Pesticides are widely used in agriculture for increased production of food and fibre, amelioration of vector-borne diseases. This has resulted in serious health hazards to man and his environment. There is now overwhelming evidence that some of these chemicals do pose potential risk to humans and other life forms and unwanted side effects to the environment [1, 2, 3].

No segment of the population is completely protected against exposure to pesticide and the potentially serious health effects, though a disproportionate burden is shouldered by the people of developing countries and by high risk groups in each country [4]. The world-wide deaths and chronic illnesses due to pesticide poisoning number about 1 million per year [5].

Necessity and value of pesticide:

Pesticides constitute an important component in the development of agriculture and protection of public health in India since tropical climate is very conducive to pest breeding. There are about 20 major diseases such as malaria, filerialsisis, dengue, Japanese encephalitis, cholera, louse-borne typhus etc, which have been brought under control by the use of pesticides [6].

There is a sequential rise in the production and consumption of pesticides in the India during the last three decades [7]. The domestic demand in India accounts for about 76% of the total pesticides used in the country against 44% globally. Currently there are 165 pesticides registered for use in India [8]. At present 39 different pesticides are being manufactured in our country. In India 18.5 g/ hectare of pesticide are used [9].

In 1983, the United Nations (UN) had produced a consolidated List of Products whose consumption and/or sale have been banned, withdrawn, severely restricted or not approved by Governments. When Sunita Narain of the Centre for Science & Environment (SCSE) compared the pesticides listed in this report given below with those approved and used in India, she found that in terms of tonnage, an amazing 70% of all pesticides used on Indian farms were banned or severely restricted in Western countries and identified by WHO as hazardous.

In 1996, even after a decade of environmental regulatory institutions it has been found that the figure in agriculture was 54.25% - 46,826 tons of pesticides out of
a total of 86,311 tons used in 1994-95 has been restricted/banned in the west [10].

**Pesticides banned/restricted in the west but used in India (1994-95) in tonne**

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Use in agriculture</th>
<th>Use in public health</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene hexachloride (BHC)</td>
<td>24,000</td>
<td>6,305.00</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>280</td>
<td></td>
</tr>
<tr>
<td>Dichloro-diphenyl-trichloroethane (DDT)</td>
<td>-</td>
<td>8,181.25</td>
</tr>
<tr>
<td>2,4-D (Dichlorophenoxyacetic acid)</td>
<td>-</td>
<td>1,200</td>
</tr>
<tr>
<td>Dichlorvos (DDVP)</td>
<td>1,500</td>
<td></td>
</tr>
<tr>
<td>Dimethoate</td>
<td>1,900</td>
<td></td>
</tr>
<tr>
<td>Endosulphan</td>
<td>4,600</td>
<td></td>
</tr>
<tr>
<td>Lindane</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Methyl parathion</td>
<td>2,600</td>
<td></td>
</tr>
<tr>
<td>Monocrotophos</td>
<td>6,296</td>
<td></td>
</tr>
<tr>
<td>Mancozeb</td>
<td>4,000</td>
<td></td>
</tr>
<tr>
<td>Paraquat</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>Total use of pesticides</td>
<td>46,826</td>
<td>14,486.25</td>
</tr>
<tr>
<td>Percentage of use consisting of banned or severely restricted pesticides</td>
<td>54.25</td>
<td>94.5</td>
</tr>
</tbody>
</table>

**Definition of pesticide:**

Harmful insects are called pests. Chemicals used to kill pests are called pesticides. The term pesticide covers a wide range of compounds including insecticides, fungicides, herbicides, rodenticides, molluscicides, nematocides, plant growth regulators and others. Among these, organochlorine (OC) pesticides, used successfully in controlling a number of diseases, such as malaria and typhus, were banned or restricted after the 1960s in most of the technically advanced countries. The introduction of other synthetic pesticides—organophosphate (OP) pesticides in the 1960s, carbamates in 1970s and the introduction of herbicides and fungicides in 1970s-1980s contributed greatly in pest control and agricultural output.
Ideally a pesticide must be lethal to the targeted pests, but not to non-target species, including man. Unfortunately, this is not. That's why the controversy of use and abuse of pesticides has emerged. The rampant use of these pesticides, under the adage, "if little is good, a lot more will be better" has played havoc with human and other life forms. In India, the first report of poisoning due to pesticides was from Kerala in 1958, where over 100 people died after consuming wheat flour contaminated with parathion [6]. Long-term, low dose exposure are increasingly linked to human health effects such as immuno-suppression, hormone disruption, diminished intelligence, reproductive abnormalities and cancer [11, 12, 13]. Nearly 40% of pesticides used in our farms are possible causative agents of Non-Hodgkin's Lymphoma (NHL). NHL implicated pesticides and their consumption in Indian agriculture (1993-94) are shown in the following diagram [10].
Acute Pesticide Poisoning:

Pesticides are toxic chemicals, by design and as such they represent risks to the users. In the developing countries, where users are often illiterate, ill-trained and do not possess appropriate protective devices, the risks are magnified [14]. The Poison Information Centre in NIOH, Ahmedabad reported that Organophosphorus (OP) compounds were responsible for the maximum number of poisoning (73%) among all agricultural pesticides [15]. In a study on patients of acute OP poisoning (N = 190), muscarinic manifestations such as vomiting (96%), nausea (82%), miosis (64%), excessive salivation (61%) and blurred vision (54%) and CNS manifestations such as giddiness (93%), headache (84%), disturbances in consciousness (44%) were the major presenting symptoms [16]. Cardiac manifestations such as sinus tachycardia (25%), sinus bradycardia (6%) and depression of ST segments with T wave inversion (6%) were also observed. The incidence of intermediate syndrome in cases of OP poisoning has also been reported [17, 18].

In humans, poisoning symptoms also include excessive sweating, lacrimation, diarrhoea, abdominal cramp, general weakness, poor concentration and tremors. In serious cases, respiratory failure and death can occur.

Other consequences may follow high acute exposures. From one to several weeks after exposure, organophosphate-induced delayed neuropathy (OPIDN) [nerve damage] may set in. This may begin with burning and tingling sensations and progress to paralysis of the lower limbs [19].

Type of Exposure and Hazards:

Different groups and segments of a population are exposed to pesticides in different ways and in different degrees. These are intentional (suicides & homicides) and unintentional exposures (occupational and non-occupational exposure from water, air and food). The occupational hazards in industrial settings and the ecological repercussions in the environment could be grouped as under:

i) Operational hazards could be during manufacture & formulation of pesticides in industrial settings and their distribution and use in field
conditions.

ii) Direct toxic effects on non-target animal life such as pollinators, predators, wild life etc. during application of pesticides.

iii) Post application hazards or indirect toxic effects which involve risk to non-target animals due to toxic residues of pesticides in food or due to pollution of the ecosystem, habitat as a whole such as water bodies or soil [8].

Amongst various groups of pesticides that are being used as pest control agent, OP compounds occupy an important position. Out of 900 pesticides in use globally, more than 250 belong to this group. These insecticides are the most commonly used chemicals which account for more than 1/3 of the market sales, an evidence of which is given in the following table:

<table>
<thead>
<tr>
<th>Class</th>
<th>Insecticide Market (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organophosphates</td>
<td>36</td>
</tr>
<tr>
<td>Pyrethroids</td>
<td>25</td>
</tr>
<tr>
<td>Carbamates</td>
<td>21</td>
</tr>
<tr>
<td>Organochlorines</td>
<td>8</td>
</tr>
<tr>
<td>Others</td>
<td>10</td>
</tr>
</tbody>
</table>

Tetraethyl pyrophosphate (TEPP) was the first OP pesticide which was developed in Germany during World War II as a byproduct of nerve gas development.

OPs are all derived from phosphoric acid. They are generally among the most acutely toxic to vertebrate animals. They are also unstable and therefore break down relatively quickly in environment. Altogether, over 100,000 OP compounds have been screened for their insecticidal properties of which 100 have been developed for commercial use.

Mode of Action:

OPs work by inhibiting several ester-splitting enzymes of the nervous system which play a vital role in the transmission of nerve impulses. Nerve impulses
travel along neurons (nerve cells) by way of electrical signals. However, at a junction between two neurons (a synapse) and between a neuron and a muscle (neuromuscular junction) the impulse is transmitted operating in the automatic nervous system, neuromuscular junctions and parts of the central nervous system by acetylcholine (a neurotransmitter) which is released by cholinergic neurons. It is broken down and inactivated in milliseconds by the enzyme acetylcholinesterase (AChE). With exposure to OPs the enzyme is unable to hydrolyze acetylcholine, which causes interference with second nerve impulse transmission at nerve endings.

OPs have high anticholinesterase activity as they are structurally similar to acetylcholine. Phosphatic compounds phosphorylate the vitally important enzymes, esterases inhibiting normal functions in mammals and insects with the accumulation of acetylcholine which disrupts the normal life and finally leads to death. The inhibition of AChE is irreversible whereas with carbamates the inhibition is reversible. For maximum efficiency, the phosphatic compounds should fit into the active centres of the esterase in a 'key in a lock' fashion. Hence the activity of the OPs depends greatly on the structure of the ester groups [20].

Binding site of OPs on AChE has been well observed in insects and hence the effect of pesticide on insects is more than mammals [21].

**Mechanisms of selectivity:**

The enzymatic hydrolysis of liberated acetylcholine seems to be essential to nerve-muscle relations in all animals. The Organophosphorus poisons may therefore seem at first to be wholly unsuitable for selective action. Selectivity, however, arises from the important complex of other reactions. A compound may be intrinsically toxic to all animals but, of similar doses externally administered, the fraction which can reach the active site sufficiently quickly can vary widely between species. In more complex compounds more possibilities of alternative reactions are opened up, leading to more competition between toxicant-producing and toxicant destroying reactions.

Most pesticides act, finally on some very general biochemical mechanism and rely for their selectivity on quantitative rather than qualitative differences. Most biochemical mechanisms are remarkably universal. Essentially quantitative differences in basically the same biochemical processes account for the difference in shape, life history and behaviour between man and his louse. They
can fairly be relied on to make a compound safe for one but lethal to the other [22].

Acetylcholine (ACh):

Acetylcholine is an ester of great biological significance. Its powerful pharmacological action, discovered in 1906 by Reid Hunt and Tavean, led some pharmacologists and physiologists to believe that the ester is a “neurohumoral transmitter” from the nerve endings to the effector organ or from the nerve ending to a second nerve cell.

The action of acetylcholine is essential for the generation of bioelectric currents which propagate impulses along nerve and muscle fibers. Esters generates these currents by changing the permeability to sodium ion across post synaptic membrane whereby ionic concentration gradients between the inside of the fiber and its outside environment, the potential sources of the electromotive force become effective. Having produced its action, the ester is inactivated by enzyme AChE. The resting condition is thus restored and the next impulse is able to pass.

The action of the enzyme AChE is thus essential for the elementary process of conduction. Owing to its vital function the enzyme has attracted the interest of many investigators and its properties have been discussed in several reviews [23].

Characteristics of AChE:

1. The enzyme has a well-defined optimum concentration for acetylcholine, which is about 4 to 7 micromoles per ml, depending on the source. At higher concentration, the activity decreases.

2. Increase in number of Carbon atoms of the acyl chain from 2 to 3 does not markedly affect the activity; however with butyryl choline as substrate the activity decreases.

3. Non-choline esters (like dimethylaminoethyl acetate) are split but the concentrations required are much higher and the maximum rates are usually much lower.

4. Particular form residing on post synaptic membrane and soluble form residing in the cytoplasm of nerve cell.
5. Diisopropylphosphoryl fluoride (DEP) irreversibly binds with AChE.

6. The active region of AChE form a gorge which contains an aromatic anionic site (near tryptophan 86) and an esteratic site formed by serine 203, histidine 447 and others.

7. AChE is a 65 KD fast acting protease type of enzyme (such as trypsin).

8. AChE's catalytic site is near the bottom of a narrow and 20 Å deep gorge that extends halfway through the protein and widens out near its base. The sides of this so called active site gorge are lined with the side chains of 14 aromatic residues that comprise 40% of its surface area. Since the side chain O-atom (oxygen atom) of the active site Ser is only 4 Å from the bottom of the gorge, ACh must bind in the gorge with its positively charged trimethylammonium group surrounded by aromatic side chains. This conclusion came as a surprise since it had been understandably expected that the trimethylammonium group would be bound at an anionic site. Perhaps the weak binding provided by the interactions of the trimethylammonium group with the electrons of the aromatic rings facilitates the rapid diffusion of ACh to the gorge, thereby accounting for the enzyme's high turnover number.

Other esterases which hydrolyze choline esters faster than non-choline esters but differ distinctly from AChE (specific in nature) can be readily distinguished by three features.

1. The rate of hydrolysis increases if the length of the acyl chain increases from 2 to 4 carbon atoms.

2. The activity substrate concentration relationship does not show a well defined optimum; i.e. there is no substrate inhibition at higher concentration.

3. It does not hydrolyze acetyl-β-methyl chain.

Since physiological substrate of these esterases is not yet established, a proper name could not be assigned at present; following usual enzyme terminology they may be referred to as cholinesterases (non specific in nature). Human and horse serum predominantly has this type of esterase.

Sources of AChE:

AChE is found in all conductive tissue throughout the animal kingdom e.g.
nerve, muscle and electric organ. The enzyme is localized exclusively in the active membrane; therefore, the concentration per gm of tissue is generally low. A high concentration of enzyme is found, for instance, in the nucleus caudatus in mammalian brain. Nucleus caudatus is easily obtained in large amount to provide a starting material for purification. Per gram fresh weight this tissue is able to hydrolyze 1.5 millimoles of acetylcholine per hour. Other sources are:

Squid head ganglia: Per gram fresh weight 15 to 30 millimole of Ach are split per hour.

Cobra snake venom: Per mg weight may split 0.5 to 1.5 millimole of Ach per hour.

Electric organ of electric fish: Per gram fresh weight hydrolyze 10 to 20 millimole.

There are various non-specific esterases other than AChE present in tissue even in brain. Therefore, to get actual interaction between AChE and OP specifically purification of AChE is necessary. Chan et. al. has done purification of AChE from brain using Sephadex G200 because of the molecular weight of AChE.

The brain and spinal cord form the central processing unit of nervous system. They receive messages via the sensory fibres from body's sense organs and receptors and analyse it, then send out signals along the motor fibres which produce an appropriate response in the muscles and glands. The four major parts of brain having the risk of being affected by OPs are Hypothalamus, Cerebral Cortex, Cerebellum and Corpus Striatum. The functions of these four parts of the brain are summarized as follows:

1. Hypothalamus: The hypothalamus has nervous pathways which connect with the limbic system which is closely connected with the smell centres of the brain. This portion of the brain also has connections with areas involved with other senses, behaviour and the organization of memory. Hypothalamus is the conductor of orchestra of hormones by sending forth releasing factors. It regulates, controls the work of other glands in the body. As for example it regulates the pituitary gland functions. For appropriate function of cerebrum contribution of hypothalamus is tremendous. It affects all kinds of metabolic activities. It regulates body temperature, food intake and thirst; plays a role in maintenance of
Internal structures of the brain
rhythms of sleep and wakefulness; functions in rage and aggression. It controls and integrates the autonomic nervous system.

2. Cerebral Cortex: The Cerebral Cortex is a 3 mm (1/2 inch) thick wrinkled layer of grey matter folded over the outside of the cerebrum. This part is folded in humans. Among all the folds there are certain grooves which divide each of the two hemispheres of the cortex into four areas called lobes. Each of the lobes serves one or more specific functions. The temporal lobes are involved with hearing and smell, the parietal lobes with touch and taste, the occipital lobes with sight and the frontal lobes with movement, speech and complicated thinking. It is the Cerebral Cortex, therefore that information received from the five senses—sight, hearing, touch, taste and smell is analyzed and processed so that other parts of the nervous system can only act on the information if necessary. In addition, the premotor and motor areas of the cerebral cortex work with other areas of the central and peripheral nervous systems to bring about coordinated movements which are vital to every conscious activity the body performs.

3. Cerebellum: It is concerned with the maintenance of equilibrium of the body, regulation of muscle tone required for posture and balance and finer adjustment of movements. It modulates time sequence of muscles contractions during movements.


Phytoremediation:

Phytoremediation is cleaning up of toxic wastes using plants. Alternatively phytoremediation may be defined as the use of plants for rehabilitation of polluted environments [24]. This is attracting a number of scientists and industry and government agencies. Phytoremediation using bacteria and fungi has evolved more than a decade ago. In the year 1991 in USA it was found that Dept. of Energy, State and private sector have spent approximately $700 billion to clean up hazardous waste. Then it has been suggested by M. Russel that proper bioremediation can save the expenses. Currently the remediation site has been planned to be converted to a biofermentor.

But there are some disadvantages of using microbial remediation—
i) Special environment is needed for a particular type of microbial species.

ii) Endogenous carbon and energy sources are to be supplied.

iii) It needs physical contact of the medium either soil or water and that may result in site destabilization, increasing water run off and increased mobilization of contaminant in leachate.

The alternative is to use plants though a few species can detoxify the wastes and are known as ‘remediation cultivators’ [24].

The advantages of using plants over microbial systems are—

i) Contaminants uptake and subsequent degradation would remain in the plant itself.

ii) It does not need endogenous supply of nutrients and energy.

iii) The rhizosphere microbial symbiosis may be an additional advantage.

iv) In waste water land discharge system trees are superior to other plants such as grass because of low establishment and maintenance costs.

v) Huge surface area, yield of biomass (wood), water pumping ability, massive transpirational stream have increased the remediation rate in trees.

vi) The ability of many wetland plants to alter pH around their roots provides oxygen into the anaerobic zone. The biotic activity increases alkalinity, sulfide ion concentration, the organic residues, the metal ion precipitation out and the water release to stream outflows.

vii) Wide range of materials which can be degraded by plants including pesticide [25], polycyclic aromatic hydrocarbons [26], chlorinated solvents [27], DDT [28], Dioxanes [29], phenols [30] etc.

Montory Pine, Donghlas Fir and *Populus* species are highly used. Always fast growing plants are chosen.

Heavy metals found in soil have been known for at least 70 years. The hypertolerance of metals is the key plant characteristic required for hyperaccumulation. The list of metal ‘hyper-accumulating’ is growing and includes—
GENERAL INTRODUCTION

a) *Viola calamimria* (can accumulate lead as 1% of its dry weight)
b) *Silene cucubalus*
c) *Haumaniastrum katangense*
d) *Sebertia acuminata*
e) *Armeria maritima*
f) *Alyssum bertolonii* and others [24].

Metal sequestration peptide in animals is metallothionein (METALOTHIONEIN). The gene is very small. Misra and Gedamu (1989) have constructed a chimeric human metallothionein gene driven by CaMV35S promoter conferring plant gene expression. This construct was used to produce transgenic plants expressing various levels of metallothionein to detoxify more and more Cd\(^{2+}\) from soil [31].

An alternative approach to create metal tolerant plant is to produce cell on culture containing heavy metal in media. A few approaches have been successful in producing plants from the cell lines.

Gene transfer method from one species to another is also utilized.

The use of plants in wastewater treatment is as old as of 300 years [32]. But in normal cases the efficiency of plant to attract is very low.

Lead, the metal contaminant of largest environmental concern is accumulated by plants like *Apocynum sp.* and *Ambrosia sp* [32].

The use of transgenic plants for cleaning up dangerous mixtures of explosives like TNT, RDX etc [33] are increasing day by day.

Expression of *merA* from mercury resistant bacteria in transgenic yellow poplar plant might provide an ecologically compatible approach for the remediation of mercury pollution [34].

Fe\(^{2+}\), Cd\(^{2+}\) and Zn\(^{2+}\) uptake by *Arabidopsis* and yeast mutants indicates strategies for developing transgenic improved phytoremediation cultivars for commercial use [35].

*Lemna minor* has been reported to be efficient in decreasing BOD, solids and nutrients from the waste-water and has high potential for treating organically
The most common parameter used in the pesticide industry to predict plant uptake from the soil is the octanol water partition coefficient ($K_{ow}$). Contaminants with a log $K_{ow}$ (=1) are considered very water soluble and would be predicted to cause ground water contamination. But plant roots do not generally accumulate these water soluble compounds at a rate surpassing passive influx in the transpiration stream. Compounds with a low log $K_{ow}$ (=1) can be accumulated in plants and many are generally considered mobile in both plant xylem and phloem. Pollutants with intermediate log $K_{ow}$ (approx. 1-4) are taken up by roots and are considered xylem mobile but generally phloem immobile unless chemically modified by plant. Compounds in this range would be expected to be good targets for phytoremediation, and the list of priority pollutants that fall in this range is extensive.

Compounds that are denser than water, have low log $K_{ow}$, and come from point sources (e.g. Leaking drums) would tend to have vertical concentration profile in the soil and make them more difficult for plant root treatment without excavation. Compounds with log $K_{ow}$ greater than 4 are greatly absorbed by roots but are substantially translocated to the shoot [32].

Phytoremediation has the potential to develop into a viable remediation option in cases where pollutants: a) are near the surface, b) are relatively non-leachable, and c) pose little eminent risk to health or the environment. Research in this area is expected to grow over the next decade as many of the current engineering technologies for cleaning surface soil of metals and non-volatile organics are clumsy, costly and physically disruptive. The Phytoremediation technology, when fully developed, could result in significant cost savings and result in the restoration of numerous sites by a relatively noninvasive method which in some form can be potentially aesthetically pleasing [32].

The uptake and phytotransformation of Organophosphorus (OP) pesticides (malathion, dementon-S-methyl, and crufomate) was investigated in vitro using the axenically aquatic cultivated plants like parrot feather (Myriophyllum aquaticum), duckweed (Spirodela oligorrhiza L.) and elodea (Elodea canadensis).
The rates of disappearance of OP compounds from the aqueous medium are in the order duckweed > parrot feather > elodea, elodea > duckweed > parrot feather, and duckweed > parrot feather > elodea for malathion, dementon-S-methyl, and crufomate respectively. The physicochemical properties of both the OP compounds and the plant species play a critical role in phytotransformation processes. The result of this study showed that selected aquatic plants have the potential to accumulate and to metabolize OP compounds. It also provided knowledge for applying phytoremediation processes to environmental problems [37].

Some investigators have shown that bacterial enzymes cause degradation of several organophosphates such as malathion [38]. Biochemical mechanisms of malathion resistance were investigated in a malathion resistant strain of the parasitoid Habrobracon hebetor [39]. Scientists have found that activation of plant enzymes may cause degradation of residual OPs like malathion and phenthoate [40].

In the present study phytoremediation efficiency of different OP pesticides would be taken into consideration.

Quantitative Structure Activity Relationship (QSAR):


The relationship between chemical structure and reactivity, both chemical and biological has been of major concern since the beginning of science. The quantitative correlation between pesticidal activities with physico-chemical parameters related to structures in the rational design of effective pesticides is often referred to as Quantitative Structure Activity Relationship (QSAR) studies. The mathematical functions could be so chosen that certain physico-chemical properties influence the bioactivity and the structural modifications which enhance such properties that would lead to generate potent compounds [20]. A number of attempts have been made from different approaches [41].

Modern chemical structure-activity work can be dated from 1869 with publication of Mendeleev’s periodic table and a century later, Crum-Brown and Frazer postulate that

\[ \Phi = f(C) \]

where, \( \Phi \) = measure of biological activity of a compound
C= chemical structure of the compound

Crum-Brown and Frazer envisioned the development of a calculus of biological structure-activity relationships (SARs) arising from the study of the effect of small changes in C on Φ [42].

Meyer and Overton [43] observed that narcotic potency of simple and neutral organic compounds (eg. Alcohol, ketone, esters etc.) paralleled their oil/water partition coefficient. Equation based on Overton's data for the narcosis of tadpoles was formulated as follows:

\[ \log \frac{1}{C} = 0.94 \log P + 0.87 \]

\[ n = 51, \ r = 0.971, \ S = 0.280 \]

C = molar concentration of chemical that stopped the movement of tadpole

P = octanol/water partition coefficient of the substance

n = number of chemicals studied

r = correlation coefficient

S = Standard deviation from the regression

The major breakthrough in this empirical approach to understanding the effect of structural changes on reaction rates and equilibria was made in about 1935 by L. P. Hammett [44] who used the ionization constants from the model system of the benzoic acids in water to devise a numerical scale σ for the electronic effect of substituents on a reaction center.

The next major advance in generalizations was that of Robert Taft [45], who defined the steric parameter E,

\[ E = \log K_X - CH_2 COOEt - \log K_H - CH_2 COOEt \]

where, K= rate of hydrolysis of esters of acetic acid

X = has no electronic effect only steric properties

Taft went on to show that a linear combination of steric and electronic parameters could be used to separate and delineate the role of the two independent factors on the reactions of organic compounds.
A hydrophobic parameter $\Pi$ was also related with octanol-water partition coefficient [46].

$$\Pi = \log P_\lambda - \log P_H$$

where, $P_H =$ octanol-water partition coefficient of a compound

$P_\lambda =$octanol-water partition coefficient of a derivative of the compound.

Similarly inductive effect, resonance parameter can be used.

Fukuto and Metcalf [47] correlated their data for the inhibition of fly head cholinesterase by a set of phenyl phosphates $\text{X—C}_6\text{H}_4\text{OP (OC}_2\text{H}_5)_2$ as

$$\log 1/C = 2.59 \sigma + 4.29$$

$n = 12; r = 0.906; S = 0.620$

$$\log 1/C = 2.42 \sigma + 0.26 \Pi - 0.60$$

$n = 8; r = 0.987; S = 0.228$

where, $C =$ molar concentration causing 50\% enzyme inhibition of 50\% of the flies

$\Pi =$hydrophobic effect in the living flies

Hydrophobic effect is very important in drug designing.

The equation used to membrane study by QSAR [48] as

$$\log 1/C = 0.87 \log P - 0.24, n = 5, r = 0.993; S = 0.100$$

where, $C =$ concentration of alcohol (ROH) necessary to produce a 10 mV change in the rest potential of a lobster axon by ROH.

The LD$_{50}$ of ROH for cats is given by

$$\log 1/C = 1.06 \log P + 1.37$$

$n = 8, r = 0.986, S = 0.134$

A QSAR can be expressed in its most general form by the following equation:

$$\text{Biological activity} = f (\text{Physicochemical and / or Structural parameters})$$

The overall objective is to find parameters from experiment or theory that when substituted into one of the many forms of the equation along with biological
activity for a series of molecules, give a statistically significant correlation. If a good model is found it may be used to predict other molecules having greater activity in the defined biological system.

For QSAR one usually describes each analogue as a parent molecule to which substituents have been added. The change in potency as substituents are changed and this is correlated with the effect of same substituents on various types of physico-chemical equilibrium constants, such as changes in logarithm of the octanol-water partition coefficient ($P_{ow}$). Therefore, for QSAR it is most logical also to describe the biological properties of the molecule in terms of some sort of equilibrium or rate constant.

Since potency is to be predicted, the relative potency of each existing compound must be supplied. The standard deviation of each potency value sets the ultimate precision that can be expected of the QSAR [41].

The nature of statistical methods of regression or partial least squares analyses is such that the best result is obtained if one includes a large number of compounds that also show a wide range of potency; the wider the potency range, the better is the result. In an ideal data set, there are an equal number of compounds tested in each potency interval. Additionally, the statistical nature of QSAR makes one aware of the advantage of testing as many compounds as possible [41].

Dihydrofolate reductase inhibition is of particular interest since such inhibitors have antibacterial, antimalarial and antitumour agents and act as starting point in drug development. The success of dihydrofolate reductase inhibitors as antimicrobial agents depends on the great differences in this enzyme from mammalian and microbial sources. A QSAR (eq 1) has been formulated for the inhibition of dihydrofolate reductase from *S. faecium* by quinazolines. This is compared with a QSAR (eq 2 and 3) for inhibition of *E. coli* dihydrofolate reductase by 2, 4-diamino-5-benzylpyrimidines. The QSAR for inhibition of bacterial enzyme is compared with QSAR (eq 4) for mammalian enzyme inhibition. A QSAR (eq 5) has also been formulated for the antimalarial action of quinazolines against *P. berghei* in mice. The antimalarial QSAR is consistent with that of the in vitro bacterial study [49].
GENERAL INTRODUCTION

\[ \log 1/C = 1.125(\pm 0.35) (\Pi-5) \]
\[ - 1.103(\pm 0.25) (\text{MR-5}) - 2.385(\pm 0.59) (I-1) \]
\[ - 4.092(\pm 0.82) (I-2) - 2.368(\pm 0.37) (I-3) \]
\[ + 8.255(\pm 0.27) \]  \hspace{1cm} (eq 1)

\( n = 67; \ r = 0.926; \ S = 0.672 \)

where, \( n \) = number of data points
\( r \) = correlation coefficient
\( S \) = standard deviation
\( \text{MR-5} \) = molar refractivity of substituents in position 5
\( \Pi-5 \) = hydrophobicity of 5- substituents
\( I \) = indicator variable assigned a value for a particular substituent

\[ \log 1/C = -1.443(\pm 0.48) \sum \sigma^+ \]
\[ + 5.865(\pm 0.38) \]  \hspace{1cm} (eq 2)

\( n = 10; \ r = 0.926; \ S = 0.418 \)

\[ \log 1/C = -1.125(\pm 0.15) \sum \sigma R^+ \]
\[ + 5.538(\pm 0.19) \]  \hspace{1cm} (eq 3)

\( n = 10; \ r = 0.986; \ S = 0.182 \)

where, \( C \) = molar concentration causing 50% inhibition
\( \sigma^+ \) = Brown's parameter
\( \sigma R^+ \) = Taft's resonance parameter

\[ \log 1/C = 0.81 (\text{MR-6}) - 0.064 (\text{MR-6})^2 \]
\[ + 0.78 (\Pi-5) - 0.73 (I-1) - 2.14 (I-2) - 0.54 (I-3) \]
\[ - 1.39 (I-4) + 0.78 (I-6) - 0.20 (\text{MR-6. I-1}) \]
\[ + 4.92 \]  \hspace{1cm} (eq 4)

\( n = 101; \ r = 0.961; \ S = 0.441 \)
where, $C$ = molar concentration of quinazoline causing 50% inhibition of mammalian dihydrofolate reductase

MR-6 = molar refractivity of substituents in position 6

$\log \frac{1}{C} = 0.877 \pm 0.28 \, (\Pi\text{-sum})$

$- 0.155 \pm 0.05 \, (\Pi\text{-sum})^2 - 0.679 \pm 0.46 \, (I-6)$

$+ 0.373 \pm 0.32 \, (I-8) + 1.526 \pm 0.29 \, (I-9)$

$+ 1.185 \pm 0.40 \, (I-10) + 3.272 \pm 0.40 \, (eq \, 5)$

$n = 60; \, r = 0.906; \, S = 0.427; \, \Pi_0 = 2.82 \, (2.5-3.2)$

where, $C$ = molar concentration (mol/kg) of orally given drug producing 90% suppression of malaria

$\Pi\text{-sum} = \text{all of the substituents in the 5, 6 and 8 positions}$

Topological indices are being developed to explain the pharmacological and toxic action of drugs at the molecular level. Two newly formulated information-theoretic topological indices namely Information content (IC) and Structural information content (SIC) are used to study QSAR with three important series of bioactive agents: carbamoyl piperidines, barbiturates and alkanes. Statistical analysis reveals that IC and SIC correlate significantly with the butyrylcholinesterase-inhibiting potency ($K_i$) of carbamoyl piperidines. Isohypnotic concentrations (c) of barbiturates and toxic doses ($LD_{50}$) of alkanes. The study shows that these topological indices may be used for the prediction of the pharmacological and toxic action of molecules [50].

To further elaborate the role of molecular topology in the quantitative prediction of physicochemical and pharmacological properties, the degree of IC and SIC with the physical as well as biological properties of bioactive agents like alcohols, diphenhydramines and anti-psychotic compounds has also been investigated [51].

Correlation analysis has been used to develop Quantitative Structure Activity Relationship (QSAR) for 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-(3-substituted phenyl) S-triazine. For inhibition of dihydrofolate reductase (DHFR) from human and other species, a set of congeners is used to probe the enzyme to
obtain information about the active site in terms of its hydrophobic, steric and electronic requirements for ligand interaction. Using the QSAR approach they have formulated structures for inhibition of *Leishmania major* dihydrofolate reductase and found that maximum inhibition for promastigote cell growth is done by a particular triazine [52].

The inhibition of DHFR is best correlated by a modified variable for hydrophobicity of the 3-X substituent (IT$_3$), an alkoxy group indicator variable ($\rho$), a disposable parameter ($\beta$) obtained by iteration, and a variable that parameterizes steric effects (MR) in the equation:

$$\log \frac{1}{K_i} = 0.65 (\pm 0.08) IT_3 - 1.22 (\pm 0.29) \log (\beta, 10^{rt3} + 1) - 1.12 (\pm 0.29)\rho + 0.58 (\pm 0.16)MR + 5.05 (\pm 0.16)$$

where, $K_i$ = Michaelis inhibition constant

The EC$_{50}$ values for triazine inhibition of *L major* cell growth in culture are correlated by the equation:

$$\log \frac{1}{EC_{50}} = 0.21 (\pm 0.09) IT_3 + 0.44 (\pm 2.10)\log \frac{1}{K_i} + 0.53 (\pm 0.32)$$

where EC$_{50}$ = concentration of drug resulting in a growth rate equal to 50% of the rate in a drug free medium.

Human Immunodeficiency Virus (HIV) is the etiologic agent of Acquired Immune Deficiency Syndrome (AIDS), which expresses its effect through the genetic direction of viral polyproteins prepared by the host. HIV 1 protease is the aspartic proteinase encoded by the virus responsible for the processing of the gag and gag-pol genes polyproteins. Therapeutic intervention at this proteolytic step of viral replication has been demonstrated to yield immature and non-infectious viral progeny. Conventional Structure Activity data indicate a preference for S-hydroxyl diastereomer in the isosteric linkage of the inhibitor positioned between Asp24/125 carboxylate groups of the protease. Structure Activity studies have provided a plethora of information regarding the structural requirements of ligands for the HIV protease. Field fit minimization of neutral molecules to crystal ligands and active site minimization of protonated ligand yielded predictive correlation for HIV protease inhibitors. All
biological activities used in the above study were expressed as

\[ \text{Bio} = -\log_{10} IC_{50}, \]

where, Bio is the biological activity and IC_{50} is the micromolar (μM) concentration of the inhibitor producing 50% inhibition [53].

Analogues of classical antifolates with the 4-aminobenzoyl group replaced by 4-amino-1-naphthoyl were synthesized for study after molecular modeling indicated ample spatial accommodation for the naphthalene ring and even larger groups in models based on reported X-ray crystallographic data describing the binding of methotrexate to human dihydrofolate reductase (DHFR). Target compounds included naphthoyl analogues of aminopterin (AMT), methotrexate (MTX), 5-deazaAMT, 5-deazaMTX, 5-methyl-5-deazaAMT, 5-methyl-5-deazaMTX and 5,8-di deazaAMT. A 5,6,7,8-tetrahydronaphthoyl analogue of 5-deazaAMT was also prepared. None of the naphthoyl analogues showed loss in binding to DHFR compared with the corresponding antifolate bearing the benzoyl group, thus confirming the anticipated bulk tolerance. Only the 5,6,7,8-tetrahydronaphthoyl analogue of 5-deazaAMT analogue showed reduced antifolate effects. All calculations were performed on a DEC VAX 6420 computer system under VMS 5.4, connected to an Evans and Sutherland PS 390 graphic station, using the MACROMODEL Version 3.0 and SYBYL Version 5.41 software packages. Molecular dynamics was conducted under MACROMODEL Version 3.5 on a Silicon Graphics, Inc., IRIS 4D/35TG+. Coordinates for the human DHFR binary complex with folate were obtained from the Brookhaven Protein Databank (structure code 1 DHF). The MTX, 5-methyl-5-deazaAMT and 5-methyl-5-deazaMTX analogues were evaluated in vivo alongside MTX against E0771 mammary adenocarcinoma in mice. All three proved effective than MTX in retarding the tumor growth [54].

A series of novel 4-(3-pyridyl)-1(2H)-phthalazinone derivatives which possess dual activities of thromboxane A₂ (TX A₂) synthetase inhibition and bronchodilation was synthesized, and their pharmacological activities were evaluated. The 2-substituents polar head groups and introduction of heteroaromatic nuclei into the 4-position of phthalazinone affect the bronchodilatory activity. Additionally, the hydrophobicity of the compounds was found to exert a marked influence on the bronchodilatory activity. Although their precise mechanism of action remains unclear, this series of novel phthalazinone derivatives represents a new class of antiasthma agents with...
Gupta et al. made a QSAR study on a series of anti-tumor aniline mustards and a series of acridine-linked aniline mustards. While cytotoxic activities of the former are found to depend only upon the electronic parameter, those of the latter are found to depend upon the lipophilicity of the linker chain and its connectivity index, thus suggesting that the linker chain probably facilitates the transfer of the compounds to the DNA and helps them to bind with the DNA. The antitumor activity of aromatic mustards should be the function of the electron density on this nitrogen atom, which can be affected by an electron releasing substituent at the aryl ring. This is in full accordance with the negative dependence of cytotoxic activities of electronic factor $\sigma$. $\sigma$ was not found so important in case of acridine-linked mustards. The activities of these mustards were found to be correlated with lipophilicity ($\Pi$) and Kier’s first-order valence molecular connectivity index $^1\chi^*$ of the linker chain (Eqs 1-3) [56].

\[
\log \left( \frac{1}{IC_{50}} \right)_{A8} = 0.55 \pm 0.24 \Pi - 0.24 \pm 0.18 \Pi^2 + 6.14
\]

\[n = 16, R = 0.81, S = 0.34, F(2,13) = 12.40, \text{EV} = 0.61\] \hspace{1cm} (eq 1)

\[
\log \left( \frac{1}{IC_{50}} \right)_{P388} = 0.27 \pm 0.17 \Pi - 0.41 \pm 0.19 \ ^1\chi^* + 7.76
\]

\[n = 16, R = 0.88, S = 0.32, F(2,13) = 22.15, \text{EV} = 0.74\] \hspace{1cm} (eq 2)

\[
\log HF = 0.28 \pm 0.09 \Pi - 0.42 \pm 0.10 \ ^1\chi^* + 2.44
\]

\[n = 16, R = 0.97, S = 0.16, F(2,13) = 89.20, \text{EV} = 0.94\] \hspace{1cm} (eq 3)

where, $IC_{50}$ = molar concentration of the drug producing 50% effect on the cells.

- $HF$ = hypersensitivity factor
- $n$ = number of data points
- $R$ = multiple correlation coefficient
- $S$ = standard error of estimates
- $F$ = F-ratio between the variance, and observed activities
- $\text{EV}$ = explained variance and data within parentheses are 95% confidence interval; A8 and P388 are cell lines.
The (S)-(−)-isomer of trimetoquinol is a potent β-adrenergic receptor (AR) agonist in heart and lung tissues whereas the (R)-(+) -isomer acts as a selective and highly stereospecific thromboxane A₂/prostaglandin H₂ (TP) receptor antagonist. The site-selective β-(AR) agents have potential in the treatment of cardiopulmonary diseases, non-insulin-dependent diabetes and obesity whereas highly selective TP receptor antagonists have value in the treatment of thrombolytic disorders. Trimetoquinol and its derivatives (iodinated analogues), reported to be highly potent β₂ adrenoceptor (AR) and site-selective thromboxane A₂/prostaglandin H₂ (TP) receptor ligands are subjected to Quantitative Structure Activity Relationship (QSAR) study. From the significant correlation equation, obtained between the binding affinity, pKᵢ (β₂- AR) and the substitutional physicochemical parameters such as molar refraction (MR), hydrophobic constant (n) and resonance parameter (R), the receptor binding interactions associated with the varying sites of these compounds are discussed. The QSAR study has also explored the possibilities of having the analogues of improved binding affinities, in future synthetic efforts. Like expressed as log[Kᵢ (β₂- AR)/Kᵢ° (TP)] related to two receptors are significantly correlated with MR and n of the substituents and the relationship may, therefore, be helpful in developing the agents of greater selectivity on β₂- AR versus TP receptor and vice versa. The ratio of two binding constants obtained on negative logarithmic scale was considered as the dependent variable and the resulting correlation is shown by the following equation:

\[-\log[Kᵢ (β₂- AR)/Kᵢ° (TP)] = 2.963(± 0.346)MR(R₁) + 0.513(± 0.142)n(R₂) + 2.530\]

n = 11; R = 0.952; S = 0.332; F(2,8) = 38.598

where, Kᵢ = dissociation constants for each competing drug

R₁, R₂ = substituents on trimetoquinol derivatives.

The statistical parameters of above equation are in tune of highly significant results [57].

The enzyme acyl-CoA cholesterol acyl transferase (ACAT, EC 2.3.1.26) is primarily responsible for the intercellular esterification of cholesterol. It also plays important role in the absorption of dietary cholesterol from the intestine, the metabolism of cholesterol in the liver and the accumulation of cholesteryl esters in arterial lesion. Reportedly, the ACAT activity is enhanced in intestinal mucosal cell when cholesterol is ingested and in arterial cells undergoing atherosclerosis. Thus the inhibition of the ACAT enzyme can directly intervene
in the progression of lesions. Further, the dietary cholesterol, which contribute to the degree of hypercholesterolemic and antiatherosclerotic agents. They have synthesized different substituted imidazoles. Their aim is to establish a QSAR between the observed biological activities and parameters of different substituents present in the imidazoles they have synthesized. The best correlation that was obtained is as follows:

\[-\log IC_{50} = 6.290(\pm 0.543) \sum V_w - 1.704(\pm 0.147) (\sum V_w)^2 + 0.277(\pm 0.071)I_x + 1.738\]

n = 26, R = 0.929, S = 0.164, F(3,22) = 46.000, EV = 0.844

\[V_w = \text{van der Waals' volume}\]

\[I_x = \text{a dummy variable showing isosteric variation at the site X of the compounds [58].}\]

QSAR studies on a series of 18 piperidine derivatives, which act as acetylcholinesterase (AChE) inhibitors, have been performed using van der Waals vol (Vw) and topochemical index (I). Significant correlation have been obtained, which make it clear that AChE inhibition activity is controlled by topochemical index [59].

In industry, government and scientific research, structure activity relationships (SARs) play an expanding role in estimating the potential toxicity of chemicals. Traditionally, human experts use experience and expertise to identify structural features responsible for toxic action. Recent development in artificial intelligence research and the improvement of computational resources have led to efficient data mining methods that can automatically extract SARs from toxicity databases with structurally diverse (noncongeneric) compounds.

The basic procedure is to submit the existing experimental data (the learning set) to a machine learning program that detects relationships between chemical structures and toxic effects. This SAR model can then be used to predict the toxicity of untested compounds.

An advantage of this data-driven approach in predictive toxicology is that SAR model can be derived in an unbiased way and that it is possible to make new discoveries in already existing data. The predictive toxicology evaluation (PTE) projects demonstrated that predictions from data driven methods are at least as
accurate as those from human experts and expert systems. They rely heavily on the quality and representation of chemical and toxicologic data in the training and testing sets.

In the development and application of SARs it is essential to identify the structural or chemical properties that predict the end point of interest [60].

As a part of a composite programme of rational drug design (RDD), some substituted benzenesulphonyl glutamines [5-N-substituted-2-(substituted benzene sulphonyl)-L-glutamines] had been synthesized and their tumor inhibitory activities were evaluated against Ehrlich Ascites Carcinoma (EAC) cell line in Swiss albino mice. QSAR studies of these inhibitory activities using Fujita-Ban model as well as Modified Hansch-Fujita model gave excellent correlations (correlation coefficient, r = 0.89 and 0.82 respectively). These results could be useful in designing ‘lead’ compound with potent inhibitory activity on DNA and RNA synthesis and tumor development [61].

The observation that in a congeneric series the substituents ought to contribute constant increments or decrements to biological activity, led Free and Wilson to develop an alternative QSAR approach, which was based on additive principle [62]. This is most conveniently expressed by the following equation:

\[
BA_i = \sum a_j X_{ij} + \mu
\]

Where, \(X_{ij}\) is the \(j\)th substituent with a value of 1 if present and 0 if not,
\(a_j\) is the contribution of the substituent to biological activity and
\(\mu\) is the overall average activity.

Therefore, steps in QSAR analysis can be generalized in the following way:
1. To select a lead compound
2. To form training set i.e. derivatives having structural similarity with lead compound.
3. To find MIC, b.p, LD_{50} of lead compound or derivatives (end point)
4. To find molecular descriptors
5. To find a relationship an correlation between molecular descriptors and end
points with the help of regression equation.

\[
\text{Biological activity} = a + b \text{ (steric index)} + c \text{ (hydrophobic index)} + d \text{ (electronic factor)}, \text{ where } a, b, c, d \text{ are constants.}
\]

6. To verify the model testing set is required.

QSAR technique is adopted in Environmental Protection Agency of different countries to determine the risk assessment factor. This is done by determining the exposure time and effect due to exposure for the detrimental compound causing environment pollution at a particular place [63].

The objective of our present study is to find through QSAR the model structure of an organophosphorus pesticide, which will have minimal effect on any part of the mammalian brain.

We have seen in the above discussions that pesticides cause toxic effect on non-targets and unwanted environmental pollution. Therefore, we tried to design a model pesticide using the pesticides used in Indian agricultural fields. In this present study, we have treated rats with seven different OPs singly and in combination and have taken the help of Multiple Regression Analysis to get the model and we have used purified rat brain AChE and \textit{in vitro} OP application. Finally phytoremediation of the pesticides at different doses were studied by the popularly used aquatic plant \textit{Spirodela oligorrhiza} L. to get a predicting equation for environment friendly OP.

The following OPs having LD\textsubscript{50} for rats & bees, and their octanol water partition coefficient values (Pow) have been used in our present study since their structures have been published [64].
### General Introduction

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>LD$_{50}$</th>
<th>LD$_{50}$</th>
<th>$P_{ow}$</th>
<th>Structure</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Rat</td>
<td>Bees</td>
<td></td>
<td></td>
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<tr>
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<td>(oral &amp; topical)</td>
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References


37. Gao, J., Garrison, A. W., Hoehamer, C., Mazur, C. S. and Wolfe, N. L.,


61. Srikanth, K., Kumar, A. C., Goswami, D., De, A. U. and Jha, T., Quantitative

