drug are commonly misused. In India misuse of such drug in confined to big cities also. Misuse of insecticide/pesticide is very high in India.²

1A.4 Global Trends of Poisoning

Developing Nations:
  • Pesticide Poisoning
  • Narcotic drugs poisoning

Developed Nations:
  • Sedatives
  • Anti depressants
  • Harmful Chemicals
  • Drugs overdose & Medicine Overdose

Table No. 1 shows percentage of poisoning cases in various region of the world.

Table No. 1: Pesticide poisoning in Global

<table>
<thead>
<tr>
<th>Name</th>
<th>Percentage of pesticide poisoning cases</th>
<th>Name</th>
<th>Percentage of pesticide poisoning cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europe</td>
<td>3.0</td>
<td>Africa</td>
<td>22.9</td>
</tr>
<tr>
<td>America</td>
<td>4.9</td>
<td>S. E. Asia</td>
<td>40</td>
</tr>
<tr>
<td>E. Medeterrain</td>
<td>16.5</td>
<td>W. Pacific</td>
<td>34.8</td>
</tr>
</tbody>
</table>
Studies in developed countries have demonstrated the annual incidence rates of acute poisoning in agricultural workers to be as much as 18.2 per 100,000 full-time workers. World Health Organization (WHO) estimated 0.3 million people die every year due to various poisoning agents. Acute pesticide poisoning is one of the most common causes of intentional deaths worldwide. High doses of analgesics, tranquilizers, and antidepressants are the commonly used agents for intentional poisoning in industrialized countries and agriculture pesticides are used in Asian region for self-poisoning particularly in rural areas. Majority of pesticide exposure is seen more in middle and low income countries due to increased use of agrochemicals in agricultural sector. Table No. II shows trends of pesticide poisoning in India and neighboring countries.

Table No. 2: Trends of pesticide poisoning in India and neighboring countries

<table>
<thead>
<tr>
<th>Percentage of poisoning cases</th>
<th>India</th>
<th>China</th>
<th>Japan</th>
<th>Pakistan</th>
<th>Bangladesh</th>
<th>Thailand</th>
<th>Malaysia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pesticide</td>
<td>81</td>
<td>28</td>
<td>7</td>
<td>39</td>
<td>55</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Other than Pesticides</td>
<td>19</td>
<td>72</td>
<td>93</td>
<td>61</td>
<td>45</td>
<td>84</td>
<td>84</td>
</tr>
</tbody>
</table>
Table No 2 shows there is tremendous rise in pesticide poisoning cases due to easy availability of pesticides for crop protection. Their misuse has led in rise of suicidal, homicidal and accidental poisoning cases in India. As compared to other developed countries like Japan, percentage of pesticide poisoning is less because use of pesticides is restricted. But percentage of pesticide poisoning cases is increased in countries like Bangladesh and Pakistan as India because poverty and easy availability of pesticides.

1A.5 Pesticides

It is known fact that for our existence we have struggle hard and hard even it may be for small cause or great. It is of great importance to struggle against insects as they create havoc by destroying the crops or spreading the disease which we call it is an insect born diseases. So it becomes a must to be destroying these insects.

In 20th century the use of pesticide is tremendously increased. Integrated Pest Management (IPM) involves adoption of different technique of pest control, so as to minimize the losses of food production, replacement of older by newer pesticides and many a times combination of two or more pesticides is marketed. Unfortunately because of their ready availability these are, often misused for homicidal or suicidal purpose. Toxicologist is facing problem in identification of these compound. 4 - 9

1A.6 Definition of Pesticides
Food and Agriculture association has defined the term of pesticide as any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant or agent for thinning fruit or preventing the premature fall of fruit, and substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport.

1A.7 Classification of Pesticides

Pesticides can be classified or grouped in many different ways:

**According to pests control**

- Insecticides (insects)
- Fungicides (fungi)
- Bactericides (bacteria)
- Herbicides (weeds)
- Acaricides, miticides (mites)
- Rodenticides (rodents)

**According to source**

- Synthetic pesticides (Chemicals manufactured by humans, they do not occur in nature)
- Organic pesticides (produced from animal or plant parts)

**According to mode of action**

- Stomach poisons (they have to be eaten)
- Contact poisons (they work via the skin)
- Fumigants (they produce a vapor that kills organisms)
According to target range

- Broad spectrum pesticides (chemicals that kill a wide range of pests)
- Selective pesticides (chemicals that kill only a specific pest or group of pest)

According to its formulation

- Liquids
- Powders
- Granules
- Baits
- Dusts

According to active ingredients

Synthetic pesticides can be grouped by their active ingredient (the chemical class to which a toxic components belongs). Major chemical groups are:

- Organophosphates e.g. - Dimethoate, Phorate, Monocrotophos, etc.
- Organochlorines e.g. Endosulafan, DDT, gammaxine, etc.
- Carbamates e.g. Baygon, Carbaryl, Carbofuran, etc.
- Pyrethroids e.g. Cypermethrin, Fenvalrate, Deltamethrine, etc.
- Neonicotinoid Insecticide e.g. Imidacloprid, Acetamiprid, etc.
- Oxidizine Insecticide e.g. Indoxacarb, etc.
- Benzylurea Insecticide e.g. Difluobenzuron, Flurobenzuron, etc.

1A.8 Organophosphate Insecticides

An organophosphate (sometimes abbreviated as OP) is the general name for esters of phosphoric acid. Phosphates are probably the most pervasive organophosphorous compounds. Organophosphates are also the basis of many insecticides, herbicides, and nerve gases. Organophosphates are widely used as solvents, plasticizers, and EP additives.4
Structural features of organophosphates

Effective organophosphates have the following structural features. In spite of enormous structural diversity of organophosphorous insecticides, all the compounds can be represented by the classical hypothetical structure as:

![Structural formula]

Where R and R₁ are short chain alkyl, alkoxy, alkylthio or amine groups and X is a labile leaving group or groups that can be metabolized in vivo to labile entity. Biological activity of the organophosphorous compound is due to the capacity of central ‘P’ atom to phosphorylate the active site of the enzyme cholinesterase which is an essential constituent of the nervous system, not only of insects but also of higher animals.

Organophosphate pesticides are used extensively worldwide and poisoning by these agents, particularly in developing nations, is a serious public health problem. Various methods have been reported in literature for the detection and determination of organophosphorous insecticide. These include gas chromatography, spectrophotometry, high performance liquid chromatography, flurometry, etc. But all these methods are reported for pure compound or for formulated products. In case of biological material due to highly susceptible impurities clean up procedure is required. Here TLC and HPTLC methods are used for detection of organophosphorous compound from biological material.

1A.9 Organochlorine Insecticides

Organochlorine insecticides are neurotoxins having high lipophilicity, hydrophobic, and are chemically stable. Metabolic degradation in target and non-target organisms or environmentally by chemical, photolytic, or microbial processes is slow. As a result, organochlorine insecticides are persistent in the environment and have a long half-life. There are three major types of organochlorine insecticides.⁹
**Dichlorodiphenylethanes:** Such as DDT, DDD, TDE, Methoxychlor, Rhothane, Methlochlor, Perthane, Dicofol (Kelthane).

![DDT Structure](image)

**Chlorinated Cyclodienes:** Such as Aldrin, Dieldrin, Endrin, Heptachlor, Chlordane, and Endosulafan.

![Aldrin Structure](image)

**Chlorinated Benzenes & Cyclohexanes:** Such as Lindane, Toxaphene, Mirex, HCB, HCH, Chlordecone (Kepone).

![BHC Structure](image)

As the analytical part is concern a tedious and time-consuming clean-up procedure is required for modern analytical Instrument. Hence TLC & HPTLC is the suitable and appropriate method for the detection of chlorinated pesticides in biological and non-biological materials in forensic case work.

**1A.10 Carbamate Pesticides**

Carbamate Pesticides affect the nervous system by disputing an enzyme that regulates acetylcholine, a neurotransmitter. The enzyme effects are usually reversible. There are several subgroups within the Carbamates. The general formula of the Carbamates:

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\]
Where $R^1$ and $R^2$ are alkyl or aryl groups. Carbamates are commonly used as surface sprays or baits in the control of household pests. Carbamates are used as either dusts or sprays. They may be absorbed through the skin as well as by ingestion and inhalation. Among the various analytical methods used for the detection and determination of carbamate insecticides, TLC & HPTLC is the most useful and most applicable method for these insecticides in biological material in forensic toxicology.$^{11}$

1A.11 Pyrethroid Insecticide

Pyrethroid Pesticides were developed as a synthetic version of the naturally occurring pesticide pyrethrin, which is found in chrysanthemums. They have been modified to increase their stability in the environment. Some synthetic pyrethroids are toxic to the nervous system.

Pyrethrins were originally derived from East African chrysanthemum flowers and shown to have insecticidal activity. In a natural environment, they were chemically unstable and broke down rapidly upon exposure to air and sunlight. They are effective against a wide range of insect and mite pests and may be mixed with other pesticides for a broad spectrum of pest control. Formulations that are commercially available include aerosols, dips, emulsifiable concentrates, wet able powders, granules, and concentrates for ultra low volume applications targeting mosquitoes. Pyrethroids may be mixed with piperonyl butoxide, a synergist, which enhances the effect of the active ingredient. Their mode of action is interference with transmission of nerve impulses. Chemical Structure of Pyrethroids:
Pyrethroids are one of the least acutely toxic insecticides to mammals because they are quickly deactivated by metabolic processes. TLC & HPTLC is found to be very useful for the detection of unabsorbed quantity of these pyrethorid insecticides present in biological material.

1A.12 Neonicotinoid Insecticides

Neonicotinoids are a relatively new class of insecticides that are so named due to their similarity in structure to nicotine. Their common names are acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam. They generally have low toxicity to mammals (acute and chronic), birds, and fish. The low affinity of neonicotinoids for vertebrate relative to insect nicotinic receptors is a major factor in their favorable toxicological profile. Chemical structure of neonicotinoids:

Imidacloprid is possibly the most widely used insecticide, both within the mode of action group and in the worldwide market. Thiamethoxam (TMX) is a
second generation neonicotinoids insecticide, belonging to the thianicotinyl subclass. Thiamethoxam’s chemical structure is slightly different from other neonicotinoids insecticides, making it highly water soluble and thus it is readily translocated in plant tissue. Acetamiprid is for use against sucking insects, such as aphids and whiteflies, on leafy vegetables, Cole crops, citrus, cotton, ornamentals, and fruiting vegetables. Clothianidin was registered in 2003 by Bayer initially for corn and canola seed treatment use. Additional approved sites include grapes, pome fruit, rice, tobacco, and turf and ornamentals.

Neonicotinoids are classified by the EPA as both toxicity class II and class III agents and are labeled with the signal word “Warning” or “Caution.” Because the neonicotinoids block a specific neuron pathway that is more abundant in insects than warm-blooded animals, these insecticides are more selectively toxic to insects than mammals. Detection of these compounds from biological material TLC & HPTLC is method of choice.¹²

**IA.13 Oxidazine Insecticides**

Indoxacarb is the only chemical available in the oxadiazine class of chemistry - meaning no other insecticide offers the same mode of action. By combining an excellent environmental profile and outstanding performance attributes, this chemistry is ideal for professional pest, lawn, and golf course management. Indoxacarb is an insecticide in a new class of chemistry with a new mode of action now registered for use on apples and pears. Indoxacarb is in the oxadiazine class of chemistry and it works as a sodium channel blocker. Indoxacarb was registered in the U.S. for use on apples, pears, and many other crops in May of 2001. It is a reduced risk pesticide with very low mammalian toxicity and a benign profile for avian and aquatic toxicity.¹³
For identification of these substances have been attempted to develop new technique by using Thin Layer Chromatography (TLC), HPTLC. These methods are highly sensitive and can be used for unequivocal identification of pesticides/drugs, etc.\textsuperscript{14} these methods are discussed in this chapter. An attempt is being made to develop a new chromogenic reagent and analytical methods for selective detection of pesticides and drugs of forensic interest.

1A.14 References

CHAPTER - I

SECTION B

LITERATURE SURVEY
1B.1 Literature Survey

Poisoning due to insecticides and pesticides has an important role in crimes all over the world. Forensic toxicologists are dealing with maximum number of cases due to insecticides and pesticide poisoning involved in various types of crimes. Pesticides also cause much hazard to the surrounding environments and other organisms. Since the analysis of insecticide residue posses an entirely different types of problem for the toxicologists because these residues are present in extremely small quantity in heterogeneous materials including the biological materials. The importance of insecticide residue problem led to intensive search for analytical methods for accurate and rapid analysis was studied by Zweig\(^1\), the determination of the pesticide in various biological materials often faced with the problem of determining the minute quantity of insecticides mixed with large amount of extraneous material or intermixing. Qualitative and quantitative methods are required to be applied keeping in view the sensitivity and specificity of the methods on one hand and nature of type of pesticides on the other hand. The technique of thin layer chromatography has made a strong impact on analytical toxicology. Extensive literature on both the qualitative and quantitative analysis of insecticides/pesticides is available.\(^2\)

The literature survey reveals a large number of research activities are centered in identification of organophosphorous, organochloro, carbamate and pyrethroid insecticides. There are few references for newly invented pesticide like avant Indoxacarb) monocrotophos, carbosulfan, hydrogen cyanamide, tridemorph and pyrethroid insecticide like cypermethrin. Consequently characterization of these pesticide is necessary in forensic case work, their identification in biological material (Viscera, Stomach wash, blood, etc.) and non-biological material (Material found at the site of scene of crime e.g. container of pesticide, strip of drugs, syringes, injection, cloths, etc.) is important task in forensic case work.

For identification of these substances thin layer chromatography (TLC) is the most widely used separation technique. A modern analytical instrumental method such as spectophotometry, gas-chromatography, HPLC, HPTLC, and mass spectrometry is sensitive and they are widely used in forensic analysis. In these
methods the sample should be free from biological impurities. These methods are highly sensitive and can be used for unequivocal identification of pesticide/drugs, etc.

Most of the present techniques for isolation and identification of poisons such as insecticides (organophosphorous, organochoro, carbamates, and synthetic pyrethroid) are based on the isolation and detection of residual quantity of the original compound (Residual quantity- the original compound that remains as it is after the death). In most of the homicidal cases the quantity of ingested poisons is less and almost all the quantity consumes in the metabolic path. In such cases the detection of metabolite in biological samples is very essential to give justice to the case.

The poison isolated from biological material in poisoning cases generally is in microgram quantities. The conventional methods of chemical analysis are not feasible, micro chemical and instrumental technique have to be developed for detection, isolation and quantitation of isolated poison.

The study is undertaken for detection and estimation of pesticide/drugs using thin layer chromatographic technique and spraying with different chromogenic reagents. The extraction of pesticides and drugs from biological material using different solvent mixtures will be undertaken. The method of extraction is set up for different pesticides and drugs using Accelerated Solvent Extractor.

The traditionally used Dragandroffs like chromogenic reagent are widely used for detection of basic nitrogen containing compound\(^3\). However it is observed that there are many limitations for this reagent. Therefore there is a need to develop different chromogenic reagents and analytical method for detection of newly invented pesticides, drugs and other organic compounds of forensic interest.

Hence an attempt is being made to develop a new chromogenic reagent and analytical methods for selective detection of pesticides and drugs of forensic interest.

In parts of the developing world, pesticide poisoning causes more deaths than infectious diseases. Use of pesticides is poorly regulated and often dangerous; their easy availability also makes them a popular method of self-harm. In 1985, the UN Food and Agriculture Organization (FAO)\(^2\) produced a voluntary code of conduct for the pesticide industry in an attempt to limit the harmful effects of pesticides.
Unfortunately, a lack of adequate government resources in the developing world makes this code ineffective, and thousands of deaths continue today. WHO has recommended that access to highly toxic pesticides be restricted, where this has been done, suicide rates have fallen. Since an Essential drugs list was established in 1977, use of a few essential drugs has rationalized drug use in many regions. An analogous minimum pesticides list would identify a restricted number of less dangerous pesticides to do specific tasks within an integrated pest management system. Use of safer pesticides should result in fewer deaths, just as the change from barbiturates to benzodiazepines has reduced the number of deaths from pharmaceutical self-poisoning.

Hundreds of active ingredients and tens of thousands of formulations are used to control agricultural pests and disease-carrying vectors, 1.5 million tones of pesticides are manufactured every year, producing a business worth US$30 billion. The widespread adoption of pesticides during the 1950s was associated with increased crop yields, opening up of new agricultural land, and reductions in incidence of vector-borne diseases. However, increasing pest resistance has resulted in lower yields and a resurgence of vector-borne diseases such as malaria. At the same time, the many health and environmental costs of intensive pesticide use have become starkly apparent.4

According to the WHO, one million serious accidental and two million suicidal poisonings due to insecticides occur worldwide every year, of which 200,000 patients die with most deaths occurring in developing countries5. In India, organocompounds (OPCs) organophosphates and organocarbamates are the commonest pesticides used and due to their easy availability, there is widespread abuse of these compounds with suicidal intent.6-9

A study of organophosphorous poisoning patient was carried out in Medical College & Research Institute, (Deemed University), Department of Anesthesiology, Chennai from April 2002 to March 2003 in ICU consecutive patients of OPC poisoning admitted during this period were included in this study. In view of these conflicting studies, we elected to study the effects of repetitive nerve stimulation serially in patients with OPC poisoning in an attempt to uncover changes which might mark the onset and resolution of intermediate syndrome.10
**1B.2 Organophosphorous Insecticide**

Various reagents are reported in literature for the detection and determination of organophosphorous insecticides by TLC and HPTLC. Baumler and Hippstein have reported\(^1\) the use of Palladium (II) chloride for the detection of organophosphorous insecticides. A sensitive spray reagent 4-(P-nitrobenzyl) pyridine tetra ethylene-pentamine has been reported by Zweig, for the detection of organophosphorous insecticide. Kawale and et al. have reported\(^12\) mercury(I) nitrate reagent & Joglekar, et al. have reported\(^13\) mercury(II) nitrate-diphenyl carbazole reagent, which were used for the detection of derivatives of barbituric acid were further utilized for detection of organophosphorous and organothiophosphate insecticides. There is some exception such as corticosteroid, chloromycetin, etc.

Katkar and Barve have developed\(^14\) spray reagent mercuric nitrate followed by potassium ferrocyanide for detection of organophosphorous insecticide, bluish color spot were observed after keeping plate for about 5 min at room temp.

Patil and Padlikar have developed\(^15\) spray reagent for sulphur containing organophosphorous insecticides from biological material by using potassium periodate (KIO\(_3\)), the free iodine liberated reacts with starch solution to give blue color.

Marutoiu and Viacsa have been separated\(^16\) eight organophosphorous insecticides and identified in water by silica gel HPTLC and detection by 2-methyl thioacridone spray reagent.

Sonnenfeld and Paul have developed\(^17\) technique of organophosphorous insecticides dichrotophos, ethion or phorate, fenaulfothion, oxydemeton-methyl, phosmet, phospholan and trichlorfon have been appeared from each other on silica gel foils and detected with silver nitrate-UV reagent.

Sharma & Boymal have worked\(^18\) on environmental samples and have been quantified on organophosphorous insecticides by densitometry on C-18 layer using N,2,6-tricholorobenzoquinonaimine spray reagent for detection.

Mirashi and Patil have developed\(^19\) spray reagent for identification and determination quinolphos, disulfoton and monocrotophos by thin layer
chromatography. They have used cupric acetate in dilute hydrochloric acid followed by potassium iodide. Cupric acetate reacts with potassium iodide liberates free iodine, this liberated free iodine in presence of cu+ in acidic medium probably reacts with these insecticide forming colored complex.

Patil and Shingare have developed a spray reagent for selective detection of dichlorvos in biological materials by thin-layer chromatography. Dichlorvos in presence of moisture breaks down to dichloroacetaldehyde which in turn reacts with phenylhydrazine hydrochloride to give a yellowish red color. In acidic media the color is intensified and consequently the sensitivity of detection increases. The reagent is selective for dichlorvos, other organophosphorous insecticides failed to give a colored spot.

Deshpande and Padlikar have developed potassium triiodide as spray reagent for detection of monocrotophos. Potassium triiodide is prepared by dissolving 2gm of Iodine & 4gm of potassium Iodide in 50% ethanol and conc. HCL (1:1). Monocrotophos gave a distinct violet pink colored spot at Rf 0.6.

Patil and Shingare have developed a spray reagent for detection of monocrotophos from biological material. Monocrotophos on alkaline hydrolysis yield N-methylacetoacetamide which in turn react with diazotized sulphanilamide or sulphanilic acid to give red color. However phenolic compound and hydrolyzed product of carbamate insecticide may interfere and are differentiated from monocrotophos by Rf values.

Patil and Garad have developed another spray reagents for detection of monocrotophos from biological material. Monocrotophos on alkaline hydrolysis yields N-methyl acetoacetamide which further reacts with chloranil to give red color complex. The active methylene group reacts with the chromogenic reagent chloranil to give red color compound. Chloranil has been used for the detection of primary, secondary amine and phenol. Monocrotophos and phenol gives red and brown spot and hRf from extract of viscera at hRf 45.

Patil et al. have developed method for detection of phosphomidon and endosulfan from biological material by using Cobalt acetate as a spray reagent. Phosphomidon after hydrolysis yield 2-chloro-2-dimethylcarbamoyl group reacts with
cobalt (II) to give oxidized yellowish brown cobalt (III), which in turns oxidize with O-toluidine. Benzidine and O.T. reacts in the same way giving blue color complex.

Mali et al. have developed spray reagent for detection of dichlorovos and dimethoate using orcinol. The alkali hydrolysis product of dichlorovas and dimethoate reacts with orcinol produces a yellow fluorescent compound.

Rane et al. have developed spray reagent for detection of dichlorovas by TLC, Dichlorovas is derivative of phosphoric acid which is readily hydrolyzed in alkali producing dimethyl phosphoric acid and dichloroacetaldehyde. 2-TBA reacts with dichloroacetaldehyde to give 2,2-dichloro-5-ethylidine-2 thiobarbituric acid, a pink color compound.

Patil and Shingare have developed a spray reagent first spray 5% stannous chloride after heating for 10 minutes at 100°C. It is cooled and sprayed with a freshly prepared 5% sodium nitrite solution followed by 1-napthylamine solution as stated above. Pink-orange colored spots are obtained for methyl parathion, ethyl parathion, fenitrothion, p-nitrophenol (a metabolite of methyl and ethyl parathion), 3 methyl 4-nitrophenol (a metabolite of fenitrothion) and also other organic compounds containing nitro group and aromatic amino group.

Shivhare has reported a spectrophotometric method for the determination of methyl parathion residues in plant material and soil. The reaction is based on reduction of nitro group present in parathion methyl with Zn-HCL to form an amino group which is subsequently diazotized and coupled with guaiacol in alkaline medium to form a yellow colored azo-dye, which show $\lambda_{\text{max}}$ at 470 nm. Other commonly found pesticides do not interfere.

Similarly Dayananda et al. have reported a colorimetric enzymatic method for determination of methyl parathion and paraoxon. They used egg albumin, 20% solution as source of cholinesterase which as incubated with 2 to 28 $\mu$g of parathion methyl of 0.2 to 2 $\mu$g of paraoxon-methyl in 0.1 ml of acetone for 10 min at 20°C. A solution of 1 $\mu$ mol of 1-napthyl acetone in 0.1 ml of acetone and 0.2 ml of aqueous 0.4% fast blue were added. The mixture was diluted with water and incubated for 25 min at 32 °C. The reaction was stopped with acetic acid, and measured the absorption at 545 nm.
Sunita et al. have described a simple spectrophotometric method for determination of organophosphorous pesticide in plant material. The method is used on the reaction of organophosphorous insecticide with ammonium molybdate in 1:1 $\text{H}_2\text{SO}_4$ and succinic hydrazide solution to form molybdenum blue complex which was extracted with butanol. The absorbance of the organic phase was measured at 789 nm vs butanol.

Kaur and Garg have studied detection on the persistence of dimethoate and phosphamidon insecticides from soil and paper substrates by thin layer chromatography. Among the fifteen different solvent systems examined, it has been observed that dimethoate can be separated by using benzene:acetone (9:1) and phosphamidon by using cyclohexane:acetone:chloroform (70:25:5) followed by iodine fuming as visualizing aid. The analysis of these two compounds could be successfully performed even up to 8 weeks times from soil and paper containing these pesticides in small amounts and it remains therein.

Dhingra Vinod has studied about the acephate is an organophosphorous insecticide of broad spectrum used in field crops. He described symptoms, postmortem changes occur in a reported case of acephate insecticide and detection and identification of insecticide in visceral material by TLC.

Daundkar et al. have studied spectrophotometric and TLC detection reagent for the insecticides dichlorvos (DDVP) and diptrex (Trichlorfon) and their metabolites, in biological tissues. They developed reagent 10% sodium hydroxide, and 0.5% aqueous sodium sulfide solution. Red spots of dichlorvos and diptrex were obtained at $R_f$ 0.26 and 0.42 respectively.

Bahman Ebrahimi has studied a new changeable bioreactor for detection of organophosphate in a flow-through system. A flow-through biosensor consisting of a fixed bed bioreactor was employed to detect the insecticide paraoxon. Based on the inhibition of organophosphorous insecticide to the enzymatic activity of acetylcholinesterase (AChE), using paraoxon as a model compound, the condition for detection of the insecticide was optimized. The influence of enzyme loading on the packing surface was studied.
Sherine has studied\textsuperscript{35} fluorescent chemosensors for toxic organophosphorous pesticides. Many organophosphorous (OP) based compounds are highly toxic and powerful inhibitors of cholinesterases that generate serious environmental and human health concerns. Organothiophosphates with a thiophosphoryl (P=S) functional group constitute a broad class of these widely used pesticides. They are related to the more reactive phosphoryl (P=O) organophosphates, which include very lethal nerve agents and chemical warfare agents, such as, VX, Soman and Sarin. Thus, they design new sensors with improved analyte selectivity and sensitivity is of paramount importance, He also describes challenges and progress towards the design of future chemosensors with dual modes for signal transduction.

Eddleston et al. have studied\textsuperscript{36} poisoning with the S-Alkyl organophosphorous insecticides profenofos and prothiofos. Compared with other commonly used OP insecticides, profenofos and prothiofos are of moderately severe toxicity, causing relatively delayed respiratory failure and death. There was no apparent response to oxime therapy. The lack of correlation between red cell AChE activity and clinical features suggests that this parameter may not always be a useful marker of synaptic AChE activity and severity after OP pesticide poisoning.

Various workers have reported the gas chromatographic method for the detection and determination of residues of organophosphorous insecticides in vegetables plant, fruits, water samples, and soils and also in the formulation.\textsuperscript{37-42} Botowski and Jones have described\textsuperscript{43} a method (HPLC-MS) for analysis of organophosphorous insecticide in soil.

\textbf{1B.3 Carbamate Insecticide}

Like organophosphorous insecticide a number of chromogenic spray reagents have been reported for the detection of carbamate and organochloro insecticide by thin layer Chromatography and High performance Thin layer Chromatography. Zoun et al. have studied\textsuperscript{45} over 100 cholinesterase inhibiting pesticides have been separated by HPTLC and detected by spraying with bovine liver suspension followed by an appropriate ester and chromogenic reagent. The method was used to detect pesticides in biological and environmental samples and foods.
Sherma and Gatz have studied a novel method for producing colored zones on a white background, instead of the usual reverse situation, for cholinesterase-inhibiting organophosphorous and carbamate pesticides has been demonstrated using acetylthiocholine as the substrate for horse serum cholinesterase, and 2,6-dichloroindophenol as the redox dye. Low nanogram amount of pesticide were determined by combination of this detection techniques and reluctance scanning.

Kawale and Jogelakar have reported the used of Tollens’s reagent for thin layer chromatographic detection of carbamate and some organophosphate insecticides in biological materials. Randerath has reported that the carbamate insecticides carbaryl, carbofuran and propoxur on alkaline hydrolysis yields respective phenolic compounds which further coupled with diazotized aryl amines. Tiwari and Singh have used alkaline fast blue B salt for detection of carbamate insecticides carbaryl, carbofuran and propoxur. Rathore, et al. has used p-nitrobenzendiazonium tetrafluroborate for TLC separation, detection and quantitation of carbaryl in water.

Padalikar et al. have described two specific reagents for detection of carbaryl and its breakdown product, 1-napthol:1 % cupric chloride followed by ammonium metavanadate, and potassium hexacyanoferrate(III) in 0.5% sodium hydroxide. Fodor-Caorba and Duttka have shown the selectivity of three detection reagent silver nitrate-2-phenoxyethanol, r-(4-nitrobenzyl)-pyridine and 2,6-dibromobenzoquinone-N-chloroimine and its dichloro analog have been examined for visualization of organocloro, organophosphorous insecticides and thiocarbamate herbicide and residues in vegetables determined by densitometry.

Raju and Gupta have reported two chromogenic spray reagents viz. diazotized-4-nitroaniline and diazotized-4-aminoacetophenone have been reported for TLC detection of carbaryl in cereal extracts. Appaiah and Ramkrishna have described the spectrophotometric determination of carbaryl in grains, based on hydrolysis of carbaryl with methanolic potassium hydroxide to 1-napthol reaction with 4-aminophenazone in the presence of alkaline oxidizing agent, and spectrophotometric measurement at the absorption maximum at 475 nm.

Sastry and Manala have described three spectrophotometric method for the determination of carbaryl and propoxur in insecticidal formulation, water and grains,
based on the formation of colored species with p-aminophenol, P-N, N-
dimethylphenylene diamine dihydrochloride and 1-amino-3-napthol-4-sulphonic
acid respectively under specific experimental conditions.

De Kok has reported an improved cleanup method for the multiresidue
analysis of N-methylcarbamate in grain, fruits and vegetables by means of HPLC with
post column reaction and fluorescence detection. Ayala has reported fluorometric
method for determination of carbaryl and 1-napthol in hexadecyltrimethyl ammonium
bromide in micellar media.

Bhatia J. has studied thin layer chromatographic and spot test detection of
carbosulfan, a carbamate pesticide by alkaline fast blue-B reagent. Carbosulfan on
alkaline hydrolysis forms a phenolic product which further condenses with fast blue-B
and forms orange colored complex on silica gel-G TLC plate.

### 1B.4 Organochloro Insecticide

For the detection of organochloro insecticides by thin layer chromatography,
Kawashiro and Hosagai have reported the use of alcoholic o-tolidue and o-
diandsidine and irradiation with UV light, giving bluish spots on TLC plates. Hurter, J,
Zurrer H. et al. have developed an ammonical silver nitrate and Diemair W. et al.
have developed silver nitrate reagent for detection of organochloro insecticide by
thin layer chromatography.

Bioscnmitter and Tolg have described potassium bromide and alkaline
rhodamine B reagent for identification of chlorinated pesticide. Guven and Aktulga
have used silica gel G impregnated with copper sulphate and ammonia is used for
the preparation of TLC plate for determination of organochloro insecticides.

Coutselinist and Kentarchou have reported an ethanolic diphenylamine
reagent for the detection of organochloro insecticide by TLC. Thielemann has used sodium hydroxide followed by methonic thymol for the detection of chlorinated pesticides.

A zinc chloride diphenylamine reagent has commonly been used for the
identification of organochloro insecticides, which gives a blue green spot with this
reagent. On heating the TLC plate at 110 °C for about 15 min the formation of blue-
green coloration is based on the reaction that when organic compounds containing halogens on heating with zinc chloride, oxidative decomposition occurs and liberates free halogen. The liberated free halogen like other strong oxidants, converts diphenylamine into the blue quinoidal compound.

Patil and Katkar have described\textsuperscript{71} the reagent for detection of endosulfan and phosphamidon in biological material based on the reaction of cobalt(II) acetate and potassium iodide with endosulfan and phosphamidon, followed by starch solution yielding iodine-starch violet colored complex. Patil et al. have described\textsuperscript{72} a specific spray reagent for the detection of endosulfan by thin layer chromatography. The reagent is based on reaction that endosulfan, containing cyclic sulphite in its structure, is readily hydrolyzed by alkali. The sulphite in turn reacts with nickel(II) ammine to give grayish black nickel(IV) oxyhydrate $\text{NiO(OH)}_2$.

Patil et al. have also reported\textsuperscript{73} a thin layer chromatographic detection of endosulfan and phosphamidon by use of cobalt (II) acetate and O-tolidine reagent. Sharma has developed\textsuperscript{74} a technique for organochlorine pesticide have been extracted from water by solid phase extraction on a C-18 cartridge with ethyl acetate, hexane-benzene (1+1), and hexane-diethyl ether (1+1) as eluents, pesticides were determined by silica gel TLC with silver nitrate-UV and densitometric scanning.

Makhubalo and Mainga have described\textsuperscript{75} 3,3',5,5'-tetramethylbenzidine reagent for detection of organochlorine pesticides. Sharma has further developed\textsuperscript{76} a technique for six component of organochloro insecticide mixture containing BHC, methoxychlor, heptachlor apoxide, dieldrin and aldrin have been successfully resolved on a C-18 chemically bonded RP layer by development with acetonitrile-water (75+25). Minimum sensitivity for visual detection and densitometric scanning ranged from 300-900 ng upon detection with o-tolidine reagent.

Sharma has studied\textsuperscript{77} chlorinated insecticides chlorpyrifos and its metabolite TPC have been quantified in tap water (5ppb) and bananas (50ppb) by pre-absorbent silica gel TLC, detection with silver nitrate–UV reagent, and scanning on zones.

Deshpande and Bhende have described\textsuperscript{78} an analytical method for chlorinated and phosphate ester pesticides. They develop titrimetric and colorimetric method to
determine aldrin, endrin, DDT and BHC. They used to convert the chloro groups into NaCl by treatment with metallic sodium in propan-2-ol, and the NaCl was determined by titrating with 0.01 N Silver nitrate with an indicator \((\text{NH}_4)_2\text{SO}_4, \text{Fe}_2(\text{SO}_4)_3, 24\text{H}_2\text{O})\).

Carbaryl was hydrolyzed to give 1-naphthol which was determined with 4-aminoantipyrine \(K_3(\text{Fe}(%\text{CN}_6))\) at \(PH\)\(^10\). Phosphamidon on oxidation with iodine, \(\text{HNO}_3\) or \(\text{H}_2\text{O}_2\) produced phosphates and could be determined by titrating unreacted iodine with \(\text{Na}_2\text{S}_2\text{O}_3\). Parathion has been determined by reducing it to a primary amine and determining the amine colorimetrically after dizotisation and coupling with N-1-naphthylethylene diamine.

A gas chromatographic methods for detection and determination of organochloro pesticide in meat and meat products\(^79\) in bovine faceses,\(^80\) in mussels,\(^81\) in milk,\(^82\) in urine,\(^83\) in egg and in formulation\(^84\) were reported in the literature with their elute and cleanup procedure.

Pyrethroid insecticide is recently introduced in the market. These are effective pest control chemicals and have low mammalian toxicity. A large number of gas liquid chromatographic method\(^85-89\) or residue analysis of synthetic pyrethroid have been reported, as has auto radiographic thin layer chromatography using 14-C-labelled compounds, particularly in metabolic studies, where the unlabelled compounds were detected by visualization on silica gel 60 F\(_{254}\). Chromatographic plates under ultraviolet (UV) light.\(^90-92\) Shono, et al. have reported\(^93\) the use of phosphomolybdic acid (20% m/v in ethanol) as a chromogenic reagent for the detection of paramethrin, cypermethrin and deltamethrin whereas palladium chloride has been described for the detection and determination of pyrethroid insecticide.

Sundarrajan has described\(^94\) silver nitrate impregnated alumina G and irradiation with UV light for general pyrethroid insecticide. Patil et al. have reported\(^95\) a spray reagent for the detection of pyrethroid insecticides containing a nitrile group by thin layer chromatography. The reaction is based on the alkaline hydrolysis of pyrethroid insecticides containing a nitrile group, yields cyanide ion, which in turn reacts with copper(II) acetate and o-tolidine in an acetic acid medium to give blue color.
Akmal et al. have reported a thin layer chromatographic method for detection of pyrethroid insecticides. These insecticides, on bromination and treatment with o-tolidine, yield an intensely blue product. 2,4-Dinitrophenylhydrazine and phosphomolybdate has also been used as a chromogenic reagent for thin layer chromatographic detection of synthetic pyrethroid insecticides in biological materials. Various other workers also studied various types of reagent for detection and identification of pyrethroid insecticide in food grain and water.

Vincent Coreel has studied insecticidal resistance developed in mosquitoes of medical importance. He ruled out pyrethroid, organophosphate, and carbamate resistance is more, new neonicotinoids insecticide alternative insecticides for vector control, because it shows low mammalian toxicity and great insecticidal activity against a broad range of pests.

From the above literature survey it is observed that the various analytical methods i.e. gas chromatography, high performance liquid chromatography, spectrophotometry, polarography, fluorimetry, mass spectrometry etc. were reported for the detection and determination of residues of different pesticides and drugs involved in forensic case work. But most of these methods were applied for the residue analysis of pesticides in grains, water sample, fruit, vegetables, soil and in insecticidal formulation, where interfering substances are very less. Thus the application of above methods for the diction and determination of residues of these drugs and insecticides in biological materials such as viscera, blood, urine, stomach wash etc. are not very suitable because of the presence of interfering materials like fats, proteins, peptides, amino acids, etc. in extracted material.

However some workers have reported the use of some of the above method for detection and determination of insecticides and drugs in biological material with tedious procedure of extraction, concentration, elution and clean-up procedure where the loss of compound is not to be ruled out. However, these methods are very time consuming and hence not useful for the routine analysis in forensic case work. To overcome these difficulties thin layer chromatography is the method of choice. The major use of TLC in pesticide and drug analysis is in qualitative screening of residues and as an aid in the identification of gas chromatographic and high performance liquid chromatographic peaks. The proper application of TLC methods gives excellent
quantitative results at ppm and ppb concentration levels. Numerous quantitative analysis have already been published and many more are expected as the excellent precision and accuracy of high performance thin layer chromatographic method (HPTLC) with densitometric scanning become more widely recognized by pesticide and drug analysis.

It is also observed from the above literature survey that a very few chromogenic reagents are reported for specific and selective detection of pesticides and drugs of forensic interest by TLC & HPTLC. Among the reported reagent, most of these are described for the detection and determination of these compounds in non-biological materials like grains, vegetables, fruits, water samples, soils etc. shows the interference with co-extracted biological materials like fats, proteins peptides and amino acids etc.

Thus there is a need to develop different chromogenic reagent for specific and selective detection of drugs, insecticide and other organic compounds of forensic interest by TLC & HPTLC, having less/no interference with co-extractives.
1B.5 References


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