

## CHAPTER - I

### PESTICIDES, THEIR RESIDUES AND THE PROBLEM.

#### Pesticides:

Insects have survived for more than 250 million years, while man, as we recognize him, has been around for only one million years. Man's continuous conflict with crop pests is well documented in history. The recurrent plagues of locusts and the potato famine in Ireland illustrate what can occur when pests gain control. Pest control has always been practised but the methods employed have changed with advancing civilisation from manpower through mechanical means to chemicals or pesticides. One of the oldest records of pesticides is contained in Homer's mention of sulphur over 1000 years B.C.

Pesticide is one of the highly emotive terms of modern technology; the reaction which it involves is varied but is usually tempered by vocational interests. To the food scientist they are termed as food contaminants. Definition of pesticides is not easy and varies among countries. Pesticides are defined as substances or mixtures of substances used to control animal and plant pests by killing or repelling them.

At the out-break of the Second World War there was one insecticide which occupied a key position; that was pyrethrum, the extract from the daisy flower *Pyrethrum cinerariaefolium*<sup>-1</sup>, a substance exceedingly poisonous to insects but practically harmless to man. For many years chemists had been seeking to produce a synthetic contact insecticide, capable of being made in the laboratory and manufactured on a large scale in the chemical works, which would rival this vegetable product in effectiveness and surpass this in cheapness and availability.

In 1940 the Swiss firm of J.R. Geigy, A.O., of Basle discovered the insecticidal properties of a chemical that is 2,2-bis(Para-chlorophenyl)-1,1,1-trichloroethane or more familiarly known as dichlorodiphenyltrichloroethane, later abbreviated to DDT. The firm took patents to cover the manufacture of this chemical and its use as an insecticide, and early in 1942 the British and American branches of the firm brought this patents to the notice of the entomologists in countries, who were seeking a substitute for pyrethrum<sup>-2</sup>. Since then search for chemical pesticides had been on; currently, about 900 pesticides are used in about 60,000 formulations<sup>-3</sup> for various pests in addition to insects.

### The Chemicals:

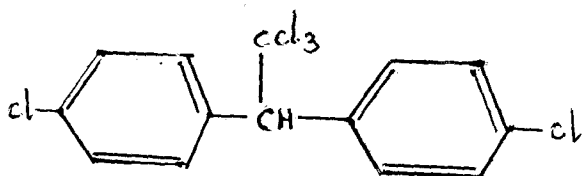
The organic chemicals those are being used as pesticides can be classified into three major groups viz. organochlorine, organophosphorus and carbamates.

Chemical compounds which are used to control insects are harmful, directly or indirectly, to man. The application of insecticides and subsequent control of insects is achieved in several ways: (i) stomach poisons for insects which eat plant leaves or cloth fabrics (ii) contact poisons which are applied directly to the body of the insects either by treating the material with which the insects come in contact or (iii) systemic insecticides in which the toxic agent is made a component of the plant itself. The perfect pesticide should achieve complete and specific control of the target pest and degrade in a specified time to harmless constituents.

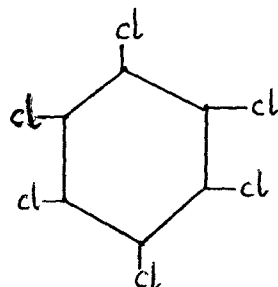
Discussion on pesticides inevitably and correctly involves consideration of the assessment of risk to man and wild-life and the benefits to public health and agriculture.

Among the three classes of pesticides viz. organochlorinated, organophosphorous and carbamate in the present discussion the pesticides belonging to organochlorinated group are found to be relatively persistent.

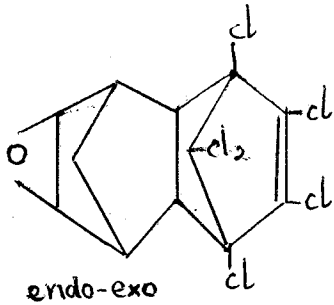
The organochlorinated pesticides viz. DDT, lindane,



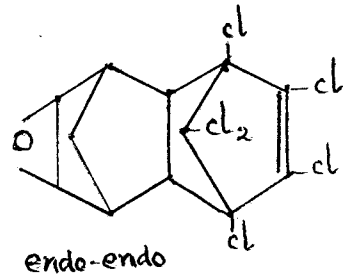
DDT



LINDANE  
(Y-BHC)



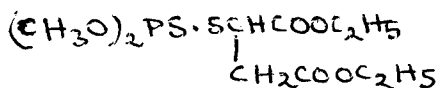
**DIELDRIN**



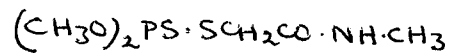
**ENDRIN**

dieldrin, endrin, etc. are commonly known. It was found that DDT in soil takes 10 years to decline to 10% of the original amount<sup>-4</sup>. The organochlorinated group of pesticides are lipophilic in nature.

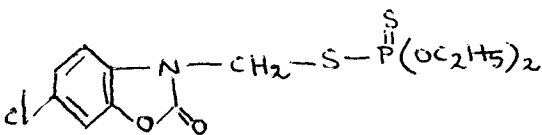
The persistence of any specific pesticide residues will be determined by many independent but interrelated factors, including the chemical characteristics of the compound and its susceptibility to radiation, hydrolysis, oxidation and reduction, and its solubility, volatility and its adsorption and partition coefficients<sup>-5</sup>. Because of the relatively low persistency and high effectiveness, organophosphorous pesticides are now used widely. Thus about 140 phosphorus compounds are or were used as practical pesticides (including plant growth regulators) in the world. The most commonly used organo-phosphorous compounds like malathion, dimethoate, phosalone, quinalphos, sumithion, thimet, ethyl-parathion, methyl-parathion, etc. are known.



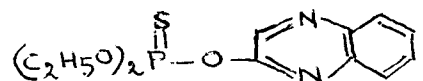
**MALATHION**



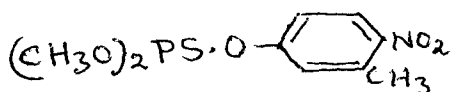
**DIMETHOATE**



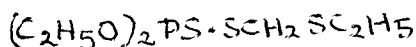
**PHOSALONE**



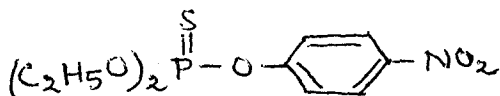
**QUINALPHOS**



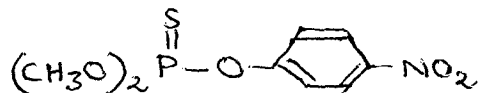
**SUMITHION  
(FENITROTHION)**



**THIMET**



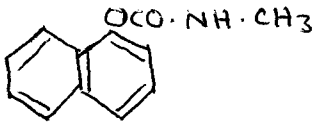
**ETHYL-PARATHION  
(PARATHION)**



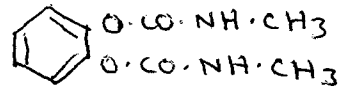
**METHYL-PARATHION**

More than 60,000 tons a year of organophosphorus pesticides are produced in the United States alone<sup>-6</sup>. Recent governmental bans and restrictions on the use of organochlorine compounds and the growing number of organophosphorus resistant strains of insects are placing a greater burden of protection into the newer major class of insect toxins, the carbamates. The first bonafide carbamate insecticide was synthesized by Dr. Hans Gysin, a senior research chemist at the Geigy Chemical Company in Switzerland<sup>-7</sup>. Because these materials were developed mostly in the 1960's, after the global distribution of DDT and during a period of environmental awareness, the carbamates tend to be more biodegradable, and some are considerably less toxic to nontarget species.

Of the thousands of methylcarbamates synthesized by various chemical companies in the last 24 years, only about a dozen have reached sizable production as commercial insecticides. Among the most widely used carbamate compounds, carbaryl (1-nepthyl N-methylcarbamate) or sevin and baygon or propoxur (2-methylcarbamate N-methylcarbamate) etc. are known.



SEVIN  
(CARBARYL)



BAYGON

Toxicity:

Almost all insecticides are toxic mainly by virtue of their ability to disrupt the transmission of impulses along the nervous system of animals. Though the mechanism of impulse transmission is apparently identical in the target insect and in other nontarget animals, fortunately there exist biochemical, physiological and morphological differences in the gross anatomy of nerves and other tissues which permit selective toxicity. That is, a chemical that is lethal to insects can be relatively nontoxic to other organisms. Further, lethal amounts in insects are much less than toxic levels in bigger forms of life.

Despite the almost countless number of experiments conducted in the research for the mode of action of DDT and other organochlorine insecticides<sup>-8</sup>, the complete mechanism

remains a mystery. There seems to be little doubt, however, that interruption of impulse transmission along the nerve is involved. The inhibitory activity of organophosphorus esters against cholinesterases was first found, in 1941, by Adrain and his co-workers<sup>9</sup>. With carbamate insecticides, the mode of action was known before their inception as insecticides. This was mostly because of prior knowledge of pharmacology of carbamate drugs and because of similarities to the known mode of action of organophosphorus insecticides. Carbamate toxicity is due to inhibition of acetylcholinesterase at certain synaptic junctions in the nervous system. All of the manifestations from this action, and the exact biochemical mechanism of inhibition, are understood, but it is very likely that cholinesterase inhibition is the *prima-facie* cause of death.

The simplest way of expressing the toxicity of such a compound is by means of an LD<sub>50</sub> values. Such a value is a statistical estimate of the dosage necessary to kill 50 percent of a large population of the test species under stated conditions (e.g. single oral dose or injection of solution). Secondly, toxicity may vary with species, age, sex, nutritional state and formulation as well as with the route of administration. An LD<sub>50</sub> value is a statistic which, in itself, gives no information on the dosage that will be fatal to a small proportion of a large group of animals. The no effect level in experimental animals and the acceptable daily intake (ADI) computed for man from it are practical parameters of assessment of toxicity.

During the discussion on pesticides we should be acquainted with following terms which may bring fourth essential ideas regarding the assessment of the use of pesticides: A pesticide residue is a residue in or on a food of any chemical used for the control of pests and the term includes derivatives or degradation products of such chemicals. The amounts are expressed in parts by weight of the chemical and/or derivatives per million parts by weight of the food (ppm). Good agricultural practice (on pesticides) is the recommended usage, of a pesticide, which is just necessary and assential for the control of a pest under all practical conditions.

The tolerance limit of a pesticide chemical is the maximum concentration of pesticide residue that is permitted in or on food at a specified stage of harvesting storage, transport, marketing or preparation of the foods, upto the final point of consumption; the concentration is expressed in ppm. A tolerance limit is then the smallest residue consistent with control of pest, but to be toxicologically acceptable.

The acceptable daily intake of a pesticide is the daily intake which, during an entire lifetime, appears to be without reasonable risk on the basis of all the known facts at the time. It is expressed in milligrams of the chemical per kilogram of body weight (mg/kg).

The "no-effect" level is the dose (presumably the highest attainable dose) in a chronic toxicity study et

which no adverse effects are observed in a group of test animals compared with the untreated control group. Estimation of the "no-effect" level of a pesticide is a critical step in arriving at the maximum acceptable dietary intake; "no-effect" implies no toxic or adverse effect.

However, the environmental contaminants spread through a variety of channels, many of which sooner or later lead to our food and drinking water. Among these contaminants, pesticides have raised strong public concern and have contributed markedly to the general uneasiness associated with the pollution boom of the last two decades.

Many ill effects with prolonged low dosage exposure of pesticides are on record. Thus DDT showed atrophy of adrenal cortex<sup>-10</sup>, hepatotoxic effect<sup>-11-14</sup>. Exposure to lindane causes blood dyscrasias like agranulocytosis<sup>-15-16</sup>, pancytopenia<sup>-17</sup>, splastic anaemia<sup>-18</sup> and leukaemia<sup>-19</sup>. Aldrin causes hepatic tumors<sup>-20</sup>. Organophosphorus compounds result in paralytic syndrome identical with carbamate compounds<sup>-21</sup>. Carbaryl may cause skin irritation as well as systemic poisoning<sup>-22</sup>. A number of investigations concluded that morbidity directly varies with the degree of pesticides<sup>-23</sup>.

#### Pesticide Residues:-

Pesticides came into extensive use both in agriculture and public health during 1944 to 1960 and, ever since, this trend has continued. The table I, II and III summarise the information regarding the amount of pesticides already consumed, being consumed and to be consumed in India<sup>-24-26</sup>.

Table - I

Consumption of Pesticides (Tech. Grade) in India

Year	Agricultural Sector	Consumption in public health	Total tonnes
1965-66	6,654	7,978	14,630
1966-67	7,442	9,695	17,137
1967-68	11,205	9,695	20,900
1968-69	14,098	14,102	28,200
1969-70	11,844	14,165	26,000
1970-71	10,164	14,156	24,320
1971-72	19,935	9,600	29,535
1972-73	25,076	10,084	35,160
1973-74	31,863	13,137	45,000
1974-75	37,523	12,341	49,864
1975-76	43,414	15,400	58,814
1976-77	36,713	15,205	51,918
1977-78	39,880	19,100	58,980

Table - II

Consumption of Pesticides in India (1977-78)

<b>Pesticides</b>	<b>Tonnes</b>
BHC	24,252
DDT	9,980
Malathion	1,732
Me-Parathion	
Metecystox	1,279
Fenitrothion	
Endrin	986
Dimethoate	347
Quinalphos	192
Aldrin	
Chlordane	317
Heptachlor	
Zineb	64
Maneb	
2,4,D	96
Other Pesticides	17,540
<b>TOTAL :</b>	<b><u>58,540</u></b>

Table - III

Requirement of Pesticides for the Year of the fifth-  
five year plan and for 1983-84 as estimated by the  
pesticides association of India.

Product	Requirements - Technical in MTS/area covered				
	1975-76	1976-77	1977-78	1978-79	1983-84
Insecticides	42,070	51,975	59,895	68,060	94,390
Fungicides	12,808	14,074	15,625	17,785	22,738
Acaricides	201	297	463	644	965
Herbicides	2,695	4,420	7,260	10,140	16,850
Plant Growth Regulators	80	160	280	400	600
Rodenticides	250	350	500	1,000	1,500
Fumigants	650	800	900	1,050	1,350
Nematicides	60	160	380	600	1,700
<b>Total :</b>	<b>58,814</b>	<b>72,236</b>	<b>84,303</b>	<b>99,679</b>	<b>1,40,090</b>

Pesticides in food are predominantly residues from their use on growing crops and, less often, from post harvest use on stored agricultural products. Awakening to the problem of toxic residues of these pesticides in food and environment came to sharp focus around 1960. The wide use of a large variety of pesticides<sup>-27</sup> has generated serious problems of their toxic residues. The justification for using pesticides in agriculture is frequently a subject of discussion; various experts have arrived at different conclusions with regard to the value of chemical crop protection in food and feed production. According to several experts the world production of food would probably be reduced by more than thirty percent if, all of a sudden, no pesticides were available<sup>-28</sup>. The world at present would be very badly off without pesticides. A world without pesticides in fact will be a world where millions of people would starve to death each year in addition to those already starving. So we have to live with the pesticides and use them so wisely that their harmful side effects are kept at an almost negligible level.

The chlorinated hydrocarbons like DDT, endrin, dieldrin, etc. are very persistent and have a high lipid/water partition coefficient. Due to these physico-chemical properties they easily penetrate biological barriers, accumulate in the lipid components of organisms and tend to be enriched by their passage upwards in the ecological food chains.

The advantages to public health and the economy of the biodegradable organophosphates and carbamates replacing the

more persistent organochlorines in agriculture and public health are assessed. For successful agriculture in the developed nations which can afford the more expensive biodegradable insecticides, organochlorine are no longer essential.

In developing countries however the replacement of the chlorinated insecticides in agriculture would retard economic development, while their elimination in public health would result in the abandonment of many vector control programmes and a resurgence of diseases. Consequently, restrictions imposed by a number of governments on the use of DDT have been a matter of concern. The Director General of WHO<sup>-29</sup> emphasized the continuing importance of DDT in operations against malaria and other vector borne diseases.

Little information is available comparing the cost involved in using organophosphates and carbamates in place of organochlorine in agriculture. In tropical countries, the use of organochlorine group of insecticides is relatively high for achieving economy, efficiency and persistency. The average persistency of most chlorinated insecticides is around 3-10 years. Recent literature reveals that parathion and diazinon are also persistent in the environment for several months. Wide-spread detection of pesticides and their toxic terminal products, in several categories of food, water, soil, air and demonstration of bioconcentration of some

pesticides through food chain has aroused considerable concern, since some of the pesticides are reported to be carcinogenic, mutagenic and teratogenic<sup>-30</sup>.

Management of Residues:-

The solution of the problem of pesticide residues starts with legislation, approval procedures, spread of correct information on the pesticides and the proper education and instruction of the users. The residues of various pesticides on crops depend on the following important factors<sup>-31</sup>:

a. Crop and its variety.

b. Climatic conditions - Dissipation of pesticides has been shown to be faster in hot and rainy season than in winter. Residues of pesticide applied to the same crop grown in different seasons but with the same dose, are different. Precipitation can wash down the residues from aerial parts of the plant, many a times to levels less than the tolerance limits fixed for the toxicant. In bright sun, some compounds are photo-degraded.

c. Management practices such as: the dose of fertilizers, water management, etc.

d. Dose of pesticide applied has a direct bearing on the amount of residue left.

e. Method of application of pesticides - The type of formulation used, nature of appliances (particularly sprayers whether high volume, low volume or ultra low volume) used, and whether it is applied on the foliage or to the soil.

f. Time interval between applications of the pesticide and between last application and harvesting.

g. Soil Type - Its pH and texture : Most of the pesticides are unstable under alkaline conditions. The persistence will be longer in acidic and clayey soils than in alkaline and sandy soils. Even the absorption of pesticides by plants is reduced by increasing clay content of the soil.

h. Treatment of the crop produce such as drying, washing, cooking, etc. which are known to decrease the residues considerably.

i. Sampling and analytical method employed - There exists different accuracy (deviation from the real value) of the residue values obtained by adopting different methods. The type of extraction and clean up procedures used, the extent of recovery obtained, etc. are important considerations. The analytical samples have to be representative of the lot.

The management of pesticide residues is then a comprehensive exercise involving selection of the appropriate pesticides (and substituting these when resistance manifests) standardising analytical methods for the pesticides and their degradation products (residues), stability or decay of the pesticides, lethality for the targets and toxicity for nontargets, surveillance and monitoring in the biosphere, in food and water and in diet and ultimately to man, properties of the residues, etc.

The present work:

The requirements of further knowledge on pesticides and

their residues, particularly in food, are many as mentioned in the foregoing paragraph. A few have been selected as follows in the present studies. These are expected to help identify the areas requiring particular attention to forming the base on which the ultimate object, "Safe use of pesticides" can be structured.

A large number of comprehensive treatises are available today which have outlined various methods and techniques for pesticide residue analysis<sup>-31-33</sup>. Official attempts have also been made for establishing unified and acceptable analytical techniques which would be adopted by all the agencies involved in this work. For instance, the Food and Drug Administration, U.S.A. has compiled adequate analytical methods for use in their analytical laboratories<sup>-34</sup>.

Similarly, final methods of analysis have also been published by AOAC<sup>-32</sup>. Codex Alimentarius Commission (FAO and WHO) at its ninth session in Rome in November, 1972 strongly felt the need for "Codex referee methods".

The importance of pesticide residue problem led to intensive search for analytical methods for accurate and rapid analysis of residues in diverse commodities and materials. This effort has not only been directed towards improvement of the existing analytical techniques and development of new instrumental methods but also for developing expertise for adequate separation of micro-quantities of residues from bulk of the material under test<sup>-35</sup>. In fact, the final analytical methods alone have been found incapable of giving

consistent and accurate results; suitable techniques for quantitative extraction of residues and clean up of the extractives are instituted as first basic steps. Briefly speaking, the following steps are necessary for adequate analysis of pesticide residues in any material:-

- (i) Sampling and sample storage.
- (ii) Preparation of the sample.
- (iii) Solvent extraction procedure<sup>-36</sup>.
- (iv) Clean-up of extractives<sup>-34,-35,-37,-38</sup>.
- (v) Analytical techniques (both for qualitative and quantitative terminal analysis):-
  - (a) Gas liquid chromatography<sup>-39</sup>.
  - (b) Wet technical analysis, including<sup>-39-41</sup> visible and ultraviolet spectrophotometry (includes total chlorine and total phosphorus method).
  - (c) Chromatography - in respect of column, paper and thin layer<sup>-36,-38,-42</sup>.
  - (d) Polarography.
  - (e) Infra-red spectroscopy<sup>-43</sup>.
  - (f) Fluorimetry.
  - (g) Mass spectroscopy.
  - (h) Neutron activation analysis.
  - (i) Enzymatic (cholinesterase inhibition) method.

There are already indications that in absence of agreed methods, widely divergent results may be obtained even when the same sample has been analysed in more than one centre.

Residue analysis laboratories in India are few and have yet to acquire the entire range of sophisticated equipments which are usually imported, expensive and are continually being replaced by newer and better models. We shall have to tailor our techniques and methods according to our resources with minimum sacrifice of accuracy and speed. We may not use the most sensitive instruments of analysis always but we shall find out the concentration as per requirements. Analytical complexities are associated with even the best of the methods available to-day. The serious analytical errors have even been observed in the estimation of organochlorine pesticides with the use of the most sophisticated and costly instrument like GLC<sup>-44</sup>.

So simple methods for extraction, cleanup and determination are needed. A small number of studies<sup>-45-46</sup> have been done in India to generalise a multiresidue routine or to reduce the steps of determination of pesticide residues. Elimination of a step from the extraction to determination can reduce or minimise the operational errors.

Although a large number of pesticides chemicals are being used now-a-days, attention has been concentrated on a limited number of widely used pesticides belonging to organochlorinated, organophosphorous and carbamate groups in the recent study. Some approaches to generalisation and development of new methodology or improvement of the steps used in residue analysis of foods have been made.

Degradation of pesticides has been studied by a number of workers<sup>-47-49</sup>. But there is a strong need to get information on residues of various pesticides on different crops or commodities from supervised trials at different parts of any country. Such studies are helpful for comparing the published results, which are tried under highly heterogeneous and localised conditions.

Study on the decay of pesticides in water, variety of food articles and also loss due to processing have been estimated. Stability in the solvent generally used in formulations has been studied.

The chemistry<sup>-50-53</sup> or the chemical nature of the pesticides in relation to commodities or environment or to some specific chemicals or in some reactions like those occurring in bio-systems should be known. It may help one to know of the formation of toxic materials and properties to be used for analytical purposes.

Effects of a few inorganic salts on pesticides are also included in the study. The observed changes can be used in the identification and determination, or for the specification, of a single component (pesticide) even in a mixture.

Determination of a few individual pesticide residues has been done; gel electrophoresis method has been proposed successfully for the determination of dimethoate (an organophosphorus compound). Also the gas liquid chromatographic method of analysis has been examined for the analysis of pesticides.

For an assessment of the residual pesticides in raw foods market survey have been done. The residual levels were compared with the residue limit as required by Prevention of Food Adulteration Rules (Ministry of Health and Family Welfare, Govt. of India).

Environment consists in a number of micro-organisms. There are some examples regarding the effect of micro-organism on pesticides or vice-versa<sup>-54-56</sup> and also of no effect in this regard<sup>-57</sup>. As soil harbours a number of organisms, it is very important to study the microbial influence on pesticides and vice-versa. This has been done.

The pesticides those are known as possessing less toxicity and high efficacy may be tried for application to some desired commodities for protection. A short but very interesting study has been done regarding the application of phosalone (an organophosphorus compound) in controlling the insect in dry fish.

Spectacular development in analytical techniques has been done and extensive literature has been made available on residue analysis<sup>-31-39</sup>. However, more studies are necessary for suitably adopting the existing, or establishing new, analytical techniques for the pesticide residue analysis under Indian conditions.

Pesticide residue survey all over the India should be done as there is an heterogeneous climatic condition, though some studies have already been done<sup>-58</sup> except the eastern part of India.

As various items of food contaminated with pesticides, the body tissues are likely to contain pesticides, particularly the organochlorine ones which tend to accumulate in the body fat. Dale et al.<sup>-59</sup> reported results of analysis of body fat from 104 samples taken in 1964 from people without any known occupational exposure to insecticides. Three insecticides, viz. DDT, BHC and dieldrin, were detected. A comparative values of DDT in human fat in different countries have been reported in the Special Committee on Harmful Effects of Pesticides<sup>-60</sup>. There is little information regarding the residue level in human tissues in India except the report of Dale et al. Hence extensive work is needed on this subject. Some amount has been done here.

The formation of nitrosamines by some of the pesticides has also been studied in some details.

Though mostly thin layer chromatographic method of determination have been followed in most of the cases the results were authenticated by GLC.