Chapter One

Introduction and Review
Chapter-One

1.  INTRODUCTION AND REVIEW

1.1  PESTICIDES

1.1.a Pesticides: Chemistry and History

Pesticides are chemical compounds or mixture of compounds, which are intended for preventing, destroying, repelling or mitigating any pest. These pests can be insects, rodents or birds, unwanted plants (weeds), fungi, or microorganisms such as bacteria and viruses. Pesticides are, by their mere purpose, designed to kill or damage living things and they are, perhaps the only toxic substances that are consciously applied to the environment. Though often misunderstood to refer only to insecticides, the term pesticide also applies to herbicides, fungicides, micro biocides, rodenticides and various other substances used to control pests. A pesticide is also any substance or mixture of substances intended for use as an insect or plant growth regulator, insect mating disruptor or egg sterilant, defoliant, or desiccant.

The "first generation" pesticides were largely inorganic, such as arsenic and copper compounds. Their use was largely abandoned because they were either too ineffective or too toxic. The "second generation" pesticides largely included synthetic organic compounds. The synthetic organochlorine compounds were the first to be used as pesticides after Paul Muller discovered dichlorodiphenyltrichloroethane (DDT) in 1939, and was awarded the Nobel Prize in the year 1944 for it (Raju, 1999). The hunt for new chemical pesticides began and pesticides from different chemical classes such as Organophosphates, Organometallics (tin/mercury), Carbamates, Pyrethroids and Phenolic/phenoxy compounds became popular because of following reasons

1. They are broad-spectrum chemicals i.e., in terms of activity they are toxic to a wide range of insects/pests, yet appear to have low toxicity to mammals.
2. They are effective in very low quantities.
3. They are persistent (do not break down rapidly in the environment by the action of soil microbes) so that they don’t have to be applied often.
1.1.b Pesticides: Types and Classification

Today pesticides have become indispensable tools in the efficient control of animals, insects, plants and fungi which otherwise have a detrimental effect on the crop production. There are over 800 pesticides and 20,000 pesticide products, which are currently in use. Apart from being classified according to the chemical class the pesticide belongs to, they can also be classified into different groups (Sanborn et al., 2002) as

**Insecticides** are usually organophosphorous (e.g. malathion, chlorpyriphos etc), organochlorine (e.g. DDT) or carbamates (e.g. carbofuran, carbaryl etc). Other insecticide chemicals include Pthalates and hydrazines, Pyrezoles and Pyrethroids etc.

**Herbicides** can be either organic compounds such as Ureas and sulfonylureas, Chlorophenoxy acids, Triazines, Carbamates, benzoic acid, dinitrophenols and Naphthalene acetic acid derivatives (e.g. atrazine, 2,4-D, metachlor etc.) or inorganic compounds (e.g. Arsenicals, Sodium Chlorate).

**Fungicides** are sulphur and copper salts (copper sulfate etc) or organic compounds such as Azoles, Benzimidazoles, Carboxyimides, Dithiocarbamates and substituted Benzenes (e.g. Captan, Mancozeb etc.)

**Antimicrobials:** Pesticides commonly used as sterilizers, disinfectants, sanitizers, antiseptics and germicides intended to kill or inhibit growth of microorganisms such as bacteria (Bactericides/bacteriostat) include organic chemicals such as Phenols and chlorinated phenols, Hyadantoins, Isothiazolones and Quaternary ammonium salts.

**Rodenticides:** Used to control rodents and related species. Organic chemicals such as Coumarins and Indandiones are used as rodenticides world over.

Other Pesticide products include acaricide/miticide (for mites and ticks), aphicide (to kill aphids, which are small soft-bodied insects. They are one of the most common pest groups of ornamental plants.), fumigant (these pesticides produce vapours which are toxic if adsorbed or inhaled by pests), insect growth regulator, ixodicide (tick control), larvicide (pesticide for killing pests larvae), molluscicides (for killing mollusks), nematocides, plant growth regulator, repellant (species), synergist (these are chemicals which when applied with Insecticides may reduce resistance development by interfering with the detoxifying enzymes in the pest that allow it to survive an insecticide treatment (http://www.epa.gov/pesticides).
**1.2 PESTICIDES PRODUCTION AND USE: GLOBAL AND INDIAN ECONOMICS**

**1.2.a Global Scenario:**

World population is now 6 billion and currently there are more than 815 million people undernourished and 777 million of them live in developing countries, 27 million in transition countries and only 11 million people in developed countries (Anonymous, 2003). Agriculture is the main source of income for more than 2.5 billion people and 96% of the farmers are living in developing countries. World population is projected to grow to 8.3 billion by 2030. This would require a 40-45% increase in food production. But, overall, the rate of growth of both population and demand for food is expected to slow. To meet the demand of increase in food production, pesticides are a major farm input with many commercial cash crop operations spending 5-10% of cash operating expenses on these products. The world pesticide market is valued at about US $37 billion and India accounts for about 2.5% of this amount. India is normally ranked 12th in the world pesticide market while the Europe is ranked 1st with close to 33% followed by US with nearly 20% of the global market share. World Pesticide consumption is about 2.5 million tones and Europe is the largest consumer with over 32% followed by the US with around 20%, Asia accounts for about 12% pesticide usage and Canada and Africa are the lowest consumers with about 4% share each, the rest of the world makes for the remaining 20% pesticide consumption (Pimentel, 1995).

The world pesticide, agrochemicals and agriculture trade industry is dominated by a relatively small number of corporations; manufacturers (approximately 15) supplying a large number of active ingredients. According to UN food and Agriculture organization studies (http://apps.fao.org/faostat/default.jsp) it is estimated that 10 of these companies produce 90% of the world’s active ingredients (Table 1.1). Just four companies, based in the US and linked in two alliances (Cargill/Monsanto and Novartis/ADM) control over 80% of the world seed market and 75% of the world agrochemical market. Only 6 corporations handle about 85% of world trade in grain. The companies are generally vertically integrated since they produce formulations as well as make the basic materials, however, increasingly; the formulation process is being tendered out to specialized large scale, low cost formulators.
Table 1.1: Major Pesticide Producers of the world along with their Flagship products

<table>
<thead>
<tr>
<th>Serial Number</th>
<th>Company</th>
<th>Prime Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AgrEvo</td>
<td>Liberty</td>
</tr>
<tr>
<td>2</td>
<td>Cyanamid</td>
<td>Pursuit</td>
</tr>
<tr>
<td>3</td>
<td>Monsanto</td>
<td>RoundUp</td>
</tr>
<tr>
<td>4</td>
<td>Dow Elanco</td>
<td>2,4-D</td>
</tr>
<tr>
<td>5</td>
<td>Novartis</td>
<td>Dual</td>
</tr>
<tr>
<td>6</td>
<td>BASF</td>
<td>Banvel</td>
</tr>
<tr>
<td>7</td>
<td>Zeneca</td>
<td>Achieve</td>
</tr>
<tr>
<td>8</td>
<td>Bayer, Ontario</td>
<td>Furadan</td>
</tr>
<tr>
<td>9</td>
<td>Rhone-Poulenc</td>
<td>Select</td>
</tr>
<tr>
<td>10</td>
<td>Bayer</td>
<td>Admire</td>
</tr>
</tbody>
</table>

1.2.b Indian Scenario

Pesticides use started in India in the year 1947 with the introduction of DDT for Malaria control and the agricultural use started in 1949 with introduction of Benzene hexachloride (BHC) for Locust control. The production of pesticides started in the year 1952 with production of BHC followed by DDT production plants. In the year 1958 the production of Basic Pesticides was 5000 MT, with the onset of Green Revolution in 1960, which targeted food self-sufficiency there was tremendous scope for growth of pesticide industry in India, which has now grown to produce a total of 90,000 MT per year. Pesticide Industry in India is largest in Asia and twelfth largest in World. In the year 2003 the net sales value of the pesticide market was approx. Rs. 45000 million.

As shown in Table 1.2 the current trend in consumption of pesticide in India is showing a slight decline, probably due to the increased toxicity of the pesticide formulations low quantities are required for efficient pest control, a shift of farmers toward biopesticides, natural plant sources and other alternative methods such as use of genetically modified crop varieties (Das *et al.*, 2002; Gupta, 2004; Kumar and Kumar, 2004; Raghuram, 2002). Despite such a large consumption of pesticides, it is estimated
that crop losses vary between 10–30% due to pests alone. In monetary terms, these losses amount to Rs. 290,000 million per year (Agnihotri, 1999).

Table 1.2: GroupWise consumption of pesticides (MT) during 1995-2001 in India

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Insecticide</td>
<td>38,788</td>
<td>34,665</td>
<td>33,379</td>
<td>30,469</td>
<td>28,926</td>
<td>26,756</td>
</tr>
<tr>
<td>Fungicide</td>
<td>10,563</td>
<td>9,969</td>
<td>10,054</td>
<td>10,428</td>
<td>8,435</td>
<td>8,307</td>
</tr>
<tr>
<td>Herbicides</td>
<td>6,040</td>
<td>7,060</td>
<td>7,103</td>
<td>7,292</td>
<td>7,369</td>
<td>7,299</td>
</tr>
<tr>
<td>Others</td>
<td>5,869</td>
<td>4,420</td>
<td>1,703</td>
<td>968</td>
<td>1,465</td>
<td>1,222</td>
</tr>
<tr>
<td>Total</td>
<td>61,260</td>
<td>56,114</td>
<td>52,239</td>
<td>49,157</td>
<td>46,195</td>
<td>43,584</td>
</tr>
</tbody>
</table>

1.3 PESTICIDE POLLUTION AND PUBLIC HEALTH

1.3.a Pesticide Pollution: A Global Concern

Pesticide use has gained popularity due to their ability to control the pest problem at a very reasonable cost thereby increasing food supplies at lower prices and also in preventing transmission of diseases such as malaria through insects. Pesticides can be applied as dust or granules, as vapors or in presence of a liquid (water or oil) and thus enter into the environment as aerial drifts or as runoff through intentional applications. Pesticides, depending upon their water solubility can either remain in the soil and are broken down by action of microorganisms, or washed off, eventually into surface and ground waters (Dennison and Turner, 1995; Rehana et al., 1996; Agarwal, 1999). The persistency of pesticides and their degradation products in the geosphere causes environmental problems. The transfer of pesticides from treated soil to surface and ground water leads to contamination of drinking water resources and to subsequent intake of pesticides by human populations. They may also present hazards to human health directly as in spray drift of pesticides or indirectly as accumulated pesticide in edible plant or animal tissue (Suri et al., 2002). Pesticides are chemical compounds, which
merely by their design are intended to be biologically active. As such exposure to pesticides leads to manifestation of many health disorders in humans.

Rachel Carson was the first to predict a massive destruction of planet’s fragile ecosystem through her book ‘Silent Spring’ (1962). The title of the book was based on the fact that birds died more in the area where pesticides were being used through aerial spraying. She was the first to point out the damage being done to non-target species by uncontrolled use of pesticides through direct and indirect toxicity. Working on chlorinated pesticide she showed that DDT was harmful to fish and crabs and not only to the insects for which it was being used. Indirect toxicity on the other hand was due to the persistency of the pesticides. **Bioaccumulation**, is the tendency of the persistent pesticides to accumulate and get concentrated in an organisms tissue and **Biomagnification**, is the increase in the concentration of pesticides higher up in the food chain, which leads to indirect toxicity. Carson’s work disclosed the fact that even when the pesticide DDT had a very low concentration in water 3 ppt (0.000003 ppm) the concentration in zooplanktons was 0.04 ppm, was found to be 0.5 ppm in minnows and further increased to 2 ppm in fish and the birds feeding on fish were found to have a concentration of 25 ppm. In most countries DDT was banned in 1972 but it can still be found in various ecosystems even 3 decades after the ban on it’s use in seals, fishes, invertebrates, amphibians, birds and human (Ramakant, 2004; Muir et al., 2003; Minh et al., 2002; Wong et al., 2002). It is now evident that even in small amounts these pesticides can cause death, irritate eyes and skin, damage nervous system, disrupt hormonal balance and immune system, effect ability to reproduce and can cause cancer (Hooghe et al., 2000; Garry et al., 1996; Mattison, 1990; Echobichon, 1990). Based on these factors **EEC (1980)** has set the maximum permissible limits for individual pesticides to 0.1ng/mL and for all pesticides collectively to 0.5 ng/mL in drinking waters. Insecticides and herbicides are the two main classes of pesticides, which are most commonly used and are found in environment as pollutants. Due to a large number of chemicals which are being used and deposited in the environment, WHO recommended the classification of pesticides based on the threat it poses to human race, calculated on the basis of Oral and Dermal LD50 values (Copplestone, 1988). Table 1.3 shows the
classification categories in which harmful chemicals are divided according to the WHO recommendations.

**Insecticides** are the major cause of concern because the effect produced by their mode of action such as enzyme inhibition, hormonal imbalance etc on the pest animals and insects is also applicable on the humans. Organophosphates are a class of pesticides having an adverse affect on the nervous system. These pesticides irreversibly inhibit a class of enzyme called Cholinesterase, which is essential for the normal transmission of nerve impulses from one nerve to another and thus affects the central nervous system (Rosentock et al., 1991). Acute and long-term exposure to organophosphates leads to elevated neurological, psychiatric symptoms and poor neuropsychological response (London et al., 1998). Acute Organophosphate toxicity is one of major modes of committing suicide (Pickett et al., 1998). Organochlorine pesticides were used extensively till 1970’s but the health hazards associated with their use implied severe restrictions on their use. Organochlorines are known to disrupt hormonal balance, retard growth in children and also adversely affect the immune system (Colborn et al., 1993; Vine et al., 2000; Karamus et al., 2002).

**Herbicides** owe their indiscriminate use to their effectiveness in controlling weeds and also to their apparent tolerability by animal species including farm animals and humans. Recent studies on long-term exposure to different types of organic herbicides in uses such as heterocyclic aromatic compounds (e.g. Triazines etc.) or phenoxy acids (e.g. 2,4-D etc.) have been reported to cause great concern for human health (Cox, 2001; Narotsky et al., 2001; Kniewald et al., 2000). Herbicides have been shown to be immunotoxic and according to a US National Toxicology Program (1994), triazines are shown to adversely affect the immune system function system in mice (http://ntp-server.niehs.nih.gov/htdocs/IT-studies/IMM940032.html). Atrazine is reported to be among the five pesticides to which immune system is most sensitive. Phenoxy and triazine herbicides are known to disrupt hormonal balances (Babic, 1989; Kniewald et al., 1995; Eubanks, 1997; Cooper et al., 1996; Rowlings et al., 1998). Herbicides are linked to increased incidence of cancer and chromosomal breakage in various studies (Taets et al., 1998; Weininger et al., 1987; Kettles, 1997; Donna et al., 1989; Van-Leeuwen, 1999). Degeneration of various organs such as Kidney and Liver (Santa-Maria et al.,
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1987; 1986) increased incidence of birth defects, complete pregnancy loss in experimental laboratory animals and human populations exposed to water supplies from a contaminated source has been reported in various studies (Munger et al., 1997). In an in vitro study triazines were found to change the production of two chemicals, Dopamine and Norepinephrine, both of which act as neurotransmitters in the central nervous system and are thus potential neurotoxic agents (Das et al., 2000, 2001).

Table 1.3: WHO recommended classification of pesticides by hazard

<table>
<thead>
<tr>
<th>Class</th>
<th>Hazard level</th>
<th>LD₅₀ for the rat, oral (mg kg⁻¹ body mass)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Solids</td>
</tr>
<tr>
<td>Ia</td>
<td>Extremely hazardous</td>
<td>≤5</td>
</tr>
<tr>
<td>Ib</td>
<td>Highly hazardous</td>
<td>5-50</td>
</tr>
<tr>
<td>II</td>
<td>Moderately hazardous</td>
<td>50-500</td>
</tr>
<tr>
<td>III</td>
<td>Slightly hazardous</td>
<td>&gt;500</td>
</tr>
<tr>
<td>III+</td>
<td>Unlikely to present hazard in normal use</td>
<td>&gt;2000</td>
</tr>
</tbody>
</table>

1.3.b Pesticide Pollution: Indian perspective

Despite the fact that the consumption of pesticides in India is very low, about 0.5 kg/ha of pesticides against 6.60 and 12.0 kg/ha in Korea and Japan, respectively, there has been a widespread contamination of food commodities with pesticide residues, basically due to non-judicious use of pesticides. In India, 51% of food commodities are contaminated with pesticide residues and out of these, 20% have pesticide residues above the maximum residue level values on a worldwide basis. It has been observed that their long-term, low-dose exposure are increasingly linked to human health effects such as immune-suppression, hormone disruption, diminished intelligence, reproductive abnormalities, and cancer (Gupta, 2004).

The first report of poisoning due to pesticides in India came from Kerala in 1958 where, over 100 people died after consuming wheat flour contaminated with parathion (Karunakaran, 1958). Since then several cases of mass poisoning have been reported but minor case still go unreported. Table 1.4 summarizes the known cases of poisoning occurred in India and deaths due to negligence of pesticide use.
Several studies have shown that pesticide pollution is a widespread problem in India, which deserves much more attention than it currently gets. Pesticides have been found in foodstuffs (Kannan, 1992), human blood, milk and fat (Bhatnagar, 2001). The poison information center in National Institute of Occupational Health, Ahmedabad, Gujarat (India) reported that organophosphates were responsible for the maximum number (73%) of pesticide poisonings among all other agrochemicals (Dewan and Sayed, 1998). A study on 356 workers in four different manufacturing units of Hexachlorohexane (HCH) showed enhanced neurological symptoms (21%) along with significant increase in detoxifying enzyme levels related to the degree of exposure (Nigam et al., 1993). Another study revealed increased level of abortions, still births, neonatal deaths and congenital birth defects in a survey of 1106 farm worker couples involved in spraying of organochlorine, organophosphate and carbamate pesticides as compared to 1020 couples not linked with pesticide use (Rupa et al., 1991).

Rajendran and co-workers (1999) reported the presence of Chlorinated pollutants in air from a tropical coastal environment (at Parangipettai - southeast coast of India). DDT and HCH ranged in concentrations from 0.16 to 5.93 ng m$^{-3}$ and 1.45 to 35.6 ng m$^{-3}$ respectively. The ban on DDT in agriculture is reflected from the low residue levels recorded, predominantly by metabolites other than the parent compounds. DDT, BHC, aldrin, endrin and dieldrin at concentrations of 1.36, 1.38, 0.95, 0.61 and 0.41 ppb respectively. The organophosphorous pesticides such as dimethoate and methyl parathion appear to be present at concentrations of 0.20 and 0.41 ppb respectively were detected by Rehana and coworkers (1996) in the river Ganges. Excessive use of agrochemicals has definitive environmental consequences that have reached alarming levels in Panjab and Haryana, which are also the highest user of agrochemicals per hectare in India (Singh, 2000; Agarwal GD, 1999).

The world’s worst industrial disaster occurred in India on the night of December 2-3, 1984, at the Union Carbide plant in Bhopal (population: 900,000), the capital city of Madhya Pradesh—one of the largest states in India. Introduction of water into a methyl isocyanate (MIC) [CH$_3$N=O] storage tank resulted in an uncontrollable reaction, with liberation of heat and MIC gas. Safety systems—such as a flare tower (for the burning of excess gas), a caustic soda scrubber (for neutralization), and a refrigeration unit did not
contain the reaction. MIC is an intermediate product in the manufacture of carbaryl, a
carbamate pesticide. More than 2,500 people died almost instantly, and over 16,000
people have died as a result of health problems related to their exposures to MIC; 50,000
people are still suffering significant long term health impacts such as ocular, respiratory,
reproductive, immunological, genetic, and psychological (Mehta et al, 1990). More than
500,000 people filed injury claims with the Bhopal Compensation Courts (Dhara et al, 2002). Most can no longer work, or eke out a meager living and despite all the
death and suffering, people are still struggling for justice (http://www.bhopal.org/).

Table 1.4: Mass pesticide poisonings in India

<table>
<thead>
<tr>
<th>Place</th>
<th>Year</th>
<th>People Affected</th>
<th>Deaths</th>
<th>Causative agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kerala</td>
<td>1958</td>
<td>360</td>
<td>100</td>
<td>Parathion</td>
</tr>
<tr>
<td>Madhya Pradesh</td>
<td>1967-68</td>
<td>57</td>
<td>17</td>
<td>Malathion, Aldrin and HCH</td>
</tr>
<tr>
<td>Uttar Pradesh</td>
<td>1977-78</td>
<td>258</td>
<td>4</td>
<td>HCH</td>
</tr>
<tr>
<td>Madhya Pradesh</td>
<td>1978</td>
<td>?</td>
<td>6</td>
<td>Phosgene</td>
</tr>
<tr>
<td>Madhya Pradesh</td>
<td>1984</td>
<td>250000</td>
<td>8000</td>
<td>Methyl isocyanate</td>
</tr>
<tr>
<td>Uttar Pradesh</td>
<td>1992</td>
<td>8</td>
<td>6</td>
<td>Aluminum Phosphide</td>
</tr>
</tbody>
</table>

1.4 CURRENT METHODS FOR PESTICIDE DETECTION.

The conventional methods for detection and analysis of pesticide residues include
physico-chemical techniques like Gas Chromatography (GC), High-pressure liquid
chromatography (HPLC), Capillary electrophoresis (CE), Mass spectrometry (MS) and
their combinations thereof such as GC-MS, LC-MS, GC-MS-MS etc (Osselton and
Snelling, 1986).

1.4.a. Chromatographic Techniques.

Gas Chromatography:

Gas Chromatography (GC) is most commonly used for separation of thermostable
pesticides. Various kinds of fused-silica capillary columns with bonded phases of
different polarities are commercially available. The popularity of GC is based on a
favorable combination of very high selectivity and resolution, good accuracy and
precision, wide dynamic concentration range and high sensitivity (Santos and Galceran, 2003). While using GC one can use a number of detectors depending upon the nature of the analyte. Flame photometric detector (FPD) is based on the element specific luminescence where a sulfur/ phosphorus containing compound is burnt in an Hydrogen rich flame eg. for Phosphorous the luminescence is measured at 526 nm. Nitrogen phosphorous Detector (NPD) is based on the fact that the presence of an alkali salt in FPD enhances the ionization of N and P containing compounds. Electron Capture Detector is highly sensitive for the detection of halogen and nitro group containing compounds.

Combination of GC with detection methods such as flame ionization detection (FID) and nitrogen–phosphorus detection (NPD) are most popular; FID gives universal response to organic compounds, while NPD is selective for compounds containing nitrogen or phosphorus and gives much lower detection limits than FID. GC–NPD thus is very suitable for detection of amino group containing pesticides. Electron-capture detection (ECD) is used for sensitive determination of the compound containing halogen or nitro group(s) in a molecule. As such Gas chromatography has been used for detection of various environmental contaminants such as volatile organic compounds (VOC’s), polycyclic aromatic hydrocarbons, polychlorinated biphenyl, terphenyls, naphthalenes, alkanes and pesticides including organochlorine, organophosphate and organonitrogen compounds (Stalikas and Konidari, 2001).

**Liquid Chromatography:**

Liquid Chromatography (LC) is the method of choice for highly polar, thermolabile and/or high-molecular mass compounds, which are not amenable to GC. The compatibility of the water samples with the reversed-phase chromatographic separation systems and the possibility of performing derivatization in aqueous solution made LC the preferred technique. Liquid chromatography approach for analysis of pesticides use various detectors such as ultraviolet (UV), fluorescent Detector (FD) and Refractive index detector (RID) (Kumazawa and Suzuki, 2000). Mass selective detection (MS) is now used more often with GC and LC after the mass detectors such as ion trap detectors and benchtop quadrupole instruments were improved in their detector design.
and operation & acquisition software. These techniques though highly sensitive suffer from several drawbacks

1. Complex sample preparation: Derivatization of the sample to be analyzed is a prerequisite for most of the pesticides, before or after using any particular technique for their efficient detection for example some pesticides such as Glyphosate, bialaphos which contain phosphonic and amino acid groups needs to be derivatized to be less polar and more volatile before they can be detected using GC. The derivatization process may be very lengthy and include the use of highly toxic chemicals (Stalikas and Konidari, 2001). A post chromatographic derivatization to get a fluorophore or chromophore attached to such pesticides for detection has also been reported for detection by HPLC (Oppenhuizen et al., 1991). A pre-column derivatization method for detection of amine containing pesticides utilizes a highly amine reactive compound 9-fluorenylmethyl chloroformate which can be later detected using fluorescent detector at an emission maxima of 315 nm (Falcó et al., 1997).

2. Large time for sample analysis: The chromatographic methods used for the final determination require extraction of the residues from the matrix and subsequent clean-up procedure before they become suitable for analysis (Bruzzoniti et al., 2000). Over 60% of the analysis time is used for sample preparation (Balinova, 1996). Specimens are typically subjected to a complex series of discrete processing steps using liquid/liquid (Smith, 2002), solid phase extraction (Krutz et al., 2003) or other clean up steps (Eskilsson and Bjorklund, 2000).

3. Sophisticated instrumentation and trained personnel to operate: Even with conventional techniques there are several detection methods available, which can be used for detection of specific compounds e.g., while using GC one can use a number of detectors depending upon the nature of the analyte, Flame photometric detector (FPD), Nitrogen phosphorous Detector (NPD) and Electron Capture Detector (ECD). Similarly Liquid chromatography approach for analysis of pesticides use various detectors such as ultraviolet (UV), fluorescent Detector (FD) and Refractive index detector (RID) (Kumazawa and Suzuki, 2000). Mass selective detection (MS) is now used more often with GC and LC after the mass
detectors such as ion trap detectors and bench top quadrupole instruments were improved in their detector design and operation and acquisition software. Thus the personnel performing the analysis should be highly trained so as to correctly determine which detector to use for which compound (Stalikas and Konidari, 2001).

4. **Unsuitable for field studies and in situ monitoring of samples:** These techniques due to their bulky and sophisticated instrumentation are limited to the lab and are unsuitable for field applications. Furthermore, collection, transport and storage can affect sample characteristics, and subsequently, the validity and hence usefulness of analysis. Field analytical methods in combination with an appropriate number of laboratory analyses for field data validation could provide a good monitoring strategy (Mallat et al., 2001; McMohan, 1993; Giese, 2000; Suri et al., 2002).

5. **Large cost of analysis per sample:** The main driving force in the emergence of alternate technology is the increasing cost and number of samples associated with environmental compliance. Therefore, more cost effective tools for water monitoring are urgently needed (Mallat et al., 2001, Nister and Emneus, 1999).

1.4.b **Electrophoretic Techniques.**

Recent advances in technology have found application of electrophoresis in the field of environmental pollution monitoring. Small amounts of pollutants can be detected using Capillary electrophoresis, where separation is performed in fused-silica capillaries with internal diameters of 25-100 μm and they provide very high theoretical plate numbers and along with sensitive detection methods such as UV-absorbance and fluorescence make it a highly promising technique. This technique is fast gaining popularity because of its various modes, which can be used for separation of polar as well as non-polar compounds (Martinez et al., 2000).

In capillary zone electrophoresis (CZE) separation is based on differences in the electrophoretic mobility (determined by size and charge) of charged analytes in an electric field. However, since many environmental pollutants are uncharged or have very similar chemical structures, they often cannot be separated from interfering components.
by CZE and Micellar electrokinetic capillary chromatography (MEKC) has to be used. MEKC separations are based on the differential solubilization of the analytes in the micellar phase. So, they are partitioned between the micelle (the so-called pseudostationary phase) and the aqueous phase, which means that they can be retained differently and then resolved even when they have no charge or when the charged compounds are badly resolved by CZE (Altria, 2000). Capillary electrochromatography (CEC) uses an electric field and an electro-osmotic flow to drive solutes through a capillary column packed with a chromatographic type stationary phase. Separation is based on partitioning the analytes between the running buffer and the stationary phase, and, in the case of charged analytes, on their electrophoretic mobility.

1.4.4 Mass spectrometry

The analysis of organic compounds by mass spectrometry (MS) first involves producing gas phase charged molecular ions and fragment ions of the parent molecules in the ion source of the instrument, and subsequently separating these ions according to their mass-to-charge ratio (m/z), and finally measuring the intensity of each of these ions. A mass spectrometer accomplishes this through a sequence of events within its three main subsystems namely: (i) the ion source in which the ionization of the organic molecules takes place, and from which the ions are then accelerated (or extracted) into (ii) the mass analyzer which separates the ions according to their m/z values, and finally (iii) the detector where the relative intensities (abundances) of the separated ions are determined. The instrument is maintained at low pressure (high vacuum) by a system of turbo molecular or oil diffusion pumps. The low pressure in the instrument permits the ions to travel from the ion source to the detector virtually unimpeded with minimal interaction with other gas phase molecules which might otherwise scatter or fragment the ions and cause a reduction in sensitivity. Mass spectrometry is capable of detecting small molecules in attomole range (10^-18 mol) (Niwa, 1995). A mass spectrum displays the values of the m/z ratio for molecular ions and fragment ions along the X-axis, increasing in value from the origin. The relative intensities of the detected ions are displayed on the Y-axis, generally as the % relative abundance compared to the most intense ion in the
spectrum. Since each organic compound produces a characteristic and often a unique mass spectrum, MS can be effectively used for structure elucidation or confirmation. MS has gained popularity more as a detector for specific compounds separated using one of the separation techniques i.e., GC, LC and CE (Careri et al., 1996; Pico et al., 2004).

1.5. ALTERNATIVE TECHNIQUES

1.5.a Biomolecules based analytical assays.

Biomolecules

Biomolecules such as proteins, nucleic acids, carbohydrates and lipids are large complex molecules with well-defined stoichiometry (chemical composition) and geometry (structure) and biological function. The process of evolution has selected these molecules to carry out their functions with a high degree of specificity, sensitivity without being affected by the functioning of thousands of other biomolecules functioning in the same milieu. Protein molecules show the maximum variety in terms of structure, function and active range followed by nucleic acids which apart from storing and transferring the genetic information (DNA and RNA, mRNA) of the organism also act as building blocks (rRNA) and active transporters (tRNA). Carbohydrates and lipids are specialized to store energy in form of chemical bonds for efficient utilization of resources and also to act as signaling molecules.

Biomolecular interaction assays

The specific interaction of biomolecules can be utilized for development of analytical assays. From the very beginning proteins have been at the forefront for development of the biomolecular interaction assays. These assays are either based on catalytic activity or non-catalytic affinity binding interaction of biomolecules (Griffiths and Hall, 1993).

Catalytic assays: The catalytic assays are based on the activity of enzymes, which have been extensively used in development of analytical assays because of a large range of physical signals generated by the biological activity of enzymes such as protons and ions (pH change), heat, light and mass change (luong et al., 1995, Lowe et al., 1990). The most widely used enzymes belong to the class oxidoreductases (horse raddish peroxidase, glucose oxidase etc) and hydrolases (alkaline/acid phosphatase, acetyl
The advantage of using enzymes is the controllable parameters such as pH, ionic strength, temperature, presence of cofactors etc. that can be optimized for maximal enzyme activity wherever the enzyme activity is required during the assay.

**Non-catalytic assays:** Numerous biomolecules, which are not catalytic, but serve their biological purpose by specifically binding to their target molecule e.g. nucleic acids, receptors and antibodies have been used for development of analytical assays. These molecules or their targets are chemically linked to some reporter group such as enzymes, radioisotopes, fluorescent dyes etc., which can be used to determine the extent of interaction of biomolecule, with their target molecule for qualitative and quantitative determination of the target analyte in the sample.

**Nucleic acids** are capable of binding specifically to other nucleic acids having complementary base pair sequences. This property of nucleic acids is the key element in efficient transfer of genetic information from one progeny to next and is also crucial in translation of stored genetic information for normal functioning of the cell. This property is exploited in development of analytical assay mainly for detection of nucleic acids belonging to pathogenic strains of bacteria and viruses in food or other milieu (Guichon et al., 2004; Shea and Cane 2004).

**Receptors** are non-catalytic protein molecules that span the cell membranes, extending into both extra cellular and intra cellular spaces. The receptors are responsible for chemical senses such as olfaction, taste and also for the inter-cellular communication within an organism through hormones, neurotransmitters and interleukins. Receptors also provide binding sites to a lot of drugs and toxins. The reversible binding of the ligand on the extra cellular region brings about a conformational change in the intracellular region of the receptors, which cause an appropriate modification of the cellular process as a response (Wingard, 1990). A number of assays have been reported utilizing receptors for bioactive compounds such as finding antagonists for histamine receptors (anti allergic) (Crane and Shih, 2004), dopamine (Wong, 2004), growth factor receptor assay in cancer prognosis (Nicholson et al., 2001), interferons (Meagre, 2002), pesticides (Bauer et al., 2002).

**Antibodies (Ab’s)** are serum glycoproteins of the immunoglobulin (Ig) class and are produced by the vertebrate immune system against foreign materials called antigens.
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Antibodies bind to their antigens with very high specificity. This specificity is exploited in development of immunoassays (IA’s). The result of the binding reaction between the Ab and an analyte is usually made visible by means of enzymatic, chemiluminiscent, fluorescent or radioactive markers. According to the label used IA’s can be classified into enzyme immunoassays (EIAs), Radioimmunoassays (RIAs), Fluorescence immunoassays (FIAs) or chemiluminiscent immunoassays (CLIAs) (Gosling, 1990). The measuring range of most IAs for pesticides is in the parts per billion to lower parts per million ranges. A lot of samples can be analyzed within a short time, while only low sample volumes are necessary. In many cases (water, some liquid food samples) no extraction step and no cleanup are necessary. Not all assays are completely specific to one single compound. Cross-reactivities of the Ab’s with haptens similar to the analyte can be observed. In some cases, matrix effects may occur, especially with soil or colored food extracts. Therefore, validation of the assays for the matrix of interest should be carried out. As IA’s are usually targeted at a single analyte or a group of analytes, multi-analyte approaches using Ab arrays are also used for detection in a complex mixture. Immunochemical assays have gained the interest of the scientific community as a potential tool, which can overcome these difficulties encountered during pesticide detection and analysis (Mullet et al., 2000; Suri et al., 2002).

1.5.b Biosensors

A Biosensor can be defined as a quantitative or semi-quantitative analytical instrument containing a sensing element of biological origin, which is either integrated within or is in intimate contact with a physico-chemical transducer (Turner, 1987). The biocomponent can be visualized as a biochemical transducer, which converts the presence/concentration of analyte into a detectable chemical and/or physical property, which is sensed and converted into an electrical signal by the physical transducer (Fig 1.1). The biocomponent is the responsive element, which provides specificity and selectivity to the biosensor. The biological components used in the biosensor construction can in general be divided into two categories: those where the primary sensing event results from catalysis (e.g. enzymes, microorganisms, cells and tissues) and those which depend on an essentially irreversible binding of the target molecule e.g. antibodies,
receptors, nucleic acids (Dennison and Turner, 1995). Depending upon the physical/chemical change brought about by the biological element different physicochemical transducers can be used which are compatible with the change produced (Fig 1.2). These include electrochemical, optical, mechanical and thermometric transducers (Turner, 1992).

Electrochemical transducers include amperometric, potentiometric and coulometric transducers that are based on the measurement of change in electric current, total potential difference and total charge transferred on the working electrode as compared to the reference electrode respectively. Optical transducers are based on the principles of simple absorption of light by the layer of substrate/ligand bound to biomolecular layer, to other techniques such as emission spectroscopy e.g. fluorescence, phosphorescence, chemiluminiscence and bioluminiscence etc., reflectance spectroscopy e.g. Surface Plasmon Resonance [SPR] and Resonant Mirror [RM] wave guide transducers etc. Mechanical transducers are based on the piezoelectric effect where by the change in frequency of a quartz crystal oscillating in applied electric field changes on binding of ligand to the immobilized biomolecular layer e.g. Quartz Crystal Microbalance [QCM] and Surface Acoustic Wave [SAW] sensors (Suri and Mishra, 1996; Donald, 1993). Biosensors based on the change in temperature due to interaction of ligand/substrate with the biological molecules have traditionally used microcalorimeters, thermistors, peltiers and other macro devices. Temperature sensitive polymer films have been successfully used in conjunction with the optical techniques (Johansson et al., 1976; Dessy et al., 1990). An ideal biosensor should meet the following criteria---

A. **Selectivity**: The device should be highly selective for the target analyte and show minimum or no cross reactivity with moieties having similar chemical structure.

B. **Sensitivity**: The device should be able to measure in the range of interest for a given target analyte with minimum additional steps such as pre cleaning and pre concentration of the samples.

C. **Linearity and reproducibility of signal response**: The linear response range of the system should cover the concentration range over which the target analyte is to be measured.
D. **Quick response time and recovery time:** The sensor response should be prompt so that real time monitoring of the target analyte can be done efficiently. The recovery time should be small for reusability of the system.

E. **Stability and operating life:** The biological element used should be interfaced such that the activity is retained for a long time so as to make the device marketable and practically useful in the field. Table 1.5 lists some of the commercialized biosensors available in the market.

![Fig 1.1: Schematic diagram of a Biosensor Unit](image-url)
Table 1.5: List of some commercially available biosensors for environmental monitoring and medical care use

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Company</th>
<th>Transducing and recognition element</th>
<th>Web page</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIA-CORE</td>
<td>Biacore AB (Uppsala, Sweden)</td>
<td>Optical / Biomolecular Interaction</td>
<td><a href="http://www.biacore.com">http://www.biacore.com</a></td>
<td>Environmental and medical</td>
</tr>
<tr>
<td>SPR-CELLIA</td>
<td>Nippon Laser and Electronics Lab (Japan)</td>
<td>Optical/ whole cells or macromolecules</td>
<td><a href="http://www.rikei.com">http://www.rikei.com</a></td>
<td>Environmental</td>
</tr>
<tr>
<td>Cellsense</td>
<td>Euroclone Ltd. (Yorkshire, UK)</td>
<td>Electrochemical/ Escherichia coli</td>
<td><a href="http://www.euroclone.net/environ/">http://www.euroclone.net/environ/</a></td>
<td>Environmental</td>
</tr>
<tr>
<td>Rapidlab 800</td>
<td>Bayer</td>
<td>electrochemical, thick film/ Enzyme</td>
<td><a href="http://www.bayer.co.uk/products/diagnostics-nptp.html">www.bayer.co.uk/products/diagnostics-nptp.html</a></td>
<td>Medical Care/ glucose, lactate</td>
</tr>
<tr>
<td>Xpress Nova 16</td>
<td>Nova Biomedical</td>
<td>Electrochemical/ Enzyme</td>
<td><a href="http://www.pointofcare.net/vendors/nova.htm">http://www.pointofcare.net/vendors/nova.htm</a></td>
<td>Medical Care/ Creatinine</td>
</tr>
<tr>
<td>OMNI 9 OPTI</td>
<td>Roche</td>
<td>optical, single-use electrochemical, thick film/ Enzyme</td>
<td><a href="http://www.roche-applied-science.com/">www.roche-applied-science.com/</a></td>
<td>Medical care/ glucose, lactate, urea, Creatinine</td>
</tr>
<tr>
<td>I-STAT 1 and PCA</td>
<td>i-STAT</td>
<td>single-use, electrochemical, thin film/ Enzyme</td>
<td><a href="http://www.i-stat.com/">http://www.i-stat.com/</a></td>
<td>Medical care/ glucose, lactate, urea, Creatinine</td>
</tr>
</tbody>
</table>
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**Biosensors**

<table>
<thead>
<tr>
<th>Biological Recognition element</th>
<th>Physical Transducer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enzymes based:</strong></td>
<td><strong>Electrochemical Transducer</strong></td>
</tr>
<tr>
<td>• Catalytic Transformation of specific substrate. (phenolics detection using tyrosinase)</td>
<td>• Potentiometric</td>
</tr>
<tr>
<td>• Specific Inhibition of enzyme activity by the target analyte. (acetylcholinesterase inhibition by organophosphates)</td>
<td>• Amperometric</td>
</tr>
<tr>
<td>• Effect on enzyme activity by the target analyte, which acts as modulator or cofactor of enzyme (Mn(II) for HRP)</td>
<td>• Coulometric</td>
</tr>
<tr>
<td><strong>Whole Cell based</strong></td>
<td><strong>Optical/Optoelectronic</strong></td>
</tr>
<tr>
<td>• general inhibition of Cellular respiration</td>
<td>• Light addressable Potentiometric sensors</td>
</tr>
<tr>
<td>• Analyte acting as inducer of specific catalytic protein</td>
<td>• Surface Plasmon Resonance</td>
</tr>
<tr>
<td><strong>Affinity Biomolecules Based.</strong></td>
<td>• UV-Visible Absorbance</td>
</tr>
<tr>
<td>• Antibodies</td>
<td>• Luminescence and Fluorescence</td>
</tr>
<tr>
<td>• Receptors</td>
<td>• Total internal reflection</td>
</tr>
<tr>
<td>• Nucleic acids</td>
<td>Fluorescence (TIRF)</td>
</tr>
<tr>
<td><strong>Thermal sensors</strong></td>
<td><strong>Piezoelectric</strong></td>
</tr>
<tr>
<td>• Isothermal Titration Calorimetry</td>
<td>• Quartz crystal Microbalance</td>
</tr>
<tr>
<td>• Heat sensitive change in Polymer film color.</td>
<td>• Surface acoustic wave sensor</td>
</tr>
</tbody>
</table>

Fig 1.2: Various combinations of physical transducers and available biological elements that can be used for biosensor development
1.6 Problem Definition: Overall Aims and Objectives

This study was aimed at the development and characterization of a highly specific, fast, sensitive, reliable and cost effective method for detection and monitoring of pesticides in environmental samples primarily targeting aqueous environmental monitoring. Biosensors have of late become a method of choice for environmental monitoring. Consisting of a biological detection element in close association with a physical transducer gives a fast method of monitoring biomolecular interactions. The specific interaction of biomolecules with target analyte generates a physical response and the transducer detects this physical change and produces a corresponding electronic signal. We chose antibodies for use as biological recognition element because the vertebrate immune system is practically capable of producing antibodies specific for any target analyte provided it is able to initiate an immune response. Thus the overall aim of the current study was to develop and characterize immunobiosensors for detection of pesticides in environmental samples.

1.6.a Atrazine: A Model Pesticide

Pesticides are in widespread use in agriculture to increase crop yields, and triazine based compounds are commonly employed as herbicides throughout the world. The herbicidal action of triazines is attributed to the blockage of electron transfer on photosystem II (Omokawa et al., 1987). Atrazine is a widely used herbicide in the s-triazine family. Certain crops are tolerant of atrazine and thus atrazine is used to kill weeds in such crops, which primarily include corn, sorghum and sugarcane (Ware, 2000). Use of atrazine has been the subject of significant concerns because it is one of the most commonly detected pesticide contaminant in rivers, streams and wells (US Geological survey, 1999; Bester and Huhnerfuss, 1993). Concerns about their toxicity and persistence in the environment has led to a ban on use of atrazine in more than one country, for instance in Germany, France, Italy, Sweden, Norway and Switzerland (Bester and Huhnerfuss, 1993; Harris et al., 1999; Berkley, 2002). Atrazine consumption is currently on the rise in India. A seven fold increse in consumption of atrazine has been recorded over the last decade with a mere 200 MT of atrazine used in 1992-93, which increased to 1421 MT in 2001 (Bhan and Mishra, 1999; Data Scan, 2003). Table 1.6 shows the major chemical and physical properties of Atrazine.
### Table 1.6. Atrazine Fact sheet (A-Z of Atrazine)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Property</th>
<th>For Atrazine</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>CAS Number</td>
<td>1912-24-9</td>
</tr>
<tr>
<td>C</td>
<td>Chemical Class</td>
<td>s-Triazine</td>
</tr>
<tr>
<td>D</td>
<td>Chemical Formula</td>
<td>C₈H₁₄ClN₅</td>
</tr>
<tr>
<td>E</td>
<td>Molecular Weight</td>
<td>215.68</td>
</tr>
<tr>
<td>F</td>
<td>IUPAC Name</td>
<td>6-chloro-N-ethyl-N'-isopropyl-1,3,5-triazine-2,4-diamine or 2-chloro-4-ethylamino-6-isopropylamino-1,3,5-s-triazine.</td>
</tr>
<tr>
<td>G</td>
<td>Appearance</td>
<td>White, Crystalline, Colorless Powder</td>
</tr>
<tr>
<td>H</td>
<td>Odor</td>
<td>Odorless</td>
</tr>
<tr>
<td>I</td>
<td>Melting Point</td>
<td>175–177 °C</td>
</tr>
<tr>
<td>J</td>
<td>Density</td>
<td>1.187 g/cm³ at 20 °C</td>
</tr>
<tr>
<td>K</td>
<td>Water solubility</td>
<td>30 mg/liter at 20 °C (30 ppm)</td>
</tr>
<tr>
<td>L</td>
<td>Solubility in other solvents:</td>
<td>Chloroform vs.; diethyl ether vs.; dimethyl sulfoxide vs., Methanol 18000mg/L.</td>
</tr>
<tr>
<td>M</td>
<td>Log octanol–water partition coefficient</td>
<td>2.3</td>
</tr>
<tr>
<td>N</td>
<td>Vapor pressure</td>
<td>40 × 10⁻⁶ Pa at 20 °C</td>
</tr>
<tr>
<td>O</td>
<td>Major Use</td>
<td>As a pre/post emergence herbicide mainly in crops like maize, sorghum and sugarcane, pineapple and conifer forests.</td>
</tr>
<tr>
<td>P</td>
<td>Mode of action:</td>
<td>Blocks photosynthesis pathway in non-tolerant plant species. It inhibits the photosystem II photosynthesis by blocking the reoxidation of Qₐ, similar to urea herbicides</td>
</tr>
<tr>
<td>Q</td>
<td>Formulations</td>
<td>More than 328 products that contain atrazine as main component are registered. It is available as dry flow able, flow able liquid, liquid, water dispersible granular, and wettable powder formulations.</td>
</tr>
<tr>
<td>R</td>
<td>Use Rate</td>
<td>Used at 1-10 pounds of active ingredient/acre</td>
</tr>
<tr>
<td>S</td>
<td>Environmental Distribution:</td>
<td>91.1% of all atrazine distributed in the environment is found in water. 4.41% of atrazine is present in soil and 4.12% in sediments. Rest in air, suspended soils, aquatic biomass and vegetal biomass in very small quantities.</td>
</tr>
<tr>
<td>T</td>
<td>Environmental Fate:</td>
<td>Atrazine is degraded to hydroxy atrazine by tolerant plant species and atrazine degrading bacteria which then broken down further by de-alkylation and hydrolysis.</td>
</tr>
<tr>
<td>U</td>
<td>Related Chemicals:</td>
<td>Didealkyl atrazine, Deethyl Atrazine, Deisopropyl Atrazine, Hydroxy Atrazine, Dealkylated Hydroxy Atrazine, Diaminochlorotriazine</td>
</tr>
<tr>
<td>V</td>
<td>Health Hazard</td>
<td>Atrazine is a US EPA Group C classified pesticide and Class III WHO classified chemical (possible human carcinogen). Known toxicity to humans, including carcinogenicity, reproductive and developmental toxicity, neurotoxicity and acute toxicity (depression, reduced respiratory rate, motor discoordination and clonic &amp; tonic spasm)</td>
</tr>
<tr>
<td>W</td>
<td>Toxicity Oral LD₅₀</td>
<td>Rat (1870-3080 mg/Kg Body weight), Mice (1750 mg/Kg Body weight), Rabbit (750 mg/Kg Body weight)</td>
</tr>
<tr>
<td>X</td>
<td>Ban or Restricted Use</td>
<td>Many countries have banned or restricted the use of Atrazine, which include Angola, South Africa, Austria, Denmark, Germany, Norway, Slovenia, Sweden and USA.</td>
</tr>
<tr>
<td>Y</td>
<td>Annual Global Production</td>
<td>Approx. 70000 MT</td>
</tr>
<tr>
<td>Z</td>
<td>Chemical Structure</td>
<td><img src="image" alt="Chemical Structure" /></td>
</tr>
</tbody>
</table>
1.6.b Methodology

Development of an Immunobiosensor starts with selection of target molecule. As shown in Fig 1.3, generation of antibodies specific to the target analyte is the first goal. Small molecules such as pesticides, do not initiate an immune response on their own. To make them antigenic they are coupled to some large protein molecule called as carrier. To carry out coupling these small molecules are generally modified to incorporate a functional group that can be used for chemical conjugation of these chemically modified structures of pesticides (Haptens); with a suitable carrier. This Hapten-carrier conjugate is used to initiate production of antibodies in vertebrate animals such as mice, rabbits, chickens etc. The antibodies are produced against the whole conjugate i.e., hapten as well as the carrier protein. Careful selection after thorough characterization of the hapten protein conjugate leads to production of qualitatively and quantitatively better antibodies specific for the hapten component of the conjugate. These antibodies are then used for development of a sensitive immunoassay that forms the basis for development of immunosensor, using these antibodies with a suitable physical transducer system. This study has been divided into two parts,
Part I: Immunoreagents Preparation and Characterization

The first part of the current study aims at producing thoroughly characterized immunoreagents that can be used to produce highly specific antibodies and immunoassays for target pesticide Atrazine. The structure of Atrazine does not provide for a functional group that can be used for conjugation with carrier protein. This part of the study deals with proposal, chemical synthesis and spectroscopic characterization of various hapten structures for Atrazine that can be conjugated to carrier protein and expected to give antibodies specific for the target pesticide. The preparation and biophysical characterization of immunogens and labeled immunoreagents using these hapten molecules were also undertaken in this part of the study. These immunogens and reagents are used in production of antibodies and development of immunoassays/immunosensors; which comprises the second part of the current endeavor.

Part II. Immunoassay and Immunosensor development

This part of the study encompasses the production of highly specific antibodies for the target analyte Atrazine and development of sensitive and specific immunoassay. Determination of cross reactivity of antibodies produced and optimization of an immunoassay using best possible combination of Ag-Ab with high sensitivity and selectivity was done in this part. The optimized immunoassay was used for development of different types of biosensors for detection of Atrazine. Finally the optimized biosensor and immunoassays were compared for their merits and demerits.

1.7 Bibliography

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