CHAPTER II
REVIEW OF LITERATURE
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1.0 TOXICITY STUDIES

To the human race, pesticides are the proverbial genie which can give unlimited service to the society if used with care and consideration, but once it gets out of control, can spell disaster by causing toxic effects on several non-target organisms (Mulrhead Thomson, 1971). Bioassay or acute toxicity test is perhaps the most useful technique available to the biologist for predicting the potential hazards of a chemical. Bioassay with fish or invertebrates has long been used to determine the suitability of water for aquatic life. The term bioassay is defined by number of workers. Biological assays measure the response of an organism to a biologically active substance (Allderdice, 1967) and are useful in determining safe water quality criteria. Warren (1971) defined the expression of bioassay as a test which is usually used to evaluate potency of the drugs or toxic substances. Sprague (1973) defined bioassay, as a test in which the quantity or strength of the material is determined, by the reaction of a living organism to it.

The median lethal concentration is the usual method of reporting the results of acute toxicity tests. The current trend is to use the symbol LC₅₀ (Lethal concentration for 50%
of the test species during a specified time interval), a notation which is used in most fields of biological testing. The symbol $\text{TL}_{50}$ (formerly $\text{TL}_{m}$) or median tolerance limit, has been commonly used by United States' fisheries workers. The two terms have the same numerical value. A time period must always be specified (for example 96 h $\text{LC}_{50}$). The application of the $\text{LC}_{50}$ has gained acceptance among toxicologists and is generally the most highly rated test for assessing potential adverse effects of chemical contaminants to aquatic life (Brungs and Mount, 1978; American Institute for Biological Sciences, 1978).

Several different terminologies are being used to indicate the pattern of response studied. Alderdice (1967) has suggested two basic categories, acute toxicity, which is usually lethal and chronic toxicity, which may be lethal or sub-lethal. A system of classifying different grades of toxicity has been given in a report by an international group (Joint IMCO/FAO/UNESCO/WHO Group of Experts, 1969). The categories seem useful for general description of pollutants. They are as below -

- Practically non-toxic - Acute lethal threshold above 10,000 mg/l
- Slightly toxic - Threshold 1,000-10,000 mg/l
- Moderately toxic - Threshold 100-1,000 mg/l
Toxic

- Threshold 1 - 100 mg/l

Very toxic

- Threshold below 1 mg/l

Sprague (1973) described the various commonly used terms as acute, sub-acute, chronic, lethal, sub-lethal, long term or short term toxicity tests.

In order to carry out acute to chronic bioassay studies, mainly two types of toxicity tests are in general use: (1) The static bioassay in which fish or other aquatic animals are exposed in a tank of standing water which may or may not be changed during the test periods. Henderson et al. (1959), Katz (1961), Pickering et al. (1962), Elster (1970), Macel and McAlister (1970) and Post and Schroeder (1971) determined 96 hours LC50 for various marine fishes by static test. American Public Health Association (1981) has recommended the use of static bioassay for determining acute fish toxicity. (2) the continuous flow or flow through bioassay in which the test solution is renewed continuously or by frequent periodic additions. Tooby et al. (1975) studied the 48 hours LC50 of organophosphorus pesticides to the fish, Rastoria hetrozona by continuous flow method. Bone et al. (1975) have also used continuous flow method for evaluation of fish toxicity in laboratory. A survey of literature reveals that toxicity can also be determined by exposing fishes in tanks (Udovo
and Beatty, 1979; Verma et al., 1979), glass jars (Carroll et al., 1979) and aquaria (Sharma et al., 1979) of various capacities. In order to maintain adequate levels of dissolved oxygen, air was bubbled through the aquaria water under test (Ujiovo and Beatty, 1979). By doing so, number of fishes per litre of water can be increased (Mishra and Srivastava, 1973; Verma et al., 1979). Galtsoo (1978) indicated that as much as two to three days of prefasting of fishes is necessary before they are subjected to toxicity tests. Hashimoto and Shihuchi (1981) established methods to evaluate the acute toxicity of pesticides to aquatic life and used these methods to assess the acute toxicity of 43 registered pesticides.

1.1 TOXICITY TO CRUSTACEANS

Shrimps, prawns, crabs and lobsters are the most important crustacean animals to human beings for their food value and their vital role in ecosystem and hence their survival under polluted conditions has been studied extensively by number of workers (Risler, 1969; Sundmoe et al., 1977; Goude, 1978; Nagraja and Namasundari, 1981; Pawan and Kootde, 1983a; 1983b; Kulkarni and Masurekar, 1984). Risler (1969) reported that crustacean animals, such as shrimps, are especially sensitive to organophosphate pesticides. Methyl and ethyl parathion acute lethal concentrations are much
lower for crustaceans than for most of the fishes (Elsler, 1969; 1970a, 1970b; Billard and Kirkelin, 1970; Couch, 1978). The freshwater prawns and shrimps, Macrobrachium kistnensis and Caridina ralihari were also found to be sensitive to organophosphate, organochlorine and carbamate pesticides (Pawar and Kadare, 1983a). The shrimp, C. ralihari was observed to be more sensitive than the prawn, M. kistnensis (Pawar and Kadare, 1983b). The authors reported 50% mortality in the freshwater shrimp, C. ralihari and prawn, M. kistnensis when they were exposed to 0.0002 ppm and 0.009 ppm of the organophosphate sumithion for 96 h respectively.

Malathion was found to be toxic to several species of shrimps and crabs. The LC50 for 96 h exposure at 20°C as reported by Elsler (1969) was 33 and 83 ppm for the sand shrimp, Crangon septemspinosa and grass shrimp, Palaemonetes vulgaris respectively. In another study Hansen (1973) obtained 50% mortality of C. rugosus using a 0.032 ppm concentration of this insecticide. In one investigation, Lowe et al. (1970) obtained 50% mortality in the penaeid shrimp when they were exposed to 0.0002 ppm of the parathion for 48 h.
1.2 TOXICITY TO MOLLUSCS

Toxicity studies on molluscs, particularly the commercially important species are scanty. (Eisler, 1970; Lowe et al., 1970; Schimmel et al., 1977; Rammana Rao and Ramamurthi, 1979; Halaparameswara Rao, 1981; Das et al., 1982; Panwar et al., 1982; Robert et al., 1983; Akarte et al., 1985; Kulkarni et al., 1985; Sukumar and Rao, 1985; Padmaja et al., 1988). The impact of malathion and parathion on molluscs has scarcely been studied. Only a few reports are available. Pulmonate and gastropod molluscs like Lymnea, Pila, Viviparous were used to test the toxicity of pesticides (Seuge et al., 1977; Sukai and Seuge, 1979; Negamshankar and Ramamurthi, 1981; Madhu et al., 1982; Negamshankar and Deshpande, 1982; Haidu et al., 1982; Praasad et al., 1982; Parwar and Kardare, 1983; Rao et al., 1985; Sukumar and Rao, 1985; Padmaja et al., 1988). Previous studies on toxicity revealed an h LC_{50} of methyl parathion to P. globosa to be 1.2 ppm (Silva Praasad Rao et al., 1981), 48 h LC_{50} of limnane and malathion to P. globosa was estimated to be 0.72 ppm and 1.2 ppm respectively (Madhu et al., 1982). This indicates that organochlorines are more toxic than organophosphonates. The 48 h LC_{50} of malathion to the snail, B. bengalensis as reported by Lomte and Alam (1982) was 5.6 ppm. But Sukumar and Rao (1985) have reported 96 h LC_{50} values of Y-HCH, mirex
and sevin to the small \textit{B. bengalensis} F. gournea (Amendale) to be 8.7, 0.017 and 13.5 ppm respectively and similarly the 96 h LC$_{50}$ values of the small \textit{B. bengalensis} f. amendale (Kobelt) were 9.6, 0.0037 and 9.6 ppm for the same pesticides respectively. Power and Kaldoro (1983) have reported 96 h LC$_{50}$ values of sodium to the small \textit{L. sancta} to be 1.43 ppm, LC$_{50}$ (96 h) of metocid for \textit{B. chilumilla} investigated by podmaja et al. (1988) was just 0.0047 ppm.

1.3 TOXICITY OF FISHES

Fishes are more widely used for bioassay than any other group of aquatic organisms. A series of annual literature reviews published by journal of Water Pollution Control Federation, Washington and number of workers all over the world are sufficient to indicate the magnitude of work carried out with fishes as test animals (Wood, 1953; Parkhurst and Johnson, 1955; Henderson and Pickering, 1958; Holden, 1972 and 1973; Korn and Earnest, 1974; McKim et al., 1976; Brungs et al., 1977; Koehler et al., 1979; Johnson and Findlay, 1980; Cizeck and Svobodova, 1981). Organochlorine pesticides may differ considerably in the effects on different species of fish. Linduska and Surber (1946) reported the toxicity of parathion as approximately the same for blue gills and rainbow trout (0.3 ppm), Parkhurst and Johnson
(1955) reported for malathion the 96 h LC$_{50}$ value of 0.12 ppm when using fingerling salmon, *Oncorhynchus tshawytscha* which is at wide variance with the 96 h LC$_{50}$ value of 12.5 ppm reported by Henderson and Pickering (1958), for fathead minnows. Butler (1963) reported 50% mortality of juvenile sheephead minnow when exposed to a 0.06 ppm concentration of parathion for 48 h. Jones (1964) reported TL$_{50}$ values (1.4 to 2.7 ppm of parathion) similar to that reported by Henderson and Pickering (1958) for the fathead minnow; he further indicated that its C-analog, paraoxon, was more toxic, demonstrating TL$_{50}$ of 0.33 ppm against this species at 96 h exposure. Pickering and Henderson (1966) reported 96 h LC$_{50}$ for phorate to be 0.0947 ppm for blue gills. Macik and McAllister (1970) reported 96 h LC$_{50}$ of methyl parathion to fathead minnows and carps as 8.9 and 7.1 ppm respectively. 96 h LC$_{50}$ values of ethyl parathion, diazinon, malathion and methyl parathion to rainbow trout are 0.047, 0.170, 0.196 and 2.75 ppm respectively (Pimental, 1971). LC$_{50}$ at 72 h exposure of parathion for the mosquitofish, *G. affinis* and green sunfish, *Lepomis cyanellus* as reported by Davey et al. (1976) was 0.20 and 0.02 ppm respectively.

Many workers have documented acute toxicity effects of various organophosphorus pesticides on different

The physical factors like temperature, pH, salinity, hardness, etc. influence the toxicity of the aquatic pollutants (Sprague, 1973). Similarly the references also indicate that different species respond to different portions of the pollutant load on an ecosystem. A pesticide may be highly toxic to one species and least toxic to the other. Further the wide variation in sensitivity of different species to different pesticides is dependent on various factors like age, sex, weight, physiological state of the animal and presence or absence of enzyme system that can degrade the pesticide (Sanders, 1979; Phillips, 1978; Nagarathanamma and Rammurthy, 1981).
<table>
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<td>24</td>
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<sup>a</sup> Ethyl parathion, <sup>b</sup> 95% Technical malathion, <sup>c</sup> 50% Commercial malathion, <sup>d</sup> Fry of size 25 to 30 mm., <sup>e</sup> Methyl parathion.
2.0 ACCUMULATION OF ORGANOPHOSPHORUS PESTICIDES IN AQUATIC ECOSYSTEM

The organophosphorus compounds constitute one major group of pesticides, and a certain portion thereof may be transported to the aquatic environment resulting either from the actual use on field or from unavoidable transmittance to water ways. However, possibly because of its relatively shorter persistence, the transformation and transformation of organophosphorus compounds in the aquatic environment has not been extensively investigated as compared with more persistent organochlorine compound (Miyamoto et al., 1979). The residues accumulation of organophosphorus pesticides malathion and methyl parathion in different components of freshwater aquatic ecosystem have been reviewed separately.

2.1 DETERMINATION OF PESTICIDE RESIDUES

A number of colorimetric methods have been reported for the determination of organophosphorus pesticides. Earlier methods involved the digestion of organophosphorus compounds with a mixture of perchloric acid to phosphoric acid (Laws and Webley, 1961). The phosphoric acid so produced was measured colorimetrically by molybdenum blue method (Holmann, 1943; Laws and Webley, 1959, 1961). The absorbance of molybdenum blue complex was measured at 730 nm and
Detectability of the method was 0.1 μg. The major drawback of this method is that it is time consuming and measures total organic and inorganic phosphorus.

Methyl parathion can be determined colorimetrically by recording absorbance of the sodium-nitrophenolate produced through alkaline hydrolysis of methyl parathion (Hippel and Kovac, 1968). Average recovery and detection limit of this method is 92% and 0.2 ppm in citrus fruit. Javanesvic and Prosic (1972) reported that methyl parathion can be measured by coupling with palladium chloride. Absorption was measured at 520 nm and detection limit of the method was 0.25 ppm.

Glont and Schechter (1960) developed a method for the determination of organophosphate esters, based upon the intense colour produced by the reaction of formaldehyde with aqueous chromotropic acid solution upon heating in presence of sulphuric acid. Sensitivity of the method is 0.10 ppm. The phosphomolybdenum blue method used was that of Staller and Curry (1964). An automated total pesticide procedure for residue analysis of various organophosphates was described by GAO and Gundner (1968). Kajisu et al. (1968) reported a sensitive method for the determination of organophosphorus pesticides with safranine-o-molybdate. The safranine-phosphomolybdate complex absorbs at 535 nm and recovery.
was more than 98%.

Getz and Watts (1964) reported a procedure based on the reaction of organophosphorus pesticide with 4-((P-nitro benzyl) pyridine in the presence of cyclohexylamine to yield chromophoric compounds. This method can detect 0.5 - 1.00 ppm organophosphorus compound. Jain et al. (1974) modified the method of Getz and Watts (1964) and improved the detectability of the method by 25 percent by recording absorbance at 540 nm. They validated the modified method with 24 organophosphorus pesticides in soil and plant samples.

2.2 PERSISTENCE OF ORGANOPHOSPHORUS PESTICIDES IN AQUATIC ECOSYSTEM

After the entry of pesticides into aquatic environment through direct or indirect means, they may remain in solution or may be absorbed on suspended particulate matter and onto or in plant and animal tissues, depending on their water solubility, adsorption properties, and partition coefficients.

2.2.1. PERSISTENCE OF ORGANOPHOSPHORUS PESTICIDES IN WATER

Several workers have studied the persistence of residues of malathion in water under both laboratory and field conditions. Mulla (1963) studied the stability of malathion
applied at 2 ppm to tap water (pH 8.0) in glass jars kept in the laboratory at 35°C. In this test malathion was found to be less stable. Its residue declined to < 0.27 ppm after 48 hours and were not detectable after 72 hours. In a field study (Guerrant et al., 1970), malathion was used (at 214 g/ha) as ULV aerial application to control mosquitoes in Hale Country, Texas. Water samples from different sources in the treated locality were taken at 4, 24, and 48 hours after the application. The maximum levels at 4 hour post treatment were 0.15, 0.5, 0.01, and 0.037 ppm in the stream, reservoir pond, fish pond and stock water tank samples, respectively. These levels, however, reduced to <0.001 ppm within 24 hours. Similarly, in another laboratory study (Eichelberger and Lichtenberg, 1971) malathion did not show any change in concentration in distilled water over 3 weeks test period. In the raw water, however, there was a substantial change. Malathion persisted for only two weeks.

Parathion on the other hand, has been found to persist longer than malathion in water. Nicholson et al. (1962) studied the residue levels of parathion in the pond water. Mulla et al. (1966) found that water samples taken from a parathion treated duck pond (at 1 lb/A) initially had residue of 0.40 to 0.51 ppm which subsequently was reduced to 0.01 ppm. after 8 days, approached as low as <0.01 ppm
14 days after the application, Chigareva (1973) reported that methyl parathion in water was reduced to 88 – 99% during 30 days after the initial concentration of 1.0 ppm. Kostovetska et al. (1976) reported that methyl parathion at 0.02 and 0.2 ppm disappeared from water within 2 and 8–14 days respectively. Methyl parathion was detected in tap water at 0.03 ppm for 95–65 days by Akimov and Kassmers (1978). Lichtenberg (1971) reported that 90% methyl parathion was degraded in Ohio-Miami river water over a period of two weeks. Walker (1978) observed the half life of methyl parathion as 27 days in freshwater.

2.2.2. PERSISTENCE OF ORGANOPHOSPHORUS PESTICIDES IN SOIL

Data on the behavior of organophosphates in soil is less voluminous. Of the two organophosphates malathion and parathion, which have been extensively used as pest control agents, the limited information so far available deals mainly with parathion. Parathion has been used as a soil insecticide, whereas instances of malathion application to soil seem quite rare probably due to its short life in such environment.

Studying the persistence of malathion, Lichtenstein and Schulz (1964) applied it (at 5 lb/A) to Carrington silt loam
plots under field conditions. They reported that 85% of the residue was lost during the first 3 days following application. Residue levels of approximately 0.1 ppm were reached within 8 days post-treatment.

Lichtenstein et al. (1977) studied the persistence of C-14 labeled methyl parathion in soil. 7% of methyl parathion was extractable 28 days after soil treatment, whereas C-14 bound residues amounted to 43% of the applied dose was extractable and 35% of the label was bound. Bound pesticide residues were either non-toxic or showed greatly decreased insecticidal activity.

In a field study, Nicholson et al. (1962) reported a very high concentration (1.9 ppm) of parathion in the bottom sediments of a farm pond in March 1960. Residues in the bottom mud of the pond after August 1960 were 1.13 ppm and after November 1960, they were 0.09 ppm. In another study, Mullin et al. (1966) studied the residue levels of parathion in the bottom mud of a duck pond treated directly with 1 lb/A parathion. They found that the parathion residue declined rapidly and reached 0.06 ppm in the top 2 inches of the mud 22 days after the treatment. Mud below the 2 inches level did not show any detectable residues.
The soil topography, soil type, organic matter, moisture, pH, and temperature are considered to be important factors determining the longevity, availability, and movement of pesticides in the soil (Cane et al., 1973; Majagopal and Sethunathan, 1984; Koepe and Lichtenstein, 1984; Venkarnamesh et al., 1986).

2.2.3. PERSISTENCE OF ORGANOPHOSPHORUS PESTICIDES IN AQUATIC PLANTS

Compared with terrestrial plants, there is not much research carried out on the nature and magnitude of residues of malathion and parathion in aquatic plants.

When malathion was added to the culture of the alga, Gonium pectorale at 1 ppm, about 56% of the applied dosage was metabolized by the alga in 4 days (Moore and Dorward, 1968). Parathion on the other hand, was neither metabolized by G. pectorale nor by the bluegreen alga, Anacystis nidulans or the green alga, Scenedesmus obliquus. At a concentration of 1 ppm in the cultures of A. nidulans and S. obliquus, the alga showed 50 to 72-fold accumulation of parathion when exposed for 7 days (Gregory et al., 1969).

In one field study (Fogart et al., 1974) consisting of 1 fogging (480 g/ha) and 3 biweekly ULV aerosol sprays
(57 g/ha) of malathion to a saltmarsh in Florida, the residue of this insecticide in the rush, *Juncus* Sp. was 4.1 ppm soon after these applications. The residue level declined to 0.08 from 0.1 ppm, 14 days after the last treatment.

In a mutated treatment scheme for mosquito control, Mulca et al. (1966) applied parathion to a duck pond and a borrow pit. The duck pond received 1 treatment of parathion at the rate of 1 lb/A, and the borrow pit received 5 weekly sprays of parathion at 0.1 lb/A. The residues of the insecticide in the submerged portion of the water-grass, *Echinochloa crus-galli*, were 0.5 ppm after 4 hours, falling to 0.2 ppm, 3 days after treating the pond. The submerged portion of this grass showed lower residue levels up to 14 days.

Practically no reference is available which discusses about the accumulation of methyl parathion and malathion in aquatic plants in India.

### 2.2.4. PERSISTENCE OF ORGANOPHOSPHORUS PESTICIDES IN AQUATIC ANIMALS

The impact of malathion and parathion on freshwater molluscs and fishes has scarcely been studied. Only a few reports are available that provide meaningful information on the effect of these insecticides on freshwater molluscs and
fishes and hence literature on other aquatic animals is also reviewed.

In a study of the persistence and transfer dynamics of parathion-S₁₄₂₅ (1 lb/A equivalent) in a model cranberry dog, the freshwater mussel, *Elliptio complanatus* was found to accumulate high residues (0.99 ppm) within the first 24 hours but residue levels dropped to 0.04 ppm after 144 hours of the treatment (Miller *et al.*, 1966). Miller *et al.* (1967) studied the persistence of the same pesticide in the fish, *Fundulus complanatus* and reported that the fish accumulated the residue to levels (1.66 ppm) far in excess of that (0.02 ppm) present in water. Mula *et al.* (1966) noted similar observations on the free-swimming mosquitofish, *G. affinis* grazing in experimental ponds treated with parathion at the rate of 1 lb/A. Pesticide residues in the fish reached as high as 22 ppm without causing any mortality or obvious symptoms of poisoning. The bio-accumulation of methyl parathion in various tissues of *Cyprinus carpio*, had been studied by Chigareva (1973). Methyl parathion content in various tissues i.e. liver, muscles, gills and brain of carp, detected after 2 days initial exposure of 1 ppm methyl parathion was 4.4, 1.1, 1.7 and 3.3 ppm respectively. The accumulated toxicant degraded to the tune of 35% after 20 days of treatment. Kanazawa (1975) examined uptake and excretion of the organophosphorus pesticides malathion, diazinon
and fenitrothion in minnows, *Pseudorasbora parva* at 23 ± 2°C, in aquarium water containing approximately 1 ppm of the pesticides. The concentration of the compounds decreased with the lapse of time, after 4 weeks to 0.27 ppm for diazinon and 0.02 ppm for fenitrothion. Malathion disappeared much more promptly, to less than 0.01 ppm after 1 week. The concentration of the organophosphorus compounds in fish was maximum shortly after initiation of exposure: 211 ppm for diazinon after 3 days, 162 ppm for fenitrothion in 4 days, and 3.4 ppm for malathion after 1 day. Thereafter the concentrations decreased gradually due to metabolism and excretion. After 30 days the fish contained 17 ppm and 4.9 ppm of diazinon and fenitrothion, respectively. Malathion was more unstable in fish, being 0.01 ppm after 1 week.

The bioconcentration of diazinon by fish, small and cray fish, in a flow through exposure was investigated by the same author Kanazawa (1978). The bio-concentration factor, (BCF) of diazinon by fish was generally greater than that of crayfish and snails. The BCF determined from a 7 day exposure to 10 mg/l, in a flow-through system was 152 for *Pseudorasbora parva*, 65 for *Carassius arvernus* and 18 for *Lampetra reticulata*. Pesticide levels in water, mud, zooplankton and fish from Sambhar reservoir (India) were measured by Kannan and Job (1979). Endrin, parathion, BHC and DOT
were detected at ng/g level in mud, zooplankton, and fish whereas only endrin and parathion were detected in water. The concentration of the organophosphorus pesticide fenitrothion in carp, snails, daphnids and algae decreased with time, and its bioaccumulation ratio relative to the concentration in water tended to increase gradually in snails, daphnids and algae due to lower metabolic activity or slow excretion (Miyamoto et al., 1979). Verma et al. (1979) measured the effect of accumulation of zolone, rogor and malathion on the ACHE of the brain, liver and muscle of Channa gambeh and Cirrhites mrigala. There was a significant decrease in enzyme activity in all the tissues of both fish species exposed to the pesticides.

Mukhopadhyay and Behadral (1980) studied the residual action of malathion in various tissues of catfish by gas chromatography analysis. Accumulation occurred largely in gills at the rate of 78.6 μg/g wet tissues, whereas in liver and kidney only minute quantities were present while no trace of malathion was observed in intestine and body muscles. Sahu and Mukhopadhyay (1983) observed slight accumulation of malathion in the testis of the fish Clarius batrachus exposed to sublethal concentration (0.5 ppm) of malathion, after 30 days.
3.0 DEGRADATION OF ORGANOPHOSPHORUS PESTICIDES

IN AQUATIC ECOSYSTEM

Pesticides undergo metabolic transformation in the aquatic ecosystem and its speed depends on the chemical nature of the pesticide. It is no denying the fact that the pesticides, though may be degraded, do not get totally eliminated at once and therefore, their degradation does not necessarily mean the end of the hazard which they otherwise cause. Instead, these may produce an equally or even more toxic metabolic products. (Matsunura, 1973). These products are stable and cause just as many residual problems as the original compounds. Giese et al. (1975) emphasized that there are three important phases of pesticide degradation viz., the cause of degradation, the pathways of degradation, and the rates of degradation. While talking about the degradation of pesticides in the soil, he remarked that it was important to recognize that, pesticides and their degradation products are a result of both catabolic and anabolic processes. The degradation of pesticides in the aquatic environment includes processes like hydrolysis, oxidative desulfuration, aromatic hydroxylation, aliphatic hydroxylation, oxidation, reduction etc. (Miyamoto et al., 1979). Hence the knowledge on degradation and metabolism of the pesticides in the aquatic ecosystem is important for assessing short-term and long-term impacts on the non-target organisms. Further
the knowledge of the location of the pesticide metabolites in various tissues is also important for understanding the route of detoxification and degradation (Cook and Moore, 1976).

3.1 IDENTIFICATION OF RESIDUES OF ORGANOPHOSPHORUS PESTICIDES AND THEIR METABOLITES

A number of methods have been reported for analysis of qualitative/quantitative pesticides and their metabolites. They include paper chromatography, thin layer chromatography, and gas chromatography.

Fischer and Küngelhöller (1961) used both paper and thin layer chromatography to identify metabolite residues of a number of organophosphorus pesticides extracted from animal tissues by hot ethanolic potassium hydroxide. The degradation products given by each compound gave a different spot pattern; for example, malathion yielded spots of Rf 0.32, 0.44, 0.50, and 0.66 on silica gel G developed with 20+80+1 methanol : dichloromethane : 10% ammonia solution. Uchiyama and Okui (1963) studied the thin-layer chromatography of a number of organophosphorus pesticides on silica gel developed with 4+1 n-hexane : acetone.

The use of thin-layer and gas-liquid chromatography in the detection, determination, and infrared identification
of organophosphorus pesticide residues, extracted from samples of vegetable tissue has been described by Abbott et al. (1965). Thin layer chromatography is used both as a preliminary tentative identification procedure and for clean up purposes prior to the gas chromatographic preparation of a pure specimen for confirmation of identity by infrared spectroscopy for this purpose, 500 µ thick layers of silica gel G are developed with 9+1 hexane : acetone for 40 minutes, resolved pesticide spots being eluted from the adsorbent with dichloromethane. Abbott and Thomson (1966) modified the above method in which they used 80+20 hexane : acetone as solvent system and palladium chloride as spray reagent.

3.3 DEGRADATION OF ORGANOPHOSPHORUS PESTICIDES IN AQUATIC ECOSYSTEM

The ultimate fate of pesticides in the aquatic environment depends on two processes, namely, activation and degradation. Degradation of organophosphorus pesticides in different components of aquatic ecosystem follow different routes. Further the rates of degradation also differs in water, soil, plant and animals (Fenske et al., 1979) and hence the degradation of organophosphorus pesticides (Malathion and Parathion) in water, soil, plant and animals has been reviewed in detail.
3.2.1. DEGRADATION OF ORGANOPHOSPHORUS PESTICIDES IN WATER

The short persistence of malathion in water as a function of pH has been revealed in several studies. For example, Spiller (1961) reported that malathion underwent 100% hydrolysis instantaneously at pH 12. At pH 9, its half time was 12 hours. Konrad et al. (1969) found that the rate of malathion hydrolysis at 7 days was 100% at pH 11 and 25% at pH 9. Earlier Weiss and Gatstatter (1964) had found that malathion was stable in nearly neutral and acidic water. Muhlman and Schrader (1957) reported that the rate of hydrolysis of malathion increased four-fold at each 10°C increase in temperature.

Parathion has also shown to degrade chemically in water. In laboratory studies; Ruxicka et al. (1967) found that parathion had a half-life of 43 h in ethanol-pH 6 buffer solution (20:80) at 70°C. In distilled water (at 0.4 ml of parathion / 530 ml water), 81.2% of the parathion was hydrolyzed chemically in 6 weeks (Cowart et al., 1971). Gomaa and Faust (1971) reported that parathion was less stable at alkaline pHs.

Besides pH, temperature was also reported
to affect the degradation of parathion in water (Faust and Suffet, 1966). It was found that its half-life at pH ≤ 3 from 1,000 days at 10°C decreased to 1.6 days at 70°C. Gomaa and Faust (1971) demonstrated degradation of parathion into paraoxon in water and according to him it was due to dissolved oxygen in water.

Miyamoto et al. (1979) identified 13 metabolites of the organophosphorus pesticide fenitrothion in water; they include fenitroxon, aminofenitrothion, and its N-formyl and N-acetyl derivatives as well as 3 demethylated products.

In another study, Kenses et al. (1973) detected 0, 6, 4, and 3 metabolites of trifluralin in water after 9, 22, 30 and 58 days respectively.

4.2.2. DEGRADATION OF ORGANOPHOSPHORUS PESTICIDES IN SOIL

Munkman and Schrader (1967), Walker and Stojarovic (1973) studied the degradation of malathion in soil. According to them, degradation of malathion in soil is affected by pH, temperature, moisture, soil microorganisms and organic matter present.
Yaron (1975) studied the chemical degradation of parathion in 14 different sterile soils. He found that parathion hydrolysis was influenced by soil mineralogy.

Miyamoto et al. (1979) studied the degradation of organophosphate fenitrothion in soil. He obtained 3, 5, and 7 metabolites (which were mostly amino derivatives) after 3, 7, and 21 days. Oxygen analogs were not obtained in their experiments. In another study, Isensee (1979) demonstrated 8 metabolites in varying concentrations of the organophosphate trifluralin applied in the soil.

3.3.3. DEGRADATION OF ORGANOPHOSPHORUS PESTICIDES IN AQUATIC PLANTS.

The degradation of pesticides in plants is well understood with systemic insecticides such as dimethoate, demeton, and a few others, but there are not many data available on malathion and parathion degradation in aquatic plants. Rapid metabolism of malathion by an algae (Chlorella pyrenoidosa) was demonstrated by Moore and Gorward (1968). At a 1 ppm concentration in the algal culture, 55.7% of the applied malathion was metabolised in 4 days. The products of degradation were not reported. Cotton (1983), Rowlands (1984) and Mian and Mulia (1980) demonstrated that degradation
of malathion in plants occurs through two main routes of hydrolysis, phosphatase(s) and carboxyesterase(s). In their studies the products of phosphatase activity were either dimethyl phosphorodithioate or dimethyl phosphorothioate or both, whereas carboxyesterase(s) resulted in malathion monoacid and malathion diacid.

The reduction of parathion to aminoparathion is demonstrated by Suzuki and Uchiyama (1975) in spinach. They reported that under anaerobic conditions the nitro reduction process is spinach homogenate proceeded via hydroxylaminoparathion as the intermediate metabolite. These authors suggested that besides hydroxylamino-parathion, nitrosoparathion could as well be one of the intermediate metabolites in the nitro reduction of parathion in plants.

Iseensee et al. (1979) demonstrated 4,6,3,5 and 1 metabolites of trifluralin after 2,5,15,22 and 30 days in the algae Oedogonium cardiacum. In another degradation study Miyamoto et al. (1979) obtained only one metabolite out of seven metabolites of organophosphate fenitrothion up to 7 days but after 21 days after start of experiment he recorded two metabolites.

After incubation of parathion in a pure culture
of freshwater alga Chlorella pyrenoidosa for 3 days, Ahmed and Casida (1958) recovered only 37% of the parent material. They also reported little oxidation product (Paraoxon). Mackiewicz et al., (1969) found that 57% of the applied para- thion was degraded to aminoparathion by this alga and 17% was degraded to an unknown product. Using axenic culture of this alga, Zuckerman et al. (1970) reported aminoparathion along with 3 unidentified compounds. Moore and Dorward (1968) reported that the alga Chanter pectorale did not meta- bolize para-thion.

3.2.4. DEGRADATION OF ORGANOPHOSPHORUS PESTICIDES IN AQUATIC ANIMALS.

The biodegradation of malathion and para-thion by freshwater aquatic animals like molluscs and fishes has been scarcely studied. Most of the biodegradation studies are carried out in insects, marine fishes, mammals and birds and hence literature on these animals is also reviewed.

The degradation of malathion by the white mouse was studied by Krueger and O'Brien (1969). The relative products of carboxylesterase and phosphatase actions recovered as water soluble metabolites 30 min after administering the dose (30 µg/g), were 60% and 20%, respectively. The
Fig 2. A general scheme of the metabolic fate of Malathion
A - Animal; M - Microorganisms; P - Plant (Reference: O'Brien, 1980)
Fig. 3 A General Scheme of the Metabolic Fate of Parathion.
A—Animal; M—Microorganisms; P—Plant (Reference—O’Brien, 1969)
monooacid derivative was the predominant metabolite from carboxyesterase action. Similar observations were reported by March et al. (1956) using malathion - $^3$H given to the white mouse and hen. The nature of metabolites in urine of malathion treated rat was investigated by O'Brien (1960). Urine samples analysed 48 h after the treatment contained malathion monoacid, malathion mono, dimethyl phosphorodimethylester, dimethyl phosphorothionate, dimethyl phosphate and dimethylmalathion. The author also found the same metabolites in the urine of dog. 48 h after malathion (25 mg/kg) was administered to the animal. Similarly urine was also found to be the principal route of excretion of water soluble metabolites in lactating ewes (O'Brien, 1960).

Bhagwan and Kaminchandran (1975) reported malathion A and B esterases in the liver of mouse. Cook et al. (1978) have reported that whole body analysis of fish (Lagodon rhomboids) exposed to malathion showed the presence of malathion mono and dicarboxylated. Mamlur and Deurerman (1978) also found degradation of malathion in the liver of mouse. Monds (1980) has studied the presence of five metabolites of malathion. Singh and Salei (1985) identified 3 metabolites of malathion in liver of the freshwater fish Rasbora daniconius after 30 days exposure.
The uptake, metabolism and elimination of organophosphate diazinon by rainbow trout, carp and shrimp were measured by Seguchi and Asano (1979). Equilibrium concentrations were reached in 3 days in fish exposed to 10 μg/l of diazinon for 14 days. The metabolite M-6 was found in all the three species, but M-2 was found only in rainbow trout and carp. When the fish were returned to clean water, diazinon and its metabolites were eliminated rapidly.

Diggle and Cage, (1951) studied biodegradation of methyl parathion in mammals and Metacalf and March (1953) in insects. These authors demonstrated the activation of phosphorothionates to phosphates by oxidative desulfuration. Brindley and Dahm (1964), among others, have identified the microsomal oxidation product of methyl parathion as methyl paraaxon which is ten fold more toxic than methyl parathion. Hollingsworth et al. (1967) studied the biodegradation of methyl parathion in mice and reported that 7 out of 8 metabolites which could be identified in urine were hydrolysis and oxidation products i.e. phosphoric acid, methyl phosphoric acid, dimethyl thiophosphoric acid, desmethyl phosphate, desmethyl thiophosphonate and phosphate.

Miyamoto et al. (1979) studied the biodegradation
of the organophosphorus pesticide fenitrothion in the freshwater snail *Grapocapaludina japonica* and the fish *Cyprinus carpio*. He observed 6 metabolites in soft tissues of snail and 8 metabolites in fishes.

4.0 EFFECTS OF PESTICIDES ON OXYGEN CONSUMPTION.

Measurement of oxygen consumption not only indicates metabolic rate, but it also provides an index to stress conditions as it is a valuable indicator of energy expended to meet the demands of environmental alterations (Holden, 1965; Ferguson and Bingham, 1966; Skidmore, 1970; van der Meer and Vennberg, 1970; Fry, 1971; McIntosh and Thorberg, 1973; Thorberg et al., 1974; Roberts, 1975; Phillips, 1978; Guile et al., 1980; Merysawskyj et al., 1987). The choice of indicator species is important as different species respond to different portions of the pollutant load in an aquatic ecosystem. (Skidmore and Tovell, 1972; Hughes and Ferr, 1976; Phillips, 1978; Thorberg, 1980).

Review of literature on oxygen consumption studies showed that number of different organisms have been used for evaluation of aquatic pollutants (Mount, 1962; Costa, 1965; Ferguson and Bingham, 1966; Hunter et al., 1966; Ohmaya, 1971; McIntosh and Thorberg, 1973; Saralva,

Pesticides are known as inhibitors of respiration and it has been well reviewed by Fukami (1975). The measurement of oxygen consumption of animals, therefore provide an additional clue to the physiological mode of action of the pesticide pollutants (Thurberg, 1980).

4.1 DETERMINATION OF OXYGEN CONSUMPTION

The oxygen consumption by aquatic animals can be determined by O$_2$-detecting electrode or by a titrimetric method as described by Winkler (Golterman et al., 1978). A survey of literature reveals that there are several methods (Knowles and Lawden, 1953; Rosam and Langan, 1963; Montgomery et al., 1964; Hart, 1967; Bryan et al., 1976). Various authors have also modified the Winkler's method (Pomery and Idracni, 1945; Desale, 1958; Ganapati and Rao, 1960; Walsh and Smith, 1961; Strickland and Parson,
4.2 OXYGEN CONSUMPTION BY MOLLUSCS

Because of availability, ease to maintain in the laboratory and wide range of sizes, molluscs are used for oxygen consumption studies. A brief survey of literature shows that most of the studies conducted are on marine molluscs (Melrose and Thunberg, 1972; Thunberg, et al., 1974; Iwanak and Mohamed, 1975; Roberts, 1975; Nagabhushanam and Deshpande, 1982).

The effects of exposure to sublethal concentrations of malathion on rate of oxygen consumption of the marine pteropod, Oncaea verruculatum was studied by Nagabhushanam and Deshpande (1982). The authors observed that the rate of oxygen consumption of O. verruculatum was directly proportional to the length of the exposure period than the concentration itself. Sublethal concentrations of malathion exhibited continuous increase in the rate of oxygen consumption while the lethal concentrations enhanced metabolic rate which was followed by sudden fall and consequent death of the exposed animals. Decrease in rate of oxygen consumption was observed by Mauricey et al. (1984) in freshw
water mussel, Lamellicidens marginalis treated with methyl parathion and by Kulkarni et al. (1985) in L. coromandus exposed to sublethal concentration of thioban.

4.3 OXYGEN CONSUMPTION BY FISHES

Voluminous literature on the oxygen consumption of fishes both, animal as a whole and excised tissue have been well documented. Number of organophosphates have been found to depress - the oxygen consumption in the different fish species (Pande et al., 1976; Koundinya and Rammurthi, 1978; Dalela et al., 1980; Rao et al., 1980; Natraj, 1980; Vijayakshmi, 1980; Natraj, 1981; Nagarathnamma and Ramamurthi, 1982; Rao et al., 1984; Bhushari et al., 1985; Ravi and Selvarajan, 1988).

Ferguson and Hingam (1966) observed that with the onset of symptoms of poisoning, the rate of \( O_2 \) consumption increased in mosquito fish exposed to organophosphorus pesticides, but just before the death of the fish, there was a marked decrease in \( O_2 \) consumption.

Reddy and Gomathy (1977) reported that lethal thiodon exposure increased the respiration of Mystus vitatus. Accelerated rate of oxygen consumption of Anabas scandens
after exposure to sumithion was reported by Natarajan (1980). Oxygen consumption of Labeo rohita exposed for 96 h to lethal and sublethal concentration of metamitox was measured by Bansal (1979). At the 96 h LC$_{50}$ concentration, an all or nothing response was observed. At sublethal concentrations, oxygen consumption was in three phases; sensitization, responsive, and normalization. Oxygen consumption data showed that there was a critical threshold time for the responsive phase from the beginning upto 6 h for metamitox. Rao et al. (1980) showed that in case of Labeo rohita oxygen consumption increased progressively with the increase in the concentration, upto a certain level and decreased with further increase of the toxicants until death assured. Bhusari et al. (1985) reported similar observations in the fish Barbus ticto exposed to organophosphorus pesticide etaxalux.

Sahlo et al. (1981) observed significantly greater oxygen consumption in Tilapia mossambica after 12 and 24 hour exposures to 2 mg/l of malathion but it was not different than that of control fish at 36 and 48 hours. Nagaratnamma and Ramamurthi (1982) observed decrease oxygen consumption due to damage in gill epithelium of Cyprinus carpio exposed to methyl parathion. Rao et al. (1984) studied oxygen consumption
in phosphamidon treated freshwater fish, *Tilapia mossambica* and observed decreased oxygen consumption due to excessive mucus secretion which formed a thin film over the gills, thereby preventing absorption of oxygen during the process of gaseous exchange. However Wagh and Shareef (1985) did not observe any significant change in oxygen uptake by *Rasbora daniconius* exposed to sublethal concentration of suquin. Dalela et al. (1980) evaluated the effects of lethal and sublethal concentrations of chlordane, metasystox and carbaryl on oxygen consumption of *Saccogranthus fossilis*. The authors further reported that at lower concentrations oxygen consumption was characterised by three distinct phases (a) phase of sensititization, (b) responsive phase and (c) the phase of normalization. Ravi and Selvarajan (1988) observed significant reduction in oxygen consumption in different regions of brain of the phosalone poisoned *Cyprinus carpio*. Bhattachar et al. (1988) observed increased rate of oxygen consumption in *Clarius batrachus* in toxic solutions and according to him it was due to increased opercular movements, which increased rate of intake of pesticides from the water. The authors also observed that just before death there was a sharp decrease in oxygen consumption which was indicated by the slow opercular movement in the fish.