THE FATE OF DDT AND CHLORPYRIFOS APPLIED TO A MODEL ECOSYSTEM
SIMULATING A TROPICAL MARINE ENVIRONMENT.

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A thesis submitted in partial fulfillment of the degree of Master of Science of the University of Nairobi.
DECLARATION.

This thesis is my original work and has not been presented for a degree in any other University.

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This thesis has been submitted for examination with our approval as University supervisors.

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DEDICATION.

I would like to dedicate this work to my loving mum (Moraa), dad (Mang'aa), brothers and sisters, not forgetting my dear wife.
ABSTRACT.

A study of dichlorodiphenyltrichloroethane (DDT) and Chlorpyrifos was conducted using a model aquarium simulating a tropical marine ecosystem. The ecosystem comprised sea water, sediment, oysters and three species of fish: Dory snapper fish (*Lutrinus fulviflamma*), Rabbit fish (*Siganus stellatus*) and Black emperor fish (*Lethrinus harak*). DDT and chlorpyrifos insecticides and the metabolites of DDT were then monitored over a period of 672 hours using Liquid Scintillation Counting and Gas Chromatography (GC) with Electron Capture Detector (ECD) techniques. The distribution of pesticide residues among the ecosystem components, the rate of loss of the pesticide residues from biota to sea water and the dissipation trends of the pesticides from sea water were assessed.

Water having a concentration of 1.2 ng/g of DDT was found to have lowered its DDT concentration to 0.4 ng/g in 168 hours although equal amounts of the pesticide was injected in intervals of 24 hours within the 168 hours. By contrast, oysters and sediment had their DDT concentrations rise from 126 to 1308 ng/g and 1 to 117 ng/g respectively in the same period. In another system containing water and sediment only, the DDT concentration in the former declined from 20.5 ng/g to 1.1 ng/g while in the latter it rose from 2.6 ng/g to 18.8 ng/g in a period of 168 hours.

The concentration of chlorpyrifos rose from 2 ng/g to 4 ng/g in water within a week as equal amounts of the pesticide were injected into the water at 24 hour intervals. Similarly, in the same ecosystem *Lutrinus fulviflamma*, *Siganus stellatus*, oysters, and sediment had their pesticide concentrations rise from 190 to 421 ng/g, 207 to 584 ng/g, 134 to 450 ng/g, and 14 to 20 ng/g respectively within 168 hours. In a system comprising water and sediment only, chlorpyrifos concentrations of 42.7 ng/g in water declined to 3.1 ng/g in 168 hours while that of sediment rose from 2.8 to 9.8 ng/g in the same period.
Oysters having DDT concentrations of 936 ng/g were noted to lose 34% of the pesticide when exposed to fresh sea water within 168 hours. By contrast, *Lethrinus harak*, *Siganus stellatus* and oysters containing chlorpyrifos at concentrations of 541 ng/g, 219 ng/g and 160 ng/g respectively, lost 64%, 50% and 26% respectively of the pesticide under similar conditions.

Chlorpyrifos was found to have half-lives of 4.2 days and 7 days in the two species of fish, *Lethrinus harak* and *Siganus stellatus*, respectively. By contrast, DDT at concentrations of 27 and 33 ng/g in aerated and non-aerated waters was found to have half-lives of 3 hours 20 minutes and 3 hours 45 minutes, respectively. Half-lives of 13 hours 21 minutes and 24 hours 3 minutes were observed for concentrations of 33 ng/g and 27 ng/g of chlorpyrifos in aerated and non-aerated waters, respectively.

DDT degradation in sea water gave dichlorodiphenyldichloroethylene (DDE) as the major metabolite while in sediment, dichlorodiphenyldichloroethane (DDD) was the major metabolite.

From the results obtained, it was concluded that higher concentrations of pesticide residues in a marine ecosystem are absorbed by biota (e.g fish and oysters) and to a lesser extent get adsorbed to sediment. Fish loses pesticide residues to fresh sea water at a higher rate compared to oysters. The rate of loss of pesticide from aerated water is higher than from the non-aerated. The rate of loss of DDT from water was higher than for chlorpyrifos as implied by their half-lives.
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List of abbreviations.

Ai........Active ingredient
App.......Appendix
Conc.....Concentration
CPM......Chlorinated Pesticide Mixture
DDD.......Dichlorodiphenyl dichloroethane
DDE.......Dichlorodiphenyl dichloroethylene
DDT.......Dichlorodiphenyl trichloroethane
EC.........Emulsifiable concentrate
ECD.......Electron capture detector
FID.......Flame ionization detector
Fig........Figure
FPD......Flame photometric detector
GC.......Gas Chromatography
Hrs.......Hours
IAEA......International Atomic Energy Agency
LSC ......Liquid scintillation counter
Min.......Minute
PCBs....Polychlorinated biphenyls
PPO.......2,5-Diphenyloxazole
POPOP....2,2'-p-phenyl bis(4-methyl-5-phenyloxazole)
TLC.......Thin Layer Chromatography
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CHAPTER ONE

General Introduction.

1.1 Uptake, distribution and elimination of DDT.

The uptake, distribution and excretion (pharmacokinetics) of some of the more commonly used organochlorine insecticides such as 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane \( (p,p'\text{-DDT}) \) and 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-endo-1,4-exo-5,8-dimethanonaphthalene \( \text{dieldrin} \) have been widely studied in a variety of species of mammals, birds and, fishes (Kenaga, 1972) and also in natural and model ecosystems (Kapoor et al., 1972).

In both terrestrial and aquatic ecosystems, major pharmacokinetics' determinants are the microflora and microfauna (plankton, bacteria, yeasts, fungi and protozoa) which facilitate the transfer of organohalogen pollutants into higher organisms and initiate food chain distribution.

Adsorption and desorption are crucial processes which affect the fate of pesticides in the aquatic environment. The process is correlated with the nature of solids in the system (particle size distribution, type of clay mineral, clay content, organic matter content, cation exchange capacity, etc) and the characteristics of the pesticide (water solubility, octanol-water partition coefficient, fugacity, etc). Clay and organic matter are the soil components most often implicated in pesticide adsorption. However, the individual effects are easily predicted since the organic matter in most soils is bound to clay, probably through a clay-metal-organic complex system (Stevenson, 1976). The partition coefficient for a wide range of chemicals between soil, organic matter and water, can be expressed as a function of octanol-water partition coefficient or water solubility. These chemical properties are useful in predicting the adsorption patterns of non-ionic chemicals (Briggs, 1981).

Though highest concentrations of DDT occur in the fatty tissues of animals, organisms do not on a world-wide scale, show significant effects on the distribution of DDT. This results presumably from the high affinity that DDT has for fatty tissues (Pierce, Olney and Felbeck, 1974). DDT and its metabolites being highly lipid soluble are stored in the adipose tissues of fish.
where they cannot exert their toxic effects until there is rapid mobilization as in the case of starvation. In fish DDT is metabolized to its primary degradation products 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene (DDE) and 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane \( p,p'-\text{DDD} \) both in the intestines and muscle tissues (Wedemeyer, 1968).

For poikilothermic animals, the rate of chemical uptake and metabolism is linked to the temperature of their environment. Thus fish can rapidly take up high concentrations of DDT from the water and this uptake increases with temperature (Hamelink and Spacie, 1977). Toxicity of DDT to fish has been shown to decrease with increase in temperature (Cope, 1965) and this may definitely affect the distribution of the pesticide in the ecosystem.

Volatile compounds may be excreted into the alveoli of the lungs, and expelled during exhalation. Certain compounds are excreted in saliva and sweat (Parke, 1968). Highly liposoluble substances are not usually excreted to any significant extent in their original forms. However, certain persistent liposoluble insecticides and their stable metabolites (for instance dieldrin, DDT and DDE) undergo only slow biotransformation, so that direct excretion, although slow, is of some significance in their removal. DDT and DDE are removed in the droppings of the quail over a period of weeks after intraperitonial injection (Ahmed and Walker, 1979). Dieldrin, DDT and DDE are excreted in the lipid-rich fractions of eggs' yolk of birds and milk (butter fat) of cows and humans (Still et al., 1973).

Different types of samples removed from a tidal marsh habitat in Florida, U.S.A, treated with 0.2 pounds per acre of DDT were found to contain the following levels of DDT: surface water and ditch, 0.3 to 4.04 \( \mu \text{g/g} \), sediments 3.5 \( \mu \text{g/g} \) (dry weight) and fish (five species), 4-58 \( \mu \text{g/g} \) (wet weight) (Croker and Wilson, 1965). By contrast sediments from Lake Michigan, U.S.A. on a wet weight basis averaged 0.014 \( \mu \text{g/g} \) of DDT and DDE. While amphipod \( \text{pontoporeia affinis} \) averaged 0.41 \( \mu \text{g/g} \) for DDT and its related metabolites. Various fish removed from the same lake had residues of 3.35 \( \mu \text{g/g} \) \( \text{alewives} \), 4.52 \( \mu \text{g/g} \)
(chub), and 5.60 µg/g (whitefish); while breast muscle had over 27 times the level of DDT found in the alewives (Hickey et al., 1966).

1.2 Uptake and distribution of chlorpyrifos.

Equilibrium for o,o-diethyl o-3,5,6-trichloro-2-pyridyl phosphorothioate (chlorpyrifos) between water and sediments in an ecosystem was established in about the seventh day post treatment. About ninety percent of the chlorpyrifos still remaining in the system was found accumulated in the top 5.0 cm layer of sediments, which corresponds to 0.35 mg/kg of pesticide in sediments (Hulbert et al., 1970).

Dissolved organophosphate was taken up rapidly by goldfish (from water) over an initial ten hour period and then, following a period of equilibrium (1.5 days) the concentrations of the organophosphate in the water began to increase indicating that water-soluble, detoxification products were excreted. The fish died when exposed to the insecticide in a model ecosystem containing plants and soil (Smith et al., 1966). Tissues of fish were found to contain chlorpyrifos and its metabolites.

Comparison of the \(^{14}\)C-labelled chlorpyrifos uptake by fish living in tanks containing clear water and those living in tanks containing water plants and soil has been carried out. The radioactive compound was rapidly taken up from the water by plants, and soil particles. It was concluded that under natural conditions this rapid uptake will limit the amount of chlorpyrifos which can be absorbed by fish (Smith et al., 1966).

Small amounts of chlorpyrifos tend to sequester in fat tissues (for instance the half-life of chlorpyrifos in the fat of cattle is 4 to 7 days (FAO/WHO, 1973)), whereas 3,5,6-trichloro-2-pyridinol (chlorpyrifos metabolite) residues are found mainly in the liver and kidney. Chlorpyrifos, mostly as 3,5,6-trichloro-2-pyridinol is eliminated mainly through the urine and to a small extent via the faeces, and via lactation (Dishburger et al., 1972a, c; Mckeller et al., 1972c; Johnson et al., 1974). Chlorpyrifos applied to a pond at
an initial concentration of 220 ng/g in water was found to decrease to 3 ng/g within 2 weeks (Hurlbert et al., 1970).

1.3 Degradation and metabolism of pesticides.

Transformation processes convert pesticide molecules into other products and if the process is biological, the products are referred to as metabolites. There are a variety of transformation processes which, in general, tend to detoxify the pesticide, make it more water soluble, alter the carbon oxidation state or oxidize them completely to carbon dioxide and water (Goring and Hamaker, 1972).

Chemical degradation is promoted both biotically and abiotically through the processes of hydrolysis, oxidation and reduction (Drapper and Wolfe, 1987). In the aquatic environment these processes may occur in the water phase and in biota at the interfacial surfaces. Degradation by microbial action is an important route for the removal of chemicals from the environment (Torstensson, 1987) and in aquatic systems; it occurs in the water phase at rates which depend on bacterial population density (Torstensson, 1987).

Hydrolytic attack may occur in the bulk of the water phase. It can also be promoted at inorganic surfaces. Photolytic attack is also a major route for transformation. This process is dependent on the flux of incident light and will hence be influenced by climate and latitude of the location on the earth's surface, by water depth and by the optical density of water (Parlar, 1990). Temperature too could be an important factor. In a study carried out in sterile sea water under fluorescent light, chlorpyrifos degrades about 5 times faster at 32°C compared to that at 15°C (Carvalho et al., 1992). Knowledge of the degradation of pesticides is important for the understanding of the toxicity of the by-products and the relative effectiveness of the pesticide compound to the metabolites. If a pesticide is slow to degrade, it means that it will remain as the parent or active molecule on a target for long and hence remain active for long periods. Some pesticides like DDT have been known to
produce more toxic by-products (e.g. DDE) to the target organisms (Karns et al., 1986).

1.3.1 Degradation and Metabolism of DDT.

Degradation of DDT in the environment can occur due to hydrolysis, photolysis, and microbial degradation (Park, 1980). The mechanism of these processes has been known to follow pseudo-first-order kinetics for the dissolved phase. The strong adsorption of DDT to particulates may affect the degradation process (Cox and Bull, 1970). It is said that in fish most of the metabolism of DDT is carried out by intestinal microflora which can convert DDT to DDE or DDD (Wedemeyer, 1968).

A survey of literature suggests that the main pathways of DDT metabolism in vertebrates are:

$$\begin{align*}
R - C - R & \quad \text{R - CH - R} \quad \text{R - CH - R} \\
\text{Cl}_2 & \quad \text{Cl}_3 \quad \text{HCl}_2 \quad \text{COOH} \\
\text{DDE} & \quad \text{DDT} \quad \text{DDD} \quad \text{DDA}
\end{align*}$$

Where R is $C_6H_4Cl$ (p-chlorophenyl group).

Fig. 1.1: Main metabolic pathways of DDT in vertebrates.

But in invertebrates, the dehydrochlorination process seems to predominate although variations from one species to another within this pattern do occur (O'Brien, 1967). In insects, DDT is mainly converted to DDE, but in the fruit fly (*Drosophila Melanogaster*) it is reported to be converted mainly to the muticide, kelthane or dicofol (Robert and Hutson, 1990).

$$\begin{align*}
R_2CHCl_j & \quad \text{R}_2C(OH)CCl_j \\
\text{(DDT)} & \quad \text{(Dicofol)}
\end{align*}$$

Where R is $C_6H_4Cl$ (p-Chlorophenyl group)

Fig. 1.2: Metabolism of DDT in fruit fly.
1.3.2 Degradation and metabolism of chlorpyrifos.

Chlorpyrifos is stable for weeks in neutral or weakly acidic formulations stored at or slightly above normal room temperatures (20°C) but the rate of breakdown e.g by hydrolysis increases with increase in temperature (Gray, 1965, Meikle, 1972). Small quantities of the anticholinesterase agent, chlorpyrifos oxen, could be found in some commercial preparations. Between 0.014 and 0.02% chlorpyrifos oxen have been reported in chlorpyrifos formulations (Struble and McDonald, 1973; Leuck et al., 1968).

Like DDT, the degradation rate of chlorpyrifos in water also follows pseudo first-order kinetics and depends on temperature, light intensity, and chemical composition of the water. The reported half-life of chlorpyrifos associated with hydrolysis in relatively pure waters (pH 5-9) ranges from 10 to 100 days at temperature between 15°C and 35°C. In natural waters, the apparent half-life of chlorpyrifos is however, less than 10 days (Schaefer and Dupras, 1970).

Light especially at wavelengths below 40 nm is a very crucial factor contributing for the chemical transformation of pesticides within the environment (Crosby, 1969; Marshell and Roberts, 1977; Zepp et al., 1975). Metal ions and other chemicals may significantly promote the rate of hydrolysis of chlorpyrifos in natural waters. It has been observed that the divalent iron, cadmium, lead and copper ions and 1% peptone solutions increase the rate of disappearance of chlorpyrifos from water (Brown, 1975). Catalytic studies of Copper on the hydrolysis of chlorpyrifos in aqueous solutions indicate that in phosphate buffer containing 0.6-0.85 mg/l copper as copper sulphate, chlorpyrifos disappeared 19 times more rapidly than it did in the absence of copper (Meikle, 1972). Copper salts occur in natural waters only in trace amounts up to about 50 µg/ml; cupric ions introduced into natural waters at pH 7 or above will quickly precipitate as the hydroxide or as basic copper carbonate (McKee and Wolf, 1963).

The hydrolysis of chlorpyrifos follows the typical pH dependance pattern
reported for other organophosphorus pesticides. That is, the half-life reaches a maximum around pH 6.0 and declines more rapidly with increasing pH (Eto, 1974). This dependence is most clearly demonstrated in 50% (v/v) methanol-water solutions held in the dark at 25°C in which the half-lives at pH 5, 6 and 9 are reported to be 1080, 1930, and 29 days, respectively (Brast, 1966) and in phosphate-buffered distilled water (25°C, dark) where the half-lives at pH 4.7, 6.9 and 8.1 are reported to be 63, 35 and 23 days respectively (Meikle, 1972). This indicates that the half-life of chlorpyrifos in aqueous solutions is greatly influenced by pH. It is also influenced by presence of metal ions, temperature and perhaps light intensity.

The degradation pathway taken by chlorpyrifos seems to depend on the conditions and the ecosystem concerned. Hydrolysis of chlorpyrifos to 3,5,6-trichloro-2-pyridinol is the most important degradation reaction occurring in most compartments of aquatic ecosystems (Schaefer and Dupras, 1970). When chlorpyrifos is exposed to ultra-violet light, it undergoes transformation in the presence of water, with liberation of 3,5,6-trichloro-2-pyridinol (Smith, 1968).

Fig 1.3: The metabolism of chlorpyrifos in Gold fish (Smith, 1966).

1.1 ----- α,α-diethyl α-3,5,6-trichloro-2-pyridyl phosphorothioate (chlorpyrifos)
1.2 ----- Ethyl α-3,5,6-trichloro-2-pyridyl phosphate
1.3 3,5,6-trichloro-2-pyridyl phosphate
1.4 3,5,6-trichloro-2-pyridinol

Chlorpyrifos spiked to a model ecosystem comprising water, fish, plants and soil has been found to kill the fish. The majority of tissues of fish are found to contain parent compound, chlorpyrifos, although 3,5,6-trichloro-α-2-pyridyl phosphate, 3,5,6-trichloro-2-pyridinol and ethyl α-3,5,6-trichloro-2-pyridyl phosphate were also detected (Smith et al., 1966). In another study, mosquitofish (Gambusia affinis i.e Girard) exposed to 14C-chlorpyrifos for three days in a model ecosystem were found to contain accumulated chlorpyrifos and 3,5,6-trichloro-2-pyridinol (Metcalf, 1974).

Studies carried out in vivo on absorption, distribution and elimination on chlorpyrifos in both laboratory (rat and monkey) and domesticated animals (cattle, swine and sheep) revealed that chlorpyrifos is rapidly hydrolyzed to 3,5,6-trichloro-2-pyridinol, primarily in the liver and kidney. This metabolite is eliminated mainly through the urine, to a lesser extent through the faeces, and to a minor extent via milk of lactating mammals (Dishburger et al., 1972a, c; Mckeller et al., 1972c; Johnson et al., 1974). Thus in fish as in mammals and birds, detoxification of chlorpyrifos appears to proceed by hydrolysis to 3,5,6-trichloro-2-pyridinol.

Chlorpyrifos is rapidly metabolized in rats and excreted mainly in the urine (90%). The degradation route appears to be mainly via dealkylation to give largely trichlorpyridyl phosphate (Smith et al., 1967).

1.4 Dissipation and volatilization of pesticides.
1.4.1 Dissipation and volatilization of DDT.

It has been shown that DDT strongly adsorbs to particulate materials, including plankton in the water column (Cox and Bull, 1970). The subsequent settling of these materials out of the water column can constitute an important loss process. Settling of particulate from the water column was found to be
the major loss process of DDT for both lakes (Michigan and Superior in the U.S.A). Particulate settling was responsible for 61–95% of the observed DDT loss rate in lakes Michigan and Superior. Degradation and hydraulic washout were responsible for 3–35% and 2–4% respectively (Victor and Wayland, 1982).

Volatileization rate of chemicals from a completely mixed water body has been reported to be proportional to the vapour pressure and inversely proportional to the molecular weight and water solubility of the chemical and to the depth of the water body (Mackay and Wolk, 1973). Therefore, the rate of evaporation can be high even for compounds of low vapour pressure if their molecular weights are small and/or their water solubilities are low. The half-lives of DDT and \((1R,4S,4a,4aS,5S,8R,8RaR)-1,2,3,4,10,10\)-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4,5,8-dimethanonaphthalene (aldrin) in water of one metre depth at 25°C are calculated to be 3.7 and 10.1 days, respectively. Although the rate can be modified by diffusion or adsorption under environmental conditions, transfer of chemicals from the water to the atmosphere may occur much faster than has been generally recognized (Mackay and Wolk, 1973).

1.4.2 Dissipation and volatilization of chlorpyrifos.

The tendency for chlorpyrifos to evaporate from water can be compared to that of other pesticides by calculating their air-to-water partitioning coefficients which may be considered as estimates of Henry’s law constants. For chlorpyrifos, this coefficient is \(8.9 \times 10^{-4}\) at 25°C. Compounds having air-to-water partitioning coefficients between \(10^{-3}\) and \(10^{-5}\) are classified as slightly volatile from the water (Environmental Protection Agency, 1975a). Assuming the solubility of chlorpyrifos in water to be 0.4 mg/l, evaporation of chlorpyrifos from a small pond could theoretically account for as much as 11% of the total losses (Blau and Neely, 1975).

Volatilization of chlorpyrifos might be a significant mechanism contributing to its dissipation in the environment. About 80% of the labelled
chlorpyrifos applied as an acetone solution was lost from Whatman number 1 filter paper in three days, but when it was applied in a formulation containing surfacants, 52% still remained after 12 days (Smith, 1966). Treatment of the primary leaves of beans and corns with 1 mg of $^{14}$C-ring labelled chlorpyrifos resulted in a loss of 75-80% of the total radioactivity within 24 to 72 hours (Smith et al., 1967a). These losses were attributed to evaporation. Assuming a leaf surface area of 10 cm$^2$ and no degradative losses, this corresponds to an evaporation rate of about $3 \times 10^{-5}$ kg/m$^2$ per day.

1.5 Toxicity and ecological effects of DDT.

1.5.1 Bioaccumulation of pesticides.

A pesticide distributes itself among various components of an aquatic ecosystem, such as water, sediments, plants, and biota (Edwards, 1977). The movement of the chemical residues from one component to another, especially selective partitioning into living organisms is of toxicological, ecological and economic importance. The pesticide is biomagnified in the food chain hence can affect predators (Hutson and Roberts, 1990).

Pesticides can find their way into fish through their gills during the respiration process as well as orally when feeding. A dynamic equilibrium is reached between the rate at which a chemical enters (uptake) fish and that at which it is eliminated. Loss of parent compound from fish, may occur by excretion of the untransformed pesticide or by metabolism. The extent of pesticide accumulation in an organism at a given time is determined by the combined result of the competing processes of uptake, metabolism and excretion. The movement of chemicals across biological membranes depends on their lipid-solubility and water solubility, degree of ionization, chemical stability and molecular shape or size of the compound (Esser and Moser, 1982). The rate of movement is also influenced by the metabolic activity of the animal and indirectly, by factors affecting this activity, such as water temperature, pH, salinity, hardness, physiological state of the fish and the presence of
The accumulation of pesticides in fish can occur, therefore, through direct partitioning of the chemical between the fish and the water (bioconcentrations) and by uptake from food (biomagnification). Bioconcentration refers to the preferential uptake of the pesticide by the fish from its immediate environment (water), whereas biomagnification or food-chain accumulation describes uptake from food and the selective transfer of the chemical from organisms of lower trophic levels to those at higher trophic levels (predators) (Hutson and Roberts, 1990). Bioaccumulation can be conveniently used to describe the uptake of pollutants through water and food. Whereas food is usually the major source of pollutants for terrestrial species (Moriarty and Walker, 1987), in fish bioconcentration processes are thought to predominate (Bruggeman et al., 1981; Ellgehausen et al., 1980). The relative importance of bioconcentration versus biomagnification is difficult to establish, being dependent on the conditions of exposure duration, dose level and the individual fish involved (Moriarty and Walker, 1987). Bioaccumulation in an organism can be assessed from the bioconcentration factor (BCF). The BCF can be defined as the ratio of the concentration of the chemical in the organism to that in the water under steady state conditions (Huckle and Millburn, 1990). Steady state condition means when uptake and elimination are equal.

1.5.2 Bioaccumulation of DDT.

There can be little doubt that the wide spread use of DDT, as exemplified in some agricultural and forestry practices, has resulted in the reduction and contamination of fishery products, and progressive and alarming decrease in the populations of certain predatory birds. This unhappy side effects are due to the remarkable capability of living organisms to concentrate organochlorine insecticides from the physical environment. The problem is aggravated by the conversion of DDT to DDE which is an even more stable
pollutant, although less toxic (WHO, 1971).

Eastern oysters residing in flowing water containing 0.1 μg/g of DDT for 40 days concentrated DDT some 70,000 times the level in the water (Butler, 1964). Oysters exposed for ten days to a mixture of eight pesticides in water, at concentrations ranging from 0.001 to 0.05 μg/g, absorbed and accumulated the pesticide in their bodies; DDT, for instance, was concentrated 15,000 times (Wilson, 1965). Kenyan-North-coast (Gazi) oysters, have been found to concentrate DDT from water containing 1.71 ng/g of DDT some 19,293 times within a 24 hour period (Mbuvi, 1997).

DDT content in sea water at 0.1 μg/g has been reported to stop the growth of eastern oysters, and dosages as low as 0.0001 μg/g significantly reduced oyster growth (Butler, 1966b). Eastern oysters containing about 151 μg/g of DDT required approximately three months in clean water to lose 95% of their DDT load. Their growth returned to normal after only 10 days of flushing with clean water. Several other mollusc species lost about 75% of accumulated DDT after 15 days of flushing in clean water (Butler, 1966a). DDT and other organochlorine insecticides affect the nervous system, resulting in uncoordinated movements, sluggishness alternating with hyperexcitability, and difficulty in respiration (Holden, 1965). The action of DDT on fish as on insects, has a negative temperature coefficient, being more toxic to rainbow trout and bluegill sunfish at 13°C than at 19 or 23°C (Cope, 1965). The first conspicuous sign of DDT poisoning in brook trout and the Atlantic salmon is shown by the fish coming to the surface, sometimes on their side and showing occasional convulsive darting movements; this was observed in the Miramichi River (U.S.A) after the application of DDT to the New Brunswick Forest (Kerswill and Edwards, 1967).

Laboratory experiments have shown that some species of fish are extremely sensitive to DDT. For instance, the extrapolated LD₅₀ dosage for young chinook and coho salmon was 0.0275 and 64 μg/g per day, respectively. The young chinook salmon appeared to be 2 to 3 times more sensitive to DDT
than were coho salmon (Buhler et al., 1969). A spray calculated to give a DDT content of 0.09 µg/g in water was used to treat a stream (Burden, 1956). Eight miles downstream from the treated area, hundreds of fish were reported dying, and the concentration of DDT at a point 10 miles downstream was 0.017 µg/g. "Two specimens of fish examined were found to have definitely died of DDT poisoning (Burden, 1956).

1.5.3 Tolerance of DDT.

A population of the mosquito fish Gambusia affinis at Sidon, Mississippi was found in 1963 to have developed a fourfold tolerance to DDT (Vinson et al., 1963). This resistance to the pesticide can be dangerous to the predators. By 1972, populations of Gambusia affinis on the lower Brazos River of Texas, where cottonfields had been heavily treated with o,o-dimethyl o-4-nitrophenyl phosphorothioate (methyl parathion) as well as toxaphene (a reaction mixture of chlorinated camphenes containing 67-69% chlorines), showed twelvefold resistance to DDT as compared to tenfold and fivefold resistance to toxaphene and aldrin, respectively (Dzuik and Plapp, 1973).

1.5.4 Behavioral effects of DDT.

Sublethal concentrations of DDT may have behavioral changes on fish. In sublethal concentrations of DDT, pre-exposure to 20 ng/g for 24 hour is enough to make brackish-water Gambusia prefer a level of salinity twice as high as the normal referendum (Hansen, 1972). Fish sublethally poisoned have been observed to prefer warmer water, and fingerling salmon exposed to 5 ng/g for only 24 hours violently avoid entering cooler water where the temperature is less than 5°C (Ogilvei and Anderson, 1965). Exposure of goldfish to 10 ng/g for 4 days permanently abolished a locomotory pattern of turning behavior which involved a memory process (Davy et al., 1972).
1.5.5 Physiological effects of DDT.

Sublethal concentrations may cause physiological changes. Another effect of DDT is to impair osmoregulation, which in marine fishes is achieved by the adsorption of water and ions through the intestinal wall, followed by excretion of the ions through the gills. The addition of 50 \( \mu g/g \) to an intestinal preparation from the eel *Anguilla rostrata* inhibited its absorption of water by about 50% (Janicki and Kinter, 1971). It also inhibited the Na-K-Atpase activity of the intestinal mucosa, responsible for the active transport, by 60% in the eel and by 38% in preparations from two species of flounders (Janicki and Kinter, 1970). Nevertheless, young brook trout raised on a DDT-contaminated diet put on 15% more weight than normal (Macek, 1968).

1.6 Toxicity and ecological effects of chlorpyrifos.

1.6.1 Uptake and toxicity of chlorpyrifos.

It has been known for sometime that low levels of toxic contaminants occur in technical-grade organophosphorus insecticides (Gysin and Margot, 1958). They are formed either as by-products during the manufacturing process or through degradation of technical products, especially when they are stored at elevated temperatures (Milles *et al.*, 1979). The similarity of substitution at the phosphorus moiety and the use of common intermediates in the manufacture of organophosphorus insecticides result in the formation of several toxic contaminants. Their presence would be expected to change the properties of the insecticides and may enhance the hazard associated with handling, its use and toxicity (Baker *et al.*, 1978; Diggory *et al.*, 1977).

Even the compounds more toxic to fish can be used if applied several days prior to fish stocking. When *Gambusia affinis* were added to water 48 hour post treatment with chlorpyrifos at 0.5 pounds per acre, cumulative toxicity reached a maximum of 56% on the second day (Darwazeh and Mulla, 1974). While if fish are put in the water immediately after treating the water with 0.5 pound per acre with chlorpyrifos, there is 100% mortality (Mulla and
Isaak, 1961).

Chlorpyrifos is more toxic than o,o-dimethyl o-4-methylthio-m-oyl phosphorothioate (fenthion), causing 85% mortality at 1 µg/g whereas fenthion kills no Gambusia, but is usually used at one-half that dosage, at which it kills none. Applied to control ricefield mosquitoes at 0.0125 pounds per acre every 6 weeks, chlorpyrifos killed no Gambusia but killed some 35% of the bluegills exposed. On salt marshes a single application of chlorpyrifos at 0.025 pounds per acre was harmless but doubling this level caused a moderate and transitory decrease in the numbers of fundulus and cyprinodon topminnows (Ludwig et al., 1968).

Some symptoms of chlorpyrifos intoxication in fish have been reported soon after treatment: intoxicated bluegills exhibited poor coordination and loss of equilibrium, many sank to the bottom, others sought shelter in away shorelines and while some tried to hide in between aquatic plants (Byrd and Oberheu, 1966; Miller, 1966b).

1.6.2 Tolerance of chlorpyrifos.

Studies have shown that Gambusia affinis is fairly tolerant to insecticides used as larvicides and is therefore compatible with larvicides in an integrated mosquito control program (Darwazeh and Mulla, 1974; Ferguson et al., 1966). It has been suggested that tolerance varies inversely with the size of the fish. Most of the large mouth bass and bluegills affected were 2.5-10.0 cm long. Small fishes (<2.5 cm) appear to be more tolerant (Miller, 1966b).

1.6.3 Neurotoxic effects of chlorpyrifos.

Over 80% of the brain acetycholinesterase activities of bluegills and largemouth bass taken from ponds treated with chlorpyrifos at $5.6 \times 10^{-5}$ Kg/m$^2$ were inhibited and did not return to normal (control fish) levels until 28 days after the pond treatment with the insecticide. Acetycholinesterase inhibition in both species of fish from the ponds dosed at the lower treatment rate
returned to normal levels 28 days after having reached maximum inhibition levels of 20 to 60% compared to control fish (Macek et al., 1972).

Crustaceans, in general, appear to be directly and often severely affected by chlorpyrifos at dosages of $(2.8-5.6) \times 10^{-6}$ Kg/m$. Shrimps and crabs are particularly vulnerable in saltwater mashes (Wall and Marganian, 1973; Miller, 1966a); Cladocerans and copepodes generally incurred heavy losses soon after treatment of freshwater systems (Hurlbert et al., 1970, 1972; Nelson and Evans, 1973). Oligochaetes are reported not to be affected in several studies (Macek et al., 1972; Nelson and Evans, 1973). Rotifers do not appear to be directly intoxicated by chlorpyrifos (Hurlbert et al., 1972; Nelson and Evans, 1973) but certain genera (like Philoditie, Filinia, Brachionus) may undergo dramatic population increases soon after treatment. It has also been noted that the flagellates Eudorina and Pleodorina and to a lesser extent Volvox, were adversely affected by chlorpyrifos.

The anticholinesterase activities of chlorpyrifos and several of its metabolites were subjected to in vitro tests with housefly brain cholinesterase. Chlorpyrifos caused little or no inhibition whereas chlorpyrifos oxen was a potent cholinesterase inhibitor. Of the other metabolites of chlorpyrifos tested, none exhibited significant anticholinesterase activities (Smith et al., 1966). Similar in vitro results were obtained when carried out with termite cholinesterase. Chlorpyrifos, 3-dichlorpyrifos, 5-dechlorpyrifos, and 6-dechlorpyrifos were inactive whereas the negative logarithm of the concentrations of the enzyme for chlorpyrifos oxen was reported to be 6.9. In contrast to the results of these in vitro tests, chlorpyrifos and these dechlorinated derivatives of chlorpyrifos were shown to be potent cholinesterase inhibitors in vivo (Hutacherern and Kwoles, 1974).

Birds kept in pens on soil treated with four to eight pounds chlorpyrifos per acre appear to retain chlorpyrifos in the fat and the skin more than in any other tissue analyzed. Residue levels did not exceed 0.16 $\mu$g/g in any tissue after one week of treatment and decreased thereafter even
though the birds remained on the treated soil (Kenaga, 1974a).

1.7 Persistence of DDT.

Organochlorines generally belong to the list of the persistent chemicals in the environment (Edwards, 1973; White, 1974). The use of insecticides such as DDT is beneficial but one important disadvantage is their dispersal and persistence in the environment (Xiang, 1981).

The use of DDT, which is one of the organochlorines, has been banned or greatly restricted in many countries because of its persistence under temperate climates (Giddings and Monroe, 1970). The restrictions on the use of DDT have been in place in the developed world for more than thirty years now. But the concentrations from historical and current uses are still sufficiently high to pose problems to sensitive species (Fleming et al., 1983). Because of its persistence, it biomagnifies along food chains and bioconcentration factors from water predators, such as *dolphins*, can be alarming (Tanabe et al., 1984). However most chemicals are not biomagnified because of their short biological half-lives. Typical persistent orgonochlorines such as DDT and PCBs (polychlorinated biphenyls) have long half-lives (>200 days) and have been noted to be having high biomagnification and large concentration factors (Hutson and Roberts, 1990).

The biological half-lives of pesticides in fish (mainly trout, catfish, sunfish, minnow and goldfish) may vary due to fish species, body weight and water temperature. Half-life for *p*,*p*'-DDT varies from 31 to over 409 days, while that of DDE is 336 days (Niimi, 1987).

DDT residues have been detected in soil, water, air, food, wildlife and man after long periods. Detectable levels were found in soil even 50 years after application (White, 1974; De Cock and Rand, 1984). However, DDT and other organochlorine pesticides are still extensively used in developing countries including Kenya, though mainly for the control of disease vectors. Studies have shown that DDT does not persist for long periods under certain
tropical and subtropical conditions (El-Zorgani, 1976; Perfect, 1980; Wandiga and Natwaluma, 1984). Research carried out in both tropical and temperate climates indicate that organochlorines are more persistent in the latter than the former. This may be due to a relatively hotter and wetter climate which increases the volatilization rate in the tropics. Micro-organism load, soil type, moisture content, ultra-violet radiation are other factors which influence this persistence.

A number of studies on the persistence and degradation of DDT in Kenya have indicated a mean half-life of 122 days for the surface deposited and 124 days for the soil incorporated pesticide. The photodegradation of the aerated and de-aerated solutions of DDT in n-hexane in sealed quartz tubes were also investigated. The aerated former solutions gave a mean half-life value of 98 days, while the de-aerated solutions gave 74 days (Wandiga and Natwaluma, 1984). In Nigeria studies conducted indicated that the applied DDT was readily volatilized with only 2% of the total DDT applied accumulating over a 4 year period (Perfect, 1980). A half-life of 105 days for DDT was obtained in a study carried out in India (Pillai et al., 1988).

1.8 Persistence of chlorpyrifos.

Organophosphorus pesticides are generally less persistent in the environment than organochlorines. Toxaphene has a half-life in fish of up to 6 months. By contrast, the half-life of \( o,o\-\text{diethyl } o\-2\-\text{isopropyl-6-methyl pyrimidin-4-yl phosphorothioate} \) \{diazinon\}, \( S\-(3,4\-\text{dihydro-4-oxobenzo}[d][1,2,3]\text{triazin-3-ylmethyl} \) \( o,o\-\text{dimethyl phosphorodithioate} \) \{Azinphosmethyl\}, and chlorpyrifos is less than one week, while that of diethyl(dimethoxythiophosphorylthio)-succinate \{malathion\} is less than one day (Macek, 1970).

Studies carried out on small ponds and salt-marsh habitats in southern U.S.A indicated that the types of formulations as well as the type of aquatic system were critical factors determining the distribution and persistence of
chlorpyrifos in aquatic systems. There are two types of formulations, that is, the quick and slow release formulations.

Emulsifiable concentrate formulations (fast release formulation) are associated with losses of large amounts of chlorpyrifos during and after treatment applied to aquatic systems. This is probably due to degradation and volatilization processes occurring at the air-water interface as well as with losses that could occur from spray drift. As much as 40\% of the total quantity applied (15 g ai) had been lost from small shallow ponds in the first 4 hours after spraying (Hurlbert et al., 1970). A number of water and sediment systems were investigated. The apparent half-life of chlorpyrifos in the water during the first 14 days post treatment was estimated to range from 0.5 to 4 days in the various systems treated (Macek et al., 1972).

The persistence of chlorpyrifos in the water of aquatic systems treated with a number of different slow-release polymer formulations has been described by various investigators (e.g. Lawson et al., 1973; McDonald and Dickens, 1970; Miller et al., 1973a,b; Nelson and Evans, 1973; Nelson et al., 1973a,b; Roberts et al., 1973a,b). These formulations can produce chronic exposure patterns analogous to those associated with the more persistent organochlorine insecticides. Using polyethylene formulation pellet, equilibrium concentrations of 2.5 μg/l were attained within 3-4 weeks and were maintained throughout the remaining portion of the 18-month test period. During the test period the formulation released only 28.8\% of the original chlorpyrifos formulated (Roberts et al., 1973a,b). Slow-release granules and polymer formulation treatment is therefore characterized by an absence of large initial losses of insecticide and by low initial water concentrations which tend to disappear only very slowly. However granular formulations have been reported in Canada to be breaking up in 5 minutes post treatment of water (Fairbairn, 1977).

The initial sediment loads, on the other hand are much higher than those associated with emulsifiable concentrate formulations. Slow-release
formulations of chlorpyrifos indicate that 80% of the total applied quantity of a 2% (by weight) rapidly sink to the sediments. A 64.7 hectare artificial lake in California (U.S.A) with a mean depth of 3.8 meters had been treated by boat with $2.2 \times 10^{-5} \text{Kgm}^{-2}$. The rate of disappearance of the pesticide from the sediments appeared to follow zero-order kinetics. The highest concentration reported in water (0.9 µg/l) was observed in the bottom waters 1 day after treatment and reached a plateau near 0.2 µg/l after 3 weeks. The expected maximum concentration in water if all the insecticide had been released from the granule and mixed instantaneously is approximately 5.9 µg/g. A similar pattern was observed when only 26.3 hectares of this lake had been treated in an identical manner (Mulla et al., 1973).

Using similar polyethylene formulation but at one-tenth the dosage rate, the concentration of chlorpyrifos in the sediments of small woodland pools of similar dimensions ranged from non-detectable limits to 2 mg/Kg-mud throughout a 20-week test period (Nelson and Evans, 1973). These formulations are being developed specifically to provide long-term control of certain aquatic pests such as mosquitoes.

1.9 Objectives of the study.

The objectives of this project were to study:

1. the distribution of the pesticide residues in a model ecosystem comprising sediment, water, biota and simulating a tropical marine environment.
2. the rate of loss of the pesticide from biota into sea water.
3. the degradation, dissipation, and volatilization of the pesticides from treated sea water.
CHAPTER TWO.

Materials, Analytical Principles and Methods.

2.1 Chemicals and materials.

Labelled $^{14}$C-Chlorpyrifos of activity 10.482 mCi/mMol (95% pure by TLC), labelled $^{14}$C-$p,p'$-DDT of activity 12 mCi/mMol, were obtained from International Atomic Energy Agency (IAEA). Unlabelled $p,p'$-DDT ($p,p'$-DDT and 2.8% DDE), DDT metabolite standards, chlorpyrifos and its metabolite standards were supplied by Greyhound Chromatography and Allied Chemicals, United Kingdom. 2,5-Diphenyloxazole (PPO), 2,2-$p$-phenyl bis (4-methyl-5-phenyloxazole) (dimethyl POPOP), toluene, acetonitrile, anhydrous sodium sulphate and florisil were obtained from Fisher Chemicals, Fair Lawn, New Jersey, U.S.A. Triton X-100 was obtained from Rohm and Haas company, U.S.A. while mannitol was supplied by BDH Chemicals, Poole, England. Analytical reagents of analar grade; methanol, n-hexane, acetone and dichloromethane were obtained from Kobian Kenya Limited, Nairobi, Kenya. Carbon 14-cocktail was obtained from R.J Harvey Instrument Corporation.

2.2 Apparatus.

A model marine ecosystem tank was constructed with glass. It measured 68 cm by 36 cm by 36 cm and contained 9.5 Kg of sediment submerged in 52 liters of sea water sampled from the Kenyan-North-Coast (Gazi). A pump and airstones were used to provide forced aeration where necessary. The system was thermostated at 24°C.

Liquid scintillation measurements were made using Tri-Carb 1000 TR Liquid Scintillation Analyzer (Packard Canberra Company). Glass columns of internal diameter 1 cm and length 15 cm were used during the cleanup process. Gas Chromatography (GC) was carried out using a Perkin-Elmer Model 8500 Gas Chromatograph model equipped with a $^{63}$Ni-Electron Capture Detector (ECD).
2.3 Analytical Principles.

2.3.1 Biological Material Oxidizer.

The Biological Material Oxidizer in essence prepares radioactive residues in a sample for scintillation counting. A biological or organic material is combusted in a stream of oxygen gas at 900°C (IAEA, 1991) Fig. 2.1.

![Flow diagram of a biological oxidizer.](image)

The carbon and hydrogen contained in the organic matrix of sample are converted to carbon dioxide and water, respectively. The combustion products are then passed through a series of catalysts at 680°C and the carbon-14 dioxide and/or tritiated water are then trapped in an external solution. The trapped solution mixture is taken for liquid scintillation analysis.

2.3.2 Liquid scintillation counting.

Liquid Scintillation Counting (LSC) techniques have resulted in the effective application of radionuclides in biological and agricultural sciences because they have allowed much wider use of low-energy beta-particle emitters such as $^{14}$C and $^3$H to be made.

The sample to be counted is mixed with an organic scintillator (the detector material) and an organic solvent to make a solution or a suspension (uniformly distributed if it can not form a solution). The scintillation molecules surround the radioactive sample molecules, hence this reduces self-absorption.
while detection and counting efficiency do greatly increase.

The ionizing particles from the radioactive material cause excitation and ionization of the solvent molecules. These transfer their excitation energy to the scintillator molecules which in turn fluoresce (give rise to light photons) on their return to the ground state. The number of light photons emitted from the counting vial due to any ionizing particle is proportional to the energy lost by that particle in the solution. The number of light photons emitted is directly proportional to the number of beta particles emitted per second. The number of disintegrations per second can be used to determine the concentration of the sample molecules in the solution (IAEA/FAO, 1983). The counter can not differentiate between the parent pesticide molecules and those of the metabolites. This necessitates the application of other methods such as Gas Liquid Chromatography (GLC) to identify and quantify the parent pesticide and its metabolites.

2.3.3 Gas Chromatography.

Gas Chromatography (GC) has proved to be one of the most useful scientific inventions of recent times. It is based upon the partitioning of sample between a gas (mobile phase) and an immobile liquid phase (stationary phase). Gas chromatography was originated by James and Martin in 1951.

The liquid used as the stationary phase should have a high boiling point and should strongly be adsorbed onto some solid support packed in the chromatographic column. A tiny amount of sample, using a hypodermic syringe, is injected into the column and if in liquid form gets vapourized immediately. The sample analytes are adsorbed onto the immobile phase with different strengths. This enables the analytes to separate as the gas pushes them through the column. The mobile phase should be held with the least force compared to the analytes. The gas should be inert, that is, it should not react with the analytes and the detector system should not respond to it. The carrier gas normally used is nitrogen because it is inexpensive and easily
available in addition to being rather unreactive. Hydrogen is the best gas to use but expensive and poses the danger of catching fire easily. However, hydrogen is normally used in a jet where combustion of the analytes is needed depending on the detector system employed.

After the analyte has gone through the column depending on its retention time, it is passed over into the detector system. The detector response depends on the concentration of the analyte in the mobile phase. The read-out draws a peak for the analyte whereby the area of the peak is directly proportional to the concentration of the analyte in the mobile phase. Since different analytes have different retention times, standards are used to identify and quantify them.

GC is extensively used to monitor technical organophosphorus pesticides and their formulations for contaminants. However, it has limitations since some contaminants may undergo some thermal rearrangements or degradation on GC analysis (Milles et al., 1979).

2.3.3.1 Flame Photometric Detector.

Flame photometric Detector (FPD) is essentially a flame emission photometer (Natusch and Thorpe, 1973). The eluted species pass into a flame, usually hydrogen-enriched, low-temperature plasma, inside a shield jet which supplies sufficient energy first to produce atoms and simple molecular species and then excite them to a higher electronic state. The excited species subsequently return to their ground states with emission of characteristic atomic line or molecular band spectra which are measured by a photomultiplier tube. A narrow bandpass filter isolates the appropriate analytical wavelength range (Willard et al., 1986).

The most highly developed FPDs are selective for phosphorus and sulphur (Patterson et al., 1978). These elements are detected by monitoring band emissions from HPO at 526 nm and S₂ at 394 nm. Detector response to phosphorus compounds is linear, whereas that for compounds containing a
single atom of sulphur is proportional to the square of the compound concentration (Willard et al., 1986). FPD response to sulphur and phosphorus is about $10^4$ times that elicited from hydrocarbons. Sensitivities are at the subnanogram level. Discrimination between sulphur and phosphorus is less impressive due to differential response, which arises because the band spectra of HPO and $S_2$ effectively overlap within the bandpass of the two filters. Therefore sulphur-containing compounds may interfere in the detection of phosphorus compounds. It is necessary, therefore, to select a column which not only separate sulphur compounds, but also separates them from other sample components. This is not possible when S and P are present in the same compound (Willard et al., 1986).

The major field of application of the GC/FPD systems has been in the determination of pesticides and pesticide residues containing sulphur and phosphorus. For such compounds, the high sensitivity and selectivity of the FPD gives it superiority over flame ionization detector (FID) or electron capture detector (ECD) (Willard et al., 1986).

### 2.3.3.2 Electron Capture Detector.

The electron capture detector (ECD) (Burgett, 1974), "has two electrodes with the column effluent passing in between. One of the electrodes is treated with a radioisotope that emits high energy electrons as it decays. These emitted electrons produce copious amounts of low energy (thermal) secondary electrons in the GC carrier gas, all of which are collected by the other positively polarized electrode. Molecules that have affinity for thermal electrons capture electrons as they pass between the electrodes and reduce this steady-state current, thus providing an electrical reproduction of the GC peak. Radioisotope sources include tritium adsorbed in titanium or scandium, and nickel as a foil or plate on the interior of the cathode chamber. The maximum working temperature is 225°C for Ti – $^3$H and 325°C for Sc – $^3$H foils. $^{63}$Ni is a higher energy source and can be used up to 400°C (Willard et al.,
All ECDs respond to electrophillic species which give the detector its specificity. Halogenated compounds such as organochlorine pesticides give excellent response; detection is at the picogram level. The ECD responds exceptionally well to traces of oxygen, therefore leak-free systems and oxygen-free carrier gases are a necessity. The sample to be analyzed should also be free of water (Willard et al., 1986). A shortcoming of the ECD is its very narrow linear working range. Proper calibration and adjustment of the sample size remove this shortcoming.

2.4 Experimental set up.

2.4.1 The model ecosystem studies with DDT.

A solution of methanol and toluene was used to make a stock solution of 1000 µg/g of labelled $^{14}$C-DDT and unlabelled DDT. The ratio of the labelled and unlabelled DDT in the mixture was 1:1. The stock solution was used in the various treatments of the aquarium in the following experiments.

**Experiment 1**

The stock solution mixture of labelled $^{14}$C-DDT and unlabelled DDT calculated to give a concentration of 0.002 µg/g of the labelled $^{14}$C-DDT in the water was injected at 24 hour intervals for 168 hours to an aquarium containing water, sediment, 80 oysters attached to 2.5 Kg rack.

**Experiment 2**

The stock solution mixture of labelled $^{14}$C-DDT and unlabelled DDT calculated to give an initial concentration of 0.014 µg/g of the labelled $^{14}$C-DDT in water was injected to an aquarium containing water, sediment, 45 oysters, and 2 racks (5.5 Kg). The sea water was replaced with fresh sea water 24 hours after dosing. The water was replaced to enable the determination of the rate of loss of the pesticide to fresh sea water from biota and sediment.

**Experiment 3**

The stock solution mixture of labelled $^{14}$C-DDT and unlabelled DDT
Experiment 3

There was no biota. An injection of the stock solution mixture of labelled $^{14}$C-chlorpyrifos and unlabelled chlorpyrifos calculated to give an initial concentration of 0.048 μg/g in the water was made once to the aquarium containing sediment and water.

Experiment 4

The stock solution mixture of labelled $^{14}$C-chlorpyrifos and unlabelled chlorpyrifos calculated to give an initial concentration of 0.038 μg/g in the water was injected to the aquarium containing aerated water. A similar experiment with no aeration was set up. This was to determine how aeration affects the volatilization/dissipation processes of chlorpyrifos from the system.

2.5 Sampling.

The sea water used had been collected from Gazi (Kenyan-North Coast) and was stored in the dark for at least two weeks before using it in the aquarium. This was to let the algae to die. Algae could be toxic to the biota, more so in the event of not replacing the water regularly.

Sampling from the aquarium was carried out at specific times, before and after the periodical treatment of the water (i.e. 0h, 2h, 4h, 24h, 72h, 168h, 336h, 504h and 672h). The sampling schedule varied slightly depending on the objective and component concerned. For the objectives in which biota was being investigated, sampling was over by the end of the 168th hour. This was because of stress experienced by oysters and fish most probably due to non replacement of the treated water with fresh water after long hours. The sampling for fish was slightly different from the rest (blank, 0h, 2h, 4h, 8h, 24h, 48h, 72h, 120h and 168h). The sampling for fish was varied just incase they could die before the 168th hour, a sizeable number of samples would have been collected from the aquarium for analysis. The h here represent hours. Sampling was always done immediately after the indicated time and this was done in duplicates. Water samples were counted using a scintillation counter.
immediately after sampling (1 ml was used). One cylinder containing sediment was pulled out of the sediment each sampling time. Water was just scooped using a clean beaker after thorough stirring (care was taken to avoid disturbing sediment). One fish and three oysters were enough for biota sampling.

2.5.1 Pre-extraction treatment of samples.

Solid samples were ground to uniform texture using a mortar and a pestle. Organic tissues were first cut into tinny chips with a clean knife before grinding. The ground sample was weighed in duplicate and packed in a pre-extracted filter paper previously extracted with n-hexane before extraction of the solid samples.

2.5.2 Extraction of chlorpyrifos and DDT from samples.

a) Extracting solvents

Preliminary experiments showed that the efficiencies of extraction of DDT and chlorpyrifos from solid samples were highest with methanol and dichloromethane, respectively. Therefore the two solvents were used to extract the solid samples. For water samples, n-hexane and dichloromethane were the most appropriate solvents to extract DDT and chlorpyrifos samples respectively.

b) Extraction of chlorpyrifos and DDT from solid samples:

Sediment (10 g), oysters (0.4 g), rack (0.3 g) and fish (5 g)(wet weight basis), samples were extracted using Soxhlet apparatus and 120 ml of extracting solvent (methanol was used for DDT samples and dichloromethane for chlorpyrifos samples) for two and half hours. The extract was concentrated to 10 ml using a rotary evaporator. 1 ml out of the 10 ml was used for counting. The remaining 9 ml extract for DDT sample was kept for partitioning into n-hexane to await cleanup. The solid residue from the soxhlet extraction
process was kept for biological oxidation to determine the bound residues.

c) Partitioning of liquid samples:

DDT water sample (10 ml) was shaken in a separatory funnel with three 20 ml portions of n-hexane and the portions were pooled and concentrated together to 10 ml to await cleanup. Some 5 ml of water (distilled and deionized) was mixed with the 9 ml methanol DDT extract remaining from part (b) above and this mixture partitioned with three 20 ml portions of n-hexane. The portions were pooled and concentrated to 10 ml to await cleanup.

2.5.3 Sample cleanup.

A glass column was packed with pre-activated (at 180°C) anhydrous sodium sulphate (2 g) and florisil (60-100 mesh) (3 g). Glass wool was used to block the outlet of cleanup columns before packing them.

The DDT sample extract was then introduced quantitatively into the column and eluted with four 10 ml portions of solvent (hexane/acetone for DDT). The ratio of the four portions used in order were:

<table>
<thead>
<tr>
<th>n-hexane</th>
<th>acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>85</td>
<td>15</td>
</tr>
</tbody>
</table>

The cleaned sample was concentrated and evaporated to dryness to await GC analysis.

2.6 Analysis of samples.

2.6.1 Moisture and organic matter content determination.

Duplicate equal masses of sediment samples were weighed and heated to a constant weight for 24 hours in pre-weighed crucibles at a temperature of 105°C in an oven. Moisture content was determined to facilitate organic matter
content determination by further ashing at 450°C in a furnace. Ashing would convert the organic matter into CO₂ and H₂O. Any further loss in weight after moisture loss therefore would be attributed to organic matter content.

2.6.2 Liquid scintillation counting (LSC)

The liquid scintillation cocktail solution (6 ml) was mixed with 1 ml of liquid sample and the content, shaken thoroughly before counting. The cocktail solution used for counting depended on the polarity of the sample. The liquid scintillation cocktail for counting organic samples was prepared by dissolving 4 grams of 2,5-diphenyl oxazole (PPO) and 0.25 grams of 2,2-p-phenyl bis(4-methyl-5-phenol oxazol) (dimethyl POPOP) in 1 litre of toluene. The scintillation cocktail for counting water samples was prepared by dissolving 4 grams of PPO and 0.1 grams of dimethyl POPOP in one litre of 33% Triton X-100 in toluene. Counting of samples was done before cleaning to avoid loss of some of the active particles during the cleaning up process. The colouring in the extracted samples was minimal to cause significant quenching. The colour could be removed by using activated charcoal before counting but this was found to be reducing the counts even more. So counting was carried out without removing colour. However, the effects of colour were further reduced by diluting 1 ml of sample with 6 ml of cocktail before counting.

2.6.3 GC-Analysis of Organochlorine Samples.

A Perkin-Elmer 8500 Gas Chromatograph equipped with an SE - 54 capillary column measuring 30 m x 0.25 mm x 0.2 μm and a ⁶¹Ni - ECD detector was used to separate and detect the organochlorine residues. Analytical conditions were: carrier gas-Nitrogen at a flow rate of 2 ml/min, temperature settings for the capillary column was initially 260°C with isothermal time of 7 min, and a ramp rate of 2°C/min for 5 minutes to 270°C. Injector and detector temperatures were set at 320°C. 1 μl of sample in analar grade n-hexane was injected into the GC-column using a hypodermic syringe and simultaneously starting the run.
CHAPTER THREE.

Results and Discussion.

3.1 Organic matter content of sediment.

There was no observable decrease in weight of the dry sediment, therefore implying presence of negligible amounts of the organic matter present. Sediment obtained from a similar source (Gazi-Kenyan North Coast) had been analyzed using a pipette method (Mbuvi, 1997). The sediment analysed using the pipette method was found to contain 88.3% sand, 11.7% silt and 0% clay thus giving it a sandy texture (Mbuvi, 1997). Organic matter in sediment is normally bound to clay, probably as a clay-metal-organic complex (Stevenson, 1976). The 0% clay content noted by Mbuvi (1997) in the sediment suggests that there is nothing that organic matter can bind to in the sediment used in this work. This probably explains the negligible amount of organic matter content observed in the sediment.

3.2 Distribution of $^{14}$C-DDT and $^{14}$C-Chlorpyrifos residues in sediment, water, fish, oyster and rack holding oysters.

The concentrations in ng/g of $^{14}$C-DDT and $^{14}$C-Chlorpyrifos residues with time were determined and the concentration values obtained are represented in tables 3.12 to 3.19 in appendix {App.}III. The values in tables 3.12 to 3.19 are graphically represented in concentration-time graphs (Figs 3.1 to 3.8). Linear scale was used to plot the graphs where concentration values of pesticide residues were in close range while log scale was applied where concentration values of pesticide residues in different components had wide ranges.

The distribution of $^{14}$C-DDT residue concentrations with time in water, sediment and oysters is represented in Table 3.1 and App. III Table 3.12. The data collected in App. III Table 3.12 is graphically represented on Fig. 3.1. It is noted (Table 3.1 and Fig 3.1; App. III Table 3.12) that oysters and sediment are capable of accumulating most of the pesticide (DDT) from water within the
Table 3.1: Concentrations (ng/g) of \(^{14}\text{C}-\text{DDT}\) in oysters during a 24 hour exposure period.

<table>
<thead>
<tr>
<th>Time in hours</th>
<th>Water</th>
<th>Sediment</th>
<th>Oyster</th>
<th>Rack.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9.47</td>
<td>0.79</td>
<td>126.3</td>
<td>72.0</td>
</tr>
<tr>
<td>24</td>
<td>0.47</td>
<td>120.00</td>
<td>935.6</td>
<td>256.0</td>
</tr>
</tbody>
</table>

first 24 hours. Oysters are capable of bioconcentrating the DDT in water to very high values, up to 4800 times. It was also noted that the higher the concentration of DDT in water, the higher the BCF that could be obtained at any given time. It is noted (Fig 3.1; App. III Table 3.12) that with initial concentration of 1.18 ng/g in water, the BCF obtained after 24 hours was 823 while Table 3.1 suggests a BCF value of 1991 from an initial concentration of 9.47 ng/g in water, within the same period. The low BCF values obtained here could be due to the method of sampling. The muscle and shell of oyster were ground together to make the oyster sample. The BCF values for muscle alone could be much higher. Mbuvi (1997) noted that over 99% of the BCF for oysters is contributed by muscle alone while their shells account for less than 1%. The high BCF of muscle could be due to its high lipid content. The pesticide has been found to be highly lipophilic. The BCF value is also dependent on the initial concentration of the pesticide residues in water and the amounts of the components in the water environment.

The dependence of the BCF value on concentration is demonstrated by Table 3.1 and Fig 3.1; App. III Table 3.12 whereby the BCF values observed for oysters were 1991 and 823 within the first 24 hours when the initial concentrations were 9.47 ng/g and 1.18 ng/g in water respectively.
Fig 3.1: Variation of concentration of $^{14}$C-DDT with time in water, sediment and oysters (pesticide injected into water after every 24 hours).
Studies carried out by various researchers also indicate that BCF values of oysters are dependent on the initial concentration levels in water of pesticides and the time of exposure of the biota. A BCF value of 19,293 has been reported from an initial concentration of 1.71 ng/g of $^{14}$C-DDT in water within a 24 hour period (Mbuvi, 1997). Eastern oysters exposed to 10 ng/g of DDT in water for a week attained a BCF of 15,100 (Butler, 1969). Similarly, eastern oysters exposed to 100 ng/g of DDT in flowing water, were found 41 days after to have bioconcentrated DDT 70,000 times the concentration in water (Butler, 1964). Oysters exposed to a mixture of pesticides ranging between 1 ng/g to 50 ng/g in water were found to have bioconcentrated DDT by 15,000 times the level in water (Wilson, 1965). The high BCF values recorded could be due to oysters being capable of filtering large volumes of water through their gills. This is possible because of the oyster's extensive ciliary arrangements. A filtration rate of 0.5 to 0.8 litres per hour per gram of wet weight of oyster muscle has been reported (World Animal Science, 1991).

The BCF values for other organisms have also been reported. BCF values, of fish, molluscs and algae, based on extractable pesticide (DDT) have been observed to range from 4,000 to 26,000 depending on the species (Carvalho et al., 1992). Concentrations of organochlorine compounds have been noted to be higher in shellfish compared to sediments collected from the same area (Morrison et al., 1996). This variations in accumulation of organochlorines could be attributed to the high lipid content of fish.

It is noted (Fig 3.1; App. III Table 3.12) also that 64% of the initial $^{14}$C-DDT pesticide injected had been lost from water within the first 24 hours. The half-life of the pesticide in water was about 4 hours. Comparable results have been reported in a related study conducted at 27°C. The loss of DDT from water has been reported to be 97% at 27°C within 24 hours time. The half-life of DDT was 2.07 hours (Mbuvi, 1997). The difference in the half-lives reported by Mbuvi (1997) and that noted in this study could be due to the different temperatures used. The higher temperature of 27°C used by Mbuvi (1997)
unlike the 24°C used in this work could most probably increase the rate of loss of DDT from water through evaporation. This probably explains the shorter half-life of DDT obtained by Mbuvi (1997).

The distribution of \(^{14}\)C-DDT residue concentrations with time in water, sediment and oysters 24 hours after replacement of the treated water is given in App. III Table 3.13. The data in this table is graphically represented on Fig. 3.2. It is observed (Fig. 3.2; App. III Table 3.13) that the rate of loss of \(^{14}\)C-DDT residues from sediment was higher than that of oysters. Within the first 24 hours of replacing the contaminated sea water with fresh sea water, sediment had lost 78% of the \(^{14}\)C-DDT residues while oysters had lost only 14%. After 168 hours, sediment had lost 91% while oysters had increased the loss to 34%. Oysters seemed to be very good accumulators of pesticide residues but poor losers of the same. This was attributed to the high BCF values of oysters. Though the BCF value declined from 1991 within the first 4 hours to 177, it increased to 569 within the first 120 hours. The concentration of \(^{14}\)C-DDT residues in water reached a maximum of 4.4 ng/g within the first 4 hours but progressively decreased to 1.1 ng/ml in the 168 hours' period. The decline of pesticide from water was attributed to evaporation and partial accumulation by oysters (Fig. 3.2; App. III Table 3.13).
Fig 3.2: Variation of concentration of $^{14}$C-DDT residues with time in water, sediment and oysters 24 hours after replacement of contaminated water with fresh sea water.
It is noted (Fig. 3.3; App. III Table 3.14) that over 78% of the $^{14}$C-DDT spiked had been lost from water in the first 24 hours, while the concentration in sediment had increased by 333% within the same period. Within 672 hours, the % of the pesticide concentration in water had declined by 97% while that of sediment had increased by 618%. It was generally noted that the % changes within the first 24 hours were large but declined with time. The concentration of the pesticide residues in sediment reached a maximum after 504 hours and declined thereafter. The high rate of loss of DDT from water was attributed to evaporation and its low water solubility which could encourage settling down of the pesticide residues with sediment particulates.

The distribution of a pesticide between water and sediment is likely to be influenced by its water solubility and adsorption characteristics (Worthing, 1987; Sharom et al., 1980). Investigations carried out in Lakes; Michigan and Superior (U.S.A) indicated that particulate settling was responsible for 61–95% of the DDT loss rate in the lakes while degradation contributed 3–35% (Victor and Wayland, 1982).
Fig. 3.3: Variation of concentration of $^{14}$C-DDT with time in water and sediment. (Pesticide injected once into the aquarium water)
The distribution of $^{14}$C-chlorpyrifos residue concentrations with time in water and sediment is given in App. III Table 3.15. The data in the table is graphically represented on Fig. 3.4. It was found that $^{14}$C-chlorpyrifos is accumulated very rapidly by sediment. It was noted (Fig. 3.4; App. III Table 3.15) that 65% of the $^{14}$C-chlorpyrifos in water had been lost in the first 24 hours while the concentration of the pesticide in sediment had increased by 608% within the same period. The concentration had reached a peak in sediment within the first 24 hours but declined at the end of the 672$^{nd}$ hour to leave 18% of the accumulated pesticide. The rate of loss of the pesticide residues from water, after the first 24 hours, was small. This is in view of the fact that only 20.5% of the pesticide concentration in water was lost between 72$^{nd}$ hour and the 672$^{nd}$ hour unlike that lost between 0 hour and 72$^{nd}$ hour. The slight decrease observed was attributed to the loss of the pesticide residues from sediment into water to replace what had been lost. This conclusion was made because from the 72$^{nd}$ to the 672$^{nd}$ hour, 60% of the pesticide residues that had accumulated in sediment were lost.

In a study carried out to investigate the partitioning of chlorpyrifos and o,o-diethyl o-4-nitrophenyl phosphorothioate (parathion) between water and sediment phases, it was noted that two days after parathion's introduction, concentrations of chlorpyrifos and parathion in water were 15 and 66% respectively (Carvalho et al., 1992). The results obtained in this work compares well with that observed by Carvalho et al (1992). It is noted (Fig 3.3; App. III Table 3.14 and Fig 3.4; App. III Table 3.15) that the rate of accumulation or decline of a pesticide is dependent on the pesticide under investigation.

Chlorpyrifos, parathion and DDT have been noted to adsorb onto fine sediments, reaching an equilibrium state within a few hours. Owing to lower affinity for sorption to particulates, the fraction of chlorpyrifos and parathion remaining in the water phase at equilibrium, represented 40-60% of the initial. This implies that association of pesticide residues with sediment particles do
Fig 3.4: Variation of concentration of $^{14}$C-Chlorpyrifos with time in water and sediment. (Pesticide injected once into the aquarium water).
affect their fate and therefore persistence in aquatic environments (Carvalho et al., 1992).

The distribution of $^{14}$C-DDT residue concentrations with time in aerated and non-aerated water is given in App. III Table 3.16. The data in the table is graphically represented on Fig. 3.5. The rate of loss of the $^{14}$C-DDT residues from aerated water was higher than from non-aerated water as noted (Fig. 3.5; App. III Table 3.16). This probably could be due to volatilization. For the first 24 hours, 88% of the pesticide had been lost from the aerated water as opposed to 71% from non-aerated water within the same period. These percentages had increased to 97 and 85 respectively at the end of the 672$^{nd}$ hour. Half-lives of $^{14}$C-DDT in aerated and non-aerated waters were found to be 3 hours 20 minutes and 3 hours 45 minutes respectively. It is reported that volatilization is one of the key factors linked to the dissipation of DDT from the environment (Abdalla and El-Zorgani, 1988; Takeoka et al., 1991; Iwata et al., 1994). The half-lives of DDT and aldrin in water of one metre depth at 25°C are calculated to be 3.7 days and 10.1 days, respectively. Although the rate can be modified by diffusion or adsorption under environmental conditions, transfer of chemicals from the water to the atmosphere may occur much faster than has been generally recognized (Mackay and Wolk, 1973). It is therefore noted that the average half-life (3 hours 28 minutes) of DDT obtained in this study is comparable to the theoretical half-life value of 3.7 days for DDT in water at 25°C.
Fig 3.5: Variation of concentration of $^{14}$C-DDT with time in aerated water and non-aerated water. (Pesticide injected once into aquarium waters.)
The distribution of $^{14}$C-chlorpyrifos residue concentrations with time in aerated and non-aerated water is given in App. III Table 3.17. The values in the table is graphically represented on Fig. 3.6. It is noted (Fig 3.6; App. III Table 3.17) that the rate of loss of $^{14}$C-chlorpyrifos was higher in aerated than in non-aerated water. It was noted that 79% of the pesticide residues had been lost from the aerated water unlike 49% from the non-aerated water within the first 24 hours. These percentage losses had increased to over 99% and 95% respectively within the 672 hours period. This suggested half-lives of 13 hours 21 minutes and 24 hours 3 minutes for the aerated and non-aerated waters respectively. It seemed (Fig 3.5; App. III Table 3.16 and Fig 3.6; App. III Table 3.17) that at the end of the 672 hours, the percentage losses in both aerated and non-aerated waters for $^{14}$C-DDT were less than those for $^{14}$C-chlorpyrifos. This observation was attributed to the low solubility of $^{14}$C-DDT which could be enabling it to settle to the bottom of the container unlike $^{14}$C-chlorpyrifos which has a higher solubility. Probably, as more of the dissolved $^{14}$C-DDT evaporate, more of it is likely to get desorbed from the aquarium surfaces in contact with water to maintain the equilibrium between the adsorbed and the dissolved $^{14}$C-DDT.

Comparable rapid rates of disappearance of chlorpyrifos have been reported by Schaefer and Dupras (1969, 1970), though they had employed not only pasteurized but also distilled water. In a study carried out in darkness, 25°C and pH 7.7, using tapwater, half-life of chlorpyrifos was reported to be 1.5 days (Meikle and Youngson, 1971). This implies that the half-life could have been less than 1.5 days if light (radiation) conditions had been used in this work. Light conditions could probably introduce the photodecomposition effect and also increase volatilization. Evaporation has been reported to be a significant mode of loss of chlorpyrifos. Blau and Neely (1975) found that in a small pond of water, evaporation of chlorpyrifos could account for up to 11% of the total losses of the pesticide from water assuming its solubility to be 400 ng/g.
Variation of concentrations of $^{14}$C-Chlorpyrifos residues with time in aerated and non-aerated water. (Pesticide injected once to the aquarium waters).
3.3 Distribution of $^{14}$C-chlorpyrifos residues in sediment, water, oysters and fish.

The distribution of $^{14}$C-chlorpyrifos residue concentrations with time in water, sediment, oysters and fish (*Luthrinus fulviflama*, *Siganus stellatus* and *Lenthrinus harak*) is given in App. III Table 3.18. The values in the table are graphically represented on Fig. 3.7. It is noted (Fig 3.7; App. III Table 3.18) that chlorpyrifos had been slowly taken up by sediment, oysters and fish within the first 24 hours such that 65% of the pesticide was still remaining in the water after this duration. Within the first 24 hour period, the percentages of pesticide residues in sediment, oysters, *Luthrinus fulviflama*, and *Siganus stellatus* increased by 35%, 150%, 74% and 155% respectively. The increase of the pesticide residues in sediment, oysters and *Siganus stellatus* had reached 40%, 236% and 183% respectively after the 168 hour period.

The BCF values observed for oysters, *Luthrinus fulviflama* and *Siganus stellatus* after the first 24 hours were 183, 154 and 245 respectively. The BCF values for oysters *Luthrinus fulviflama* and *Siganus stellatus* had declined to 110, 103 and 143 respectively after the 168 hour period. Studies on the degradation of chlorpyrifos and parathion in untreated and formalin-treated sediments have shown that organophosphorous pesticides degrade more rapidly in both sediments. The accumulation of each pesticide by fish, molluscs and algae takes place rapidly, attaining stable values after 12 to 24 hour’s exposure. After a 120 hour exposure, BCF factors based on extractable pesticides from tissue and water ranged from 150 to 650 for chlorpyrifos and 62 to 340 for parathion depending on the concerned species of biota investigated (Carvalho et al., 1992). These BCF values are comparable to those obtained in this work. Sub-lethal concentration of o-5-chloro-1-isopropyl-1H-1,2,4-triazol-3-yl o,o-diethyl phosphorothioate {Isazofos} has been found to be accumulated rapidly (with fluctuations) within the first 8 hours and thereafter declining by 63% in the next 16 hours before rising slowly again by 2.1%. The concentration of isazofos in water declined by 15% (Mansingh and Robinson, 1997).
Fig 3.7: Variation of concentration of $^{14}$C-Chlorpyrifos with time in water, sediment, oysters, *Luthrinus fulviflama* and *Siganus stellatus* (pesticide injected into water after every 24 hours).
The distribution of $^{14}$C-chlorpyrifos residue concentrations with time in water, sediment, oysters and fish 24 hours after replacement of the treated water is given in App. III Table 3.19. The data in the table is graphically represented on Fig. 3.8. It is noted (Fig 3.8; App. III Table 3.19) that, within the first 24 hours of replacing the contaminated sea water with fresh sea water, sediment, oysters and *Siganus stellatus* and *Lethrinus harak* had lost 20%, 12%, 22% and 41% of the accumulated pesticide respectively while water had gained by 97%. After the first 72 hours, sediment, oysters *Siganus stellatus* and *Lethrinus harak* had lost 17%, 10%, 34% and 44% respectively while the water had gained by 96% of its initial concentration. After the seven day period, sediment, oysters, *Siganus stellatus* and *Lethrinus harak* had lost 31%, 26%, 50% and 64% respectively while the concentration in water had gone up by 62% of its initial value. Fish and oysters comparatively accumulate the chlorpyrifos faster than sediment (Fig 3.7; App. III Table 3.18). Fish lost the bioaccumulated pesticide at a higher rate than oysters (Fig 3.8; App. III Table 3.19). This implies that oysters are poor losers of the bioaccumulated pesticides as observed (Fig 3.2; App. III Table 3.13 and Fig 3.8; App. III Table 3.19). The half-life of chlorpyrifos in *Lethrinus harak* and *Siganus stellatus* was 4.2 days and 7 days respectively. Comparable results have been reported for other organophosphorous pesticides. Macek (1970) reported the half-lives of diazinon, azinphosmethyl and chlorpyrifos in fish of about one week, and for diethyl(dimethoxythiophosphorylthio) succinate {malathion} to be approximately one day. It was also observed (App. III Table 3.19) in the study that the half-life of chlorpyrifos in fish was dependent on the species of fish. The half-life of chlorpyrifos in *Lethrinus harak* was shorter than that noted in *Siganus stellatus*. The difference in the half-life of chlorpyrifos in the two fishes could be due to their difference in fat content, body weight and eating habits of the two species. It has been noted by Niimi (1987) that the biological half-lives of pesticides in fish may vary due to fish species, body weight and water temperature.
Variation of concentration of $^{14}$C-Chlorpyrifos with time in water, sediment, oysters, *Lethrinus harak* and *Siganus stellatus* (pesticide injected into water once and the treated water replaced after 24 hours with fresh sea water).
The BCF values observed (App. III Table 3.19) after the first 24 hours for oysters, *Lethrinus harak* and *Siganus stellatus* were 124, 283 and 151 respectively. The values had changed to 118, 248 and 120 respectively after the first 72 hours. Within 168 hours the BCF values had reached 128, 210 and 119 respectively. Of the three biota considered, the fishes were the best bioconcentraters with the *Lethrinus harak* leading (App. III Table 3.19). The oysters (muscle + shell combined) had small BCFs unlike fish probably due to the bulk of an oyster being composed of the shell. Considering muscle of oysters alone, BCF value of oysters could most probably be higher than even those of fish. It has been reported by Mbuvi (1997) that less than 1% of the BCF value of the oysters for the DDT pesticide is contributed by their shells yet the bulk of an oyster is the shell. This bulk factor of the oyster-shell could probably also influence the BCF of oysters for chlorpyrifos and this probably explains the small BCF values obtained for oysters in this experiment.

Information on the elimination of chlorpyrifos from sediment, oysters and fish is scarce though some information on the elimination and accumulation of other organophosphorous pesticide residues is available. Elimination of isazofos from red hybrid *tilapia* after a 24 hour exposure to a sub-lethal concentration has been studied. In the study, concentration in the fish declined by 42% in one hour of replacement of contaminated water with fresh water, 47% in four hours and kept on fluctuating afterwards. Residue concentration of isazofos increased by 38% in 24 hours time before declining sharply thereafter. Fluctuations of the concentrations of isazofos in water was observed later on (Mansingh and Robinson, 1997). However, elimination of another organophosphorous pesticide diazinon from red hybrid tilapia after a 24 hour exposure to a sub-lethal concentration was found to be steady-losing 44% of the pesticide residues after 24 hours and completely after the 72 hour experimental period (Mansingh and Robinson, 1997). The work indicated that it could most probably take more than 10 days for chlorpyrifos to be
dissipated completely from the species of fish considered. This implies that chlorpyrifos is more persistent compared to isazofos and diazinon.

3.4 Analytical results.

A Chlorinated Pesticide Mixture (CPM) containing \( p,p'\)-DDT, \( p,p'\)-DDE and \( p,p'\)-DDD in analar grade n-hexane was used as the standard. The individual metabolites in n-hexane were run separately to determine the retention time (Rt) of each in the CPM mixture. The retention times were found to be 8.29, 9.33 and 10.37 minutes for \( p,p'\)-DDD, \( p,p'\)-DDE and \( p,p'\)-DDT respectively. These retention times were used to identify the peaks of the metabolites in the chromatograms of the samples. All the samples were found to contain \( p,p'\)-DDE, \( p,p'\)-DDD and \( p,p'\)-DDT. The n-hexane to be used for GC-analysis was first injected into the GC-column to assess its potential of interfering with the analytes. The analar grade n-hexane gave a void peak outside the retention-time-area of interest. The void peak was clearly away from the peaks of the analytes hence could not interfere with them. The void peak could have been due to an impurity which was not identified in the solvent. The chromatogram of the solvent run alone is shown in App. II Fig 3.9. The chromatograms of the CPM and the various samples analysed are represented in Figures 3.9 to 5.3 in App. II. The peak areas of the corresponding standards and the sample peak areas were compared to determine the concentration of each metabolite in the sample under consideration. The peak areas were electronically calculated by the GC-instrument. The areas could also be obtained by multiplying the base of the peak with its corresponding half-height. The calculated concentrations are depicted in Tables 3.2 to 3.11. The tables of the GC-Analysis results give the concentration in ng/g, concentration ratio and % change of the \( p,p'\)-DDT and its metabolites at designated times. The percentage change in concentration is based on the preceding concentration value. The negative and positive % changes indicate decline and accumulation in relation to the preceding concentration values respectively. The concentration ratios
are to give a picture of what was happening to the concentration of each analyte in relation to the other analytes at the given time. This information may give leads to the major breakdown metabolite of DDT in each aquarium component.

The data collected for the distribution of \( p,p' \)-DDT and its degradation products in non-aerated and aerated waters is shown in Tables 3.2 and 3.3 respectively. It was observed (Table 3.2) that concentration of DDT and its metabolites generally declined with time. This was attributed to volatilization and adsorption (to the aquarium walls) as the most probable factors contributing to this loss. The proportions of the metabolites in water generally increased with time. This was an indication of the degradation process in non-aerated water.

### Table 3.2: The distribution of \( p,p' \)-DDT and its degradation products in non-aerated water.

<table>
<thead>
<tr>
<th></th>
<th>After 2 hours</th>
<th>After 168 hours</th>
<th>After 672 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>conc. (ng/g)</td>
<td>conc. (ng/g)</td>
<td>conc. (ng/g)</td>
</tr>
<tr>
<td>( p,p' )-DDT</td>
<td>28.750</td>
<td>22.11</td>
<td>6.759</td>
</tr>
<tr>
<td>( p,p' )-DDE</td>
<td>1.300</td>
<td>1.00</td>
<td>0.399</td>
</tr>
<tr>
<td>( p,p' )-DDD</td>
<td>3.021</td>
<td>2.32</td>
<td>3.016</td>
</tr>
</tbody>
</table>

There was decline in the concentrations of the DDT residues in the aerated water with time (Table 3.3). The proportion of DDE declined while that of DDD increased with time. According to the concentration ratios (Table 3.3), the proportion of DDT in the aquarium declined while the proportion of DDD increased with time. This was probably due to microbial action in the aerated water. Microbial action on DDT has been found to favour production of DDD as the major metabolite in water as reported by Johnsen (1976). The rate of
degradation in the aerated water (Table 3.3) was higher than that of the non-aerated water (Table 3.2) as implied by the concentration ratios and % changes. The non-aerated and aerated waters were observed to have dissipated 78% and 97% respectively of the \( p,p' \)-DDT from the 2\textsuperscript{nd} hour to 168\textsuperscript{th} hour period. The high rate of dissipation could be probably attributed to higher microbial population density and higher volatilization rate in the aerated compared to the non-aerated water environment. Torstensson, (1987) observed that degradation by microbial action is an important route for the removal of pesticides from aquatic systems; and that it occurs in the water phase at rates which are dependent on the bacterial population density.

### Table 3.3: The distribution of \( p,p' \)-DDT and its degradation products in aerated water.

<table>
<thead>
<tr>
<th></th>
<th>After 2 hours</th>
<th>After 168 hours</th>
<th>After 672 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>conc. (ng/g)</td>
<td>conc. (ng/g)</td>
<td>conc. (ng/g)</td>
</tr>
<tr>
<td>( p,p' )-DDT</td>
<td>24.551</td>
<td>19.82</td>
<td>11.255</td>
</tr>
<tr>
<td>( p,p' )-DDE</td>
<td>2.375</td>
<td>1.92</td>
<td>0.842</td>
</tr>
<tr>
<td>( p,p' )-DDD</td>
<td>1.239</td>
<td>1.00</td>
<td>0.831</td>
</tr>
</tbody>
</table>

The distribution of the \( p,p' \)-DDT and its degradation products in water, oysters and sediment contained in an aquarium into which \( p,p' \)-DDT was being injected into water after every 24 hours for 168 hours, is shown in Tables 3.4, 3.5 and 3.6 respectively. Decline in the concentrations of \( p,p' \)-DDT and \( p,p' \)-DDE with time was noted (Table 3.4). This could probably be due to accumulation by sediment and oysters. Volatilization and adsorption were other factors which may have contributed to this decline. The concentration of \( p,p' \)-DDD declined at first and then increased. The variations in the concentrations of the pesticide residues were attributed to probable shifting of equilibriums as
more of the parent pesticide was injected and the formation of the metabolites on the various components in the aquarium.

**Table 3.4:** The distribution of the \( p,p' \)-DDT and its degradation products in water in an aquarium containing water, oysters and sediment. The pesticide was injected after every 24 hours for 168 hours.

<table>
<thead>
<tr>
<th></th>
<th>After 2 hours</th>
<th>After 72 hours</th>
<th>After 168 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>conc. (ng/g)</td>
<td>conc. (ng/g)</td>
<td>conc. (ng/g)</td>
</tr>
<tr>
<td>( p,p' )-DDT</td>
<td>1.918</td>
<td>1.891</td>
<td>0.963</td>
</tr>
<tr>
<td>( p,p' )-DDE</td>
<td>0.429</td>
<td>0.380</td>
<td>0.361</td>
</tr>
<tr>
<td>( p,p' )-DDD</td>
<td>0.266</td>
<td>0.138</td>
<td>0.271</td>
</tr>
</tbody>
</table>

There was increase in oyster concentrations of \( p,p' \)-DDT and \( p,p' \)-DDE while that of \( p,p' \)-DDD generally declined (Table 3.5). The increase could be due to fresh injections of the \( p,p' \)-DDT which was containing 2.8% \( p,p' \)-DDE as an impurity. The high initial \( p,p' \)-DDT, \( p,p' \)-DDE and \( p,p' \)-DDD concentrations observed could be due to their high solubility in the adipose tissues of the oysters. The concentration of \( p,p' \)-DDD was very high compared to that of \( p,p' \)-DDE initially. This could be due to the microbial degradation of \( p,p' \)-DDT to \( p,p' \)-DDD in oysters. DDT has been reported to be highly lipophilic (WHO, 1979). The fluctuation of the concentration of DDE from 84.116 to 324.512 most probably could be due to the physiological and biochemical aspects of the oysters. Esser and Moser (1982), noted that the extent of pesticide accumulation in an organism at any given time is determined by the combined result of the competing processes of uptake, metabolism and excretion. They also noted that the movement of chemicals across biological membranes depends on their lipid-solubility and water solubility, degree of ionization, chemical stability and molecular shape and size of the compound. The rate of movement...
is also influenced by the metabolic activity of the animal and indirectly, by factors affecting this activity such as physiological state of the animal (Esser and Moser, 1982)

Table 3.5: Distribution of \( p,p'-\)DDT and its degradation products in oysters in an aquarium containing water, oysters and sediment. The pesticide was injected after every 24 hours for 168 hours.

<table>
<thead>
<tr>
<th></th>
<th>After 2 hours</th>
<th>After 72 hours</th>
<th>After 168 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>( p,p'-)DDT</td>
<td>106.308</td>
<td>407.290</td>
<td>629.621</td>
</tr>
<tr>
<td>( p,p'-)DDE</td>
<td>322.000</td>
<td>84.116</td>
<td>324.512</td>
</tr>
<tr>
<td>( p,p'-)DDD</td>
<td>931.144</td>
<td>350.188</td>
<td>128.889</td>
</tr>
</tbody>
</table>

There was general increase in the concentrations of DDT and its degradation products in the sediment with time (Table 3.6). This could be attributed to the adsorption of the metabolites onto the sediment particles. It has been reported by Stevenson, (1976) that adsorption of pesticides onto particulates and water solubility are crucial processes which affect the fate of pesticides in the aquatic environment. Since the solubility [0.001–0.006 mg/L at 25°C (WHO, 1979)] of DDT in water is low, much of it could settle down onto the sediment hence the increase in pesticide metabolite concentrations witnessed in the sediment in this work.
Table 3.6: Distribution of \( p,p'-\text{DDT} \) and its degradation products in sediment in an aquarium containing water, sediment and oysters. The pesticide was injected after every 24 hours for 168 hours.

<table>
<thead>
<tr>
<th></th>
<th>After 2 hours</th>
<th>After 72 hours</th>
<th>After 168 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>conc. (ng/g)</td>
<td>conc. (ng/g)</td>
<td>conc. (ng/g)</td>
</tr>
<tr>
<td></td>
<td>(ratio)</td>
<td>(ratio)</td>
<td>(ratio)</td>
</tr>
<tr>
<td>( p,p'-\text{DDT} )</td>
<td>7.737</td>
<td>35.961</td>
<td>50.418</td>
</tr>
<tr>
<td></td>
<td>2.58</td>
<td>13.20</td>
<td>2.82</td>
</tr>
<tr>
<td>( p,p'-\text{DDE} )</td>
<td>6.748</td>
<td>26.652</td>
<td>39.616</td>
</tr>
<tr>
<td></td>
<td>2.25</td>
<td>9.78</td>
<td>2.23</td>
</tr>
<tr>
<td>( p,p'-\text{DDD} )</td>
<td>3.003</td>
<td>2.725</td>
<td>17.865</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

The distribution of \( p,p'-\text{DDT} \) and its degradation products in water, oysters and sediment sampled from an aquarium in which the treated (contaminated) water had been replaced with fresh sea water 24 hours after treatment is represented in Tables 3.7, 3.8 and 3.9 respectively. The concentration of DDT increased earlier on then declined with time (Table 3.7). The initial increase was attributed to the loss of pesticide into water from oysters and sediment. The loss of pesticide from water experienced later on was attributed to volatilization. Decline in concentrations of DDE with time was observed. The concentration of DDD in water kept on building up (increasing) though the increase declined with time. The decline was attributed to the uptake by oysters and adsorption onto sediment and aquarium walls. Some pesticide residues could have also been lost through volatilization.
Table 3.7: The distribution of \( p,p'-\text{DDT} \) and its degradation products in water in an aquarium containing water, oysters and sediment, at various time intervals, 24 hours after replacing the contaminated water with fresh sea water.

<table>
<thead>
<tr>
<th></th>
<th>After 2 hours</th>
<th>After 72 hours</th>
<th>After 168 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>conc. (ng/g)</td>
<td>conc. (ng/g)</td>
<td>conc. (ng/g)</td>
</tr>
<tr>
<td>( p,p'-\text{DDT} )</td>
<td>3.541 4.95</td>
<td>4.464 12.40</td>
<td>1.326 8.90</td>
</tr>
<tr>
<td>( p,p'-\text{DDE} )</td>
<td>0.715 1.00</td>
<td>0.360 1.00</td>
<td>0.149 1.00</td>
</tr>
<tr>
<td>( p,p'-\text{DDD} )</td>
<td>0.736 1.03</td>
<td>1.660 4.61</td>
<td>1.107 7.43</td>
</tr>
</tbody>
</table>

The concentration of DDT declined in the oysters with time (Table 3.8). The decline of DDT was attributed to loss of pesticide to the fresh sea water. The concentrations of DDE went down and then up (slightly) with time. The initial decline of DDE was probably due to loss into water. The slight increase of DDE was attributed to probable shift of equilibrium from the water to the oysters. The concentration of DDD rose first then went down with time. The increase could be due to microbial degradation. The high % change (+79.52) in Table 3.8) for DDD confirms the high microbial degradation of DDT to DDD in the oysters.

Table 3.8: Distribution of \( p,p'-\text{DDT} \) and its degradation products in oysters in an aquarium containing water, oysters and sediment 24 hours after replacing the contaminated water with fresh sea water.

<table>
<thead>
<tr>
<th></th>
<th>After 2 hours</th>
<th>After 72 hours</th>
<th>After 168 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>conc. (ng/g)</td>
<td>conc. (ng/g)</td>
<td>conc. (ng/g)</td>
</tr>
<tr>
<td>( p,p'-\text{DDT} )</td>
<td>979.640 20.51</td>
<td>435.395 5.08</td>
<td>378.664 5.02</td>
</tr>
<tr>
<td>( p,p'-\text{DDE} )</td>
<td>239.782 5.02</td>
<td>110.181 1.28</td>
<td>148.400 1.97</td>
</tr>
<tr>
<td>( p,p'-\text{DDD} )</td>
<td>47.769 1.00</td>
<td>85.755 1.00</td>
<td>75.412 1.00</td>
</tr>
</tbody>
</table>
There was decline in DDT pesticide residues in the sediment though this reduced with time (Table 3.9). The pesticide residues lost from sediment were most probably taken up by water. This was due to the concentration of pesticide residues in water (Table 3.5) increasing though the concentrations declined later on. As the organic matter content of the sediment employed was very low, it is possible that adsorption of the metabolites onto sediment particles was poor or weak, and therefore this factor could favour desorption of the pesticide residues into fresh sea water. Stevenson, (1976) observed that desorption is one of the crucial processes which affect the fate of pesticides in the aquatic environment. Clay and organic matter are the soil components most often implicated in pesticide adsorption. Adsorption is easily predicted because the organic matter in most soils is bound to clay, probably through a clay-metal-organic complex system (Stevenson, 1976). This implies that desorption of pesticide residues from sediment was very favourable.

Table 3.9: Distribution of \( p,p' \text{-DDT} \) and its degradation products in sediment in an aquarium containing water, oysters and sediment 24 hours after replacing the contaminated water with fresh sea water.

<table>
<thead>
<tr>
<th></th>
<th>After 2 hours</th>
<th>After 72 hours</th>
<th>After 168 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>conc. (ng/g)</td>
<td>conc. (ng/g)</td>
<td>conc. (ng/g)</td>
</tr>
<tr>
<td>( p,p' \text{-DDT} )</td>
<td>976.667</td>
<td>215.433</td>
<td>191.974</td>
</tr>
<tr>
<td></td>
<td>(ratio) 16.90</td>
<td>(ratio) 7.12</td>
<td>(ratio) 8.63</td>
</tr>
<tr>
<td>( p,p' \text{-DDE} )</td>
<td>507.556</td>
<td>110.277</td>
<td>112.836</td>
</tr>
<tr>
<td></td>
<td>(8.78)</td>
<td>(3.64)</td>
<td>(5.07)</td>
</tr>
<tr>
<td>( p,p' \text{-DDD} )</td>
<td>57.794</td>
<td>30.257</td>
<td>22.254</td>
</tr>
<tr>
<td></td>
<td>(1.00)</td>
<td>(1.00)</td>
<td>(1.00)</td>
</tr>
<tr>
<td>% change cone.</td>
<td>-77.94</td>
<td>-78.24</td>
<td>-10.89</td>
</tr>
<tr>
<td>% change (ratio)</td>
<td>-47.65</td>
<td>-47.65</td>
<td>-26.45</td>
</tr>
</tbody>
</table>

Table 3.9: Distribution of \( p,p' \text{-DDT} \) and its degradation products in sediment in an aquarium containing water, oysters and sediment 24 hours after replacing the contaminated water with fresh sea water.

After 2 hours After 72 hours After 168 hours

<table>
<thead>
<tr>
<th></th>
<th>conc. (ng/g)</th>
<th>conc. (ng/g)</th>
<th>conc. (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( p,p' \text{-DDT} )</td>
<td>976.667</td>
<td>215.433</td>
<td>191.974</td>
</tr>
<tr>
<td></td>
<td>(ratio) 16.90</td>
<td>(ratio) 7.12</td>
<td>(ratio) 8.63</td>
</tr>
<tr>
<td>( p,p' \text{-DDE} )</td>
<td>507.556</td>
<td>110.277</td>
<td>112.836</td>
</tr>
<tr>
<td></td>
<td>(8.78)</td>
<td>(3.64)</td>
<td>(5.07)</td>
</tr>
<tr>
<td>( p,p' \text{-DDD} )</td>
<td>57.794</td>
<td>30.257</td>
<td>22.254</td>
</tr>
<tr>
<td></td>
<td>(1.00)</td>
<td>(1.00)</td>
<td>(1.00)</td>
</tr>
<tr>
<td>% change cone.</td>
<td>-77.94</td>
<td>-78.24</td>
<td>-10.89</td>
</tr>
<tr>
<td>% change (ratio)</td>
<td>-47.65</td>
<td>-47.65</td>
<td>-26.45</td>
</tr>
</tbody>
</table>

Tables 3.7 to 3.9, timing was started immediately after replacing the contaminated water.

The distribution of \( p,p' \text{-DDT} \) and its degradation products in water and sediment sampled from an aquarium in which \( p,p' \text{-DDT} \) had been injected into
the aquarium water is shown in Tables 3.10 and 3.11 respectively. The DDT concentration declined progressively (Table 3.10). The concentration of the DDE metabolite increased but reached a peak after 168 hours and then declined. The concentration of the DDD increased progressively but the increase declined with time. The rate of increase in water of DDE (% change was +434.70) was higher than that of DDD (% change was +182.43) as noted from Table 3.10. The high rate of increase of DDE could be due to favourable chemical/catalytic degradation of DDT to DDE in water. The loss of pesticide residues from water was attributed to adsorption onto sediment and aquarium walls and probably volatilization. The small increases of concentrations of DDT in water was probably due to desorption from sediment. This conclusion was made because the concentration in sediment of DDD declined from 20.788 ng/g (at the 168th hour) to 3.895 ng/g (at the 672nd hour) in reference to Table 3.11.

Table 3.10: The distribution of \( p,p' \)-DDT and its degradation products in water in an aquarium containing water and sediment only. The pesticide was injected once at the beginning and monitored for 672 hours.

<table>
<thead>
<tr>
<th></th>
<th>After 2 hours</th>
<th>After 168 hours</th>
<th>After 672 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>conc. (ng/g)</td>
<td>conc. (ratio)</td>
<td>conc. (ng/g)</td>
</tr>
<tr>
<td>( p,p' )-DDT</td>
<td>17.640</td>
<td>28.29</td>
<td>10.265</td>
</tr>
<tr>
<td>( p,p' )-DDE</td>
<td>0.893</td>
<td>1.43</td>
<td>4.777</td>
</tr>
<tr>
<td>( p,p' )-DDD</td>
<td>0.623</td>
<td>1.00</td>
<td>2.760</td>
</tr>
</tbody>
</table>

There was significant increase (% change values after 168 hours) in the concentrations of the DDT pesticide residues in the sediment in the first week followed by a decline (% change values after 672 hours) as noted from Table 3.11. This could be due to adsorption of the pesticide residues to the sediment.
because of their high concentrations in water initially. The concentrations of the pesticide residues declined later on probably due to desorption into water as the water lost the metabolites due to volatilization. The decline of DDT in sediment was attributed to favourable desorption into water. This was as a result of low organic matter and clay content in the sediment employed in the study.

The increase in the concentrations of DDD (% change of +477.45) and DDE (% change of +185.57) as observed from Table 3.11 implies generous bacterial (microbial) population density and significant chemical/catalytic degradation in the sediment respectively. The high percentage increase witnessed for DDT in sediment implied that the most probable major breakdown product of DDT in sediment is DDD. Investigations carried out on DDT in estuarine sediments by Albone et al, (1972) have shown that DDD is the major degradation metabolite. This explains the continuous build up of DDD in water probably because of desorption from sediments as it is formed. It has also been reported by Pfaender and Alexander, (1972) that DDT-degradation of up to 67% within 24 weeks in sediments do occur. The decline in DDE build up in sediment indicated limited chemical/catalytic degradation in sediment. Though adsorption of DDE from water onto sediments could not be discounted.

Table 3.11: Distribution of \( p,p' \)-DDT and its degradation products in sediment in an aquarium containing water and sediment only. The pesticide was injected once at the beginning and monitored for 672 hours.

<table>
<thead>
<tr>
<th></th>
<th>After 2 hours</th>
<th>After 168 hours</th>
<th>After 672 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>conc. (ng/g)</td>
<td>conc. (ratio)</td>
<td>conc. (ng/g)</td>
</tr>
<tr>
<td>( p,p' )-DDT</td>
<td>2.385</td>
<td>1.00</td>
<td>16.510</td>
</tr>
<tr>
<td>( p,p' )-DDE</td>
<td>3.546</td>
<td>1.49</td>
<td>10.126</td>
</tr>
<tr>
<td>( p,p' )-DDD</td>
<td>3.600</td>
<td>1.51</td>
<td>20.788</td>
</tr>
</tbody>
</table>
The degradation of DDT in water was generally found to be poor compared to sediments and oysters. The order of the rate of degradation was found to be sediment > oysters > water. This could be due to differences in the microbial population density and catalytic factors in the three. The rate of degradation of many pesticides in sediments has been reported to be higher than in water. The bacterial concentrations in estuarine sediments have been reported to be $10^2$-$10^4$ times higher than in the overlying water column (Carvalho et al., 1992). DDT incubated in fresh lake water samples has been reported to change to DDD significantly while sea water samples indicate poor metabolic activity with respect to DDT degradation (Johnsen, 1976).

Higher DDT pesticide residue concentrations were observed in the GC-analysis compared to the corresponding scintillation analysis. This was attributed to the injection of both the labelled and unlabelled DDT pesticide. The Scintillator detects only the labelled pesticide while the GC-system detects both the labelled and unlabelled molecules of DDT and its breakdown products. Even then, the concentrations obtained in GC-analysis were less than double the scintillation values. This could have been due to loss of pesticide residues during preparation and cleanup of samples prior to GC-analysis. It had been noted that during recovery studies for DDT, there is loss of some of the DDT pesticide residues during the process of concentrating and cleaning up of samples. Samples were counted after undergoing extraction and concentration (using the rotary evaporator) except samples of water.
Conclusions

1) Oysters were found to bioconcentrate DDT in water rapidly. A BCF of 4,800 was recorded. This value could have been even higher had oyster muscle alone been considered during sampling. This is because the bulk of an oyster is the shell. Higher BCF values for the same have been observed by other researchers before. A value of 19,293 has been reported by Mbuvi (1997). Butler, (1969) reported a BCF value of up to 15,100.

2) Sediment also do accumulate DDT from water fairly well and since the volume of sediment in seas is large, it can serve as a sizeable sink for the pesticide residues. This could be due to the low solubility of the pesticide in water which therefore settles down onto the sediment. Adsorption could also be playing significant roles in the accumulation of the pesticides by sediment.

3) The bioconcentration observed for chlorpyrifos was low compared to that of DDT. This could be due to their differences in lipophilic characteristics and their half-lives in the various components of the aquarium. Bruggeman et al (1981) and Macek et al (1970) observed that biomagnification is limited to compounds having long half-lives (a few months or more in fish) and large bioconcentration ratios (>5).

4) Chlorpyrifos had a half-life of less than 7 days in the two species of fish studied namely Lethrinus harak and Siganus stellatus. The half-life of chlorpyrifos was dependent on the species of fish. The difference in half-lives of the pesticide in fish was attributed to difference in fat content, body weight and eating habits of the two species of fish.

5) DDD was found to be a major DDT metabolite in sediment. This was attributed to high concentration of microbial population density in sediment. By contrast, DDE was the main breakdown product in water. This was
attributed to favourable chemical/catalytic degradation factors in aqueous environment.

6) The dissipation of both pesticides (chlorpyrifos and DDT) was higher in aerated water compared to non-aerated water. DDT was found to have half-lives of 3 hrs 20 min. and 3 hrs 45 min. in aerated and non-aerated waters respectively while chlorpyrifos had 13 hrs 20 min. and 24 hrs 3 min. respectively. The longer half-life of chlorpyrifos in water was attributed to its higher water solubility compared to DDT.
Recommendations.

1) DDT has been noted to have very short half-lives in the tropical region in contrast to the temperate region. In this study of the dissipation of DDT in water within the tropics, short half-lives of less than 4 hours were noted. More studies on DDT's half-life in biota and other different environments need to be carried out to ascertain whether the banning imposed worldwide on its use is justified or not.

2) Distribution and degradation of p,p'-DDT and chlorpyrifos should be followed up in a tropical marine containing water and sediment only but no aeration. The pesticide concerned could be injected at the beginning of the experimental set up. This could help to throw light on the degradation pattern of the pesticides in anaerobic environments.

3) Monitoring of the uptake, distribution, degradation and volatilization of p,p'-DDT in a tropical marine aquarium comprising oysters and sea water only need to be carried out. This could help to determine the degradation pathway taken by the pesticide in oysters.

4) Monitoring of the uptake, distribution, degradation and volatilization of p,p'-DDT in a tropical marine aquarium comprising one or two species of fish and sea water only need to be carried out. This could help to determine the degradation pathway taken by the pesticide in fish.

5) Monitoring of the uptake, distribution, degradation and volatilization of chlorpyrifos in a tropical marine aquarium comprising oysters and sea water only need to be carried out. This could help to determine the degradation pathway taken by the pesticide in oysters.
6) Monitoring of the uptake, distribution, degradation and volatilization of chlorpyrifos in a tropical marine aquarium comprising one or two species of fish and sea water only need to be carried out. This could help to determine the degradation pathway taken by the pesticide in fish.

The investigation on the recommendations (3) to (6) above could help to uncover the distribution and degradation patterns in the various components of the aquarium. This may eliminate the possible interferences from other components in the aquarium in which case it could be hard to pin-point with certainty where each metabolite or breakdown product is emanating from.

7) Though aquarium experiments do not give the exact situation in the field, they do give an approximate picture of the expectations in the field. Therefore aquarium and field studies on the fate of DDT, Chlorpyrifos and other pesticides whose usage is suspect, should be encouraged for comparison purposes in order to have a clear picture portrayed by both (laboratory and field experiments).

8) Due to breakdown of some instruments like the Biological oxidizer and GC-Flame photometric Detector, bound residues and GC-analysis of organophosphorus sample residues could not be determined. Future experiments could be directed towards bound-residue-determination of the pesticide injected and also determine the degradation pathways taken by chlorpyrifos in the various aquarium components.
REFERENCES.


Ludwig, P.D., Dishburger, H.J., Mcneill, J.C., Miller, W.D. and Rice, J.R.


McKeller, R.L., Wetters, J.H. and Dishburger, H.J. Residues of chlorpyrifos,


Nelson, L.L., Miller, T.A. and Young, W.W. Polymer formulations of mosquito larvicides. V. Effects of continuous low-level chlorpyrifos residues on
the development of *culex pipiens quinquefasciatus* Say populations in


Ogilvie, D.M. and Anderson, J.M: Effect of DDT on temperature selection by

Park, R.A. Transport and Behavior of pesticides and other trace organic
materials in Aquatic Environments; Center for Ecological Modelling,


Parlar, H. The role of photolysis in the fate of pesticides, in progress in
pesticide Biochemistry and Toxicology(eds. T.R. Roberts and D.H. Hutson),

Patterson, P.L., Howe, R.L. and Abu-Shumays, A. Dual-flame Photometric
Detector for Sulphur and Phosphorus Compounds with Gas

Perfect, J. Environmental Impact of DDT in a Tropical Agro-ecosystem,
*Ambio.* 9, 16-21.

Pfaender, F.K. and Alexander, M. Extensive microbial degradation of DDT in

Pierce, R.H., Jr.; Olney, C.E.; Felbeck, G.T. *G. Cosmochin. Acta.* 38, 1061-
1073, 1974.

Pillai, M.K.K., Samwel, T., Aggarwal, H.C. Persistence and binding of DDT
and gamma-HCH in a study of loam soil under field conditions in Delhi

Roberts, D.R., Roberts, L.W., Miller, T.A., Nelson, L.L. and Young, W.W. Polymer


Struble, D.I and MacDonald, S. Residue analysis of chlorpyrifos and its oxygen analog in field-treated wheat plants. J. Econ. Entomol. 66 (3), 1973


WHO. DDT and its derivatives. Environmental health criteria 9, 1979


Appendix I.

Common and (IUPAC) names of the pesticides quoted in the work

**Organochlorines.**

<table>
<thead>
<tr>
<th>Common name</th>
<th>IUPAC name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldrin</td>
<td>(1R,4S,4a,4aS,5S,8R,8RaR)-1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-1,4,5,8-dimethanonaphthalene.</td>
</tr>
<tr>
<td>Dicofol</td>
<td>2,2,2-Trichloro-1,1-bis(4-chlorophenyl)ethanol.</td>
</tr>
<tr>
<td>p,p'-DDA</td>
<td>2,2-Bis(4-chlorophenyl)ethanoic acid.</td>
</tr>
<tr>
<td>p,p'-DDD</td>
<td>1,1-Dichloro-2,2-bis(4-chlorophenyl)ethane.</td>
</tr>
<tr>
<td>p,p'-DDE</td>
<td>1,1-Dichloro-2,2-bis(4-chlorophenyl)ethylene.</td>
</tr>
<tr>
<td>p,p'-DDT</td>
<td>1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane.</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahyro-endo-1,4-exo-5,8 dimethanonaphthalene.</td>
</tr>
</tbody>
</table>

**Organophosphorus.**

<table>
<thead>
<tr>
<th>Common name</th>
<th>IUPAC name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azinphosmethyl</td>
<td>S-(3,4-dihydro-4-oxobenzo[d][1,2,3]triazin-3-ylmethyl) o,o-dimethyl phosphorodithioate.</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>o,o-Diethyl o-3,5,6-trichloro-2-pyridyl phosphorothioate.</td>
</tr>
<tr>
<td>Diazinon</td>
<td>o,o-Diethyl o-2-isopropyl-6-methyl pyrimidin-4-yl phosphorothioate.</td>
</tr>
<tr>
<td>Fenthion</td>
<td>o,o-Dimethyl o-4-methylthio-m-toyl phosphorothioate.</td>
</tr>
<tr>
<td>Isazofos</td>
<td>o-5-Chloro-1-isopropyl-1 H-1,2,4-triazol-3-yl o,o-diethyl phosphorothioate.</td>
</tr>
<tr>
<td>Malathion</td>
<td>Diethyl(dimethoxythiophosphorylthio)succinate.</td>
</tr>
<tr>
<td>Methyl parathion</td>
<td>o,o-Dimethyl o-4-nitrophenyl phosphorothioate.</td>
</tr>
<tr>
<td>Parathion</td>
<td>o,o-Diethyl o-4-nitrophenyl phosphorothioate.</td>
</tr>
<tr>
<td>Toxaphene</td>
<td>A reaction mixture of chlorinated camphenes containing 67-69% chlorines.</td>
</tr>
</tbody>
</table>
Appendix II: GC-Chromatograms

A sample of chromatograms of the solvent, CPM and samples analysed.

Fig. 3.9: Chromatogram of n-hexane used in GC.
Fig. 4.0: Chromatogram of chlorinated pesticide mixture (CPM).

<table>
<thead>
<tr>
<th>Conc. (ppb)</th>
<th>Rt</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP' - DDE</td>
<td>2</td>
</tr>
<tr>
<td>PP' - DDD</td>
<td>2</td>
</tr>
<tr>
<td>PP' - DDT</td>
<td>2</td>
</tr>
</tbody>
</table>
Fig. 4.1: Chromatogram of nonaerated water sampled after 2 hrs.

Fig. 4.2: Chromatogram of nonaerated water sampled after 35 days.
Fig. 4.3: Chromatogram of aerated water sampled after 2 hrs.

Fig. 4.4: Chromatogram of aerated water sampled after 35 days.
Fig. 4.5: Chromatogram of sediment sampled after 2 hrs (pesticide injected after every 24 hrs for 7 days).

Fig. 4.6: Chromatogram of sediment sampled after 7 days (pesticide injected after every 24 hrs for 7 days).
Fig. 4.7: Chromatogram of oysters sampled after 2 hrs of replacing the water (pesticide injected once and the contaminated water replaced after 24 hrs).

Fig. 4.8: Chromatogram of oysters sampled after 7 days of replacing the water (pesticide injected once and the contaminated water replaced after 24 hrs).
Fig. 4.9: Chromatogram of water sampled after 2 hrs (pesticide injected once at beginning).

Fig. 5.0: Chromatogram of water sampled after 35 days (pesticide injected once at beginning).
Fig. 5.2: Chromatogram of sediment sampled after 2 hrs (pesticide injected once at beginning).

Fig. 5.3: Chromatogram of sediment sampled after 35 days (pesticide injected once at beginning).
APPENDIX III

Tables showing data represented on figures 3.1 to 3.8

Table 3.12: Distribution of $^{14}$C-DDT residues in ng/g in water, Sediment, Oyster and Rack.

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>Water</th>
<th>Sediment</th>
<th>Oyster</th>
<th>Rack</th>
<th>BCF for Oysters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0.24</td>
<td>0.79</td>
<td>126.3</td>
<td>72.0</td>
<td>-</td>
</tr>
<tr>
<td>0</td>
<td>1.18</td>
<td>0.79</td>
<td>126.3</td>
<td>72.0</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>0.71</td>
<td>16.00</td>
<td>669.0</td>
<td>-</td>
<td>942.25</td>
</tr>
<tr>
<td>4</td>
<td>0.60</td>
<td>17.00</td>
<td>272.4</td>
<td>-</td>
<td>454.00</td>
</tr>
<tr>
<td>24</td>
<td>0.42</td>
<td>19.06</td>
<td>345.8</td>
<td>-</td>
<td>823.33</td>
</tr>
<tr>
<td>72</td>
<td>0.33</td>
<td>71.00</td>
<td>643.5</td>
<td>-</td>
<td>1950.00</td>
</tr>
<tr>
<td>120</td>
<td>0.24</td>
<td>90.08</td>
<td>1142.2</td>
<td>-</td>
<td>4759.17</td>
</tr>
<tr>
<td>168</td>
<td>0.39</td>
<td>117.00</td>
<td>1308.0</td>
<td>-</td>
<td>3353.85</td>
</tr>
</tbody>
</table>

A graphical representation of Table 3.12 above is given on Fig 3.1.

Table 3.13: The Dissipation of $^{14}$C-DDT pesticide residues from sediment and oysters exposed to sublethal concentrations of DDT.

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>Water</th>
<th>Sediment</th>
<th>Oyster</th>
<th>Rack</th>
<th>BCF for Oysters</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.3</td>
<td>1200</td>
<td>935.5</td>
<td>256.0</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>3.2</td>
<td>390</td>
<td>1234.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>4.4</td>
<td>380</td>
<td>778.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>24</td>
<td>2.6</td>
<td>260</td>
<td>805.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>72</td>
<td>1.4</td>
<td>190</td>
<td>735.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>120</td>
<td>1.2</td>
<td>130</td>
<td>683.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>168</td>
<td>1.1</td>
<td>110</td>
<td>615.5</td>
<td>69.0</td>
<td>-</td>
</tr>
</tbody>
</table>

Values on Table 3.13 are graphically represented on Fig 3.2

Table 3.14: Distribution of $^{14}$C-DDT residues in ng/g between water and sediment ecosystem.

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>Water</th>
<th>Sediment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0.274</td>
<td>2.6507</td>
</tr>
<tr>
<td>0</td>
<td>20.520</td>
<td>2.6307</td>
</tr>
<tr>
<td>2</td>
<td>9.925</td>
<td>4.8560</td>
</tr>
<tr>
<td>4</td>
<td>8.211</td>
<td>10.0010</td>
</tr>
<tr>
<td>24</td>
<td>4.424</td>
<td>11.4710</td>
</tr>
<tr>
<td>72</td>
<td>2.742</td>
<td>11.6540</td>
</tr>
<tr>
<td>168</td>
<td>1.094</td>
<td>18.8020</td>
</tr>
<tr>
<td>336</td>
<td>1.077</td>
<td>16.3220</td>
</tr>
<tr>
<td>504</td>
<td>0.816</td>
<td>25.5220</td>
</tr>
<tr>
<td>672</td>
<td>0.709</td>
<td>19.0230</td>
</tr>
</tbody>
</table>

Table 3.14 data is represented in Fig 3.3.
Table 3.15: Distribution of $^{14}$C-Chlorpyrifos residues in ng/g in water and sediment ecosystem.

<table>
<thead>
<tr>
<th>Time(hrs)</th>
<th>Water</th>
<th>Sediment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank.</td>
<td>0.448</td>
<td>2.829</td>
</tr>
<tr>
<td>0</td>
<td>42.671</td>
<td>2.829</td>
</tr>
<tr>
<td>2</td>
<td>32.743</td>
<td>6.025</td>
</tr>
<tr>
<td>4</td>
<td>26.705</td>
<td>7.625</td>
</tr>
<tr>
<td>24</td>
<td>15.140</td>
<td>20.021</td>
</tr>
<tr>
<td>72</td>
<td>3.166</td>
<td>15.702</td>
</tr>
<tr>
<td>168</td>
<td>3.081</td>
<td>9.848</td>
</tr>
<tr>
<td>336</td>
<td>2.214</td>
<td>4.385</td>
</tr>
<tr>
<td>504</td>
<td>2.819</td>
<td>4.192</td>
</tr>
<tr>
<td>672</td>
<td>2.516</td>
<td>3.598</td>
</tr>
</tbody>
</table>

Values in Table 3.15 are plotted in Fig 3.4.

Table 3.16: The loss of $^{14}$C-DDT residues in ng/g from aerated and non-aerated sea water.

<table>
<thead>
<tr>
<th>Time(hrs)</th>
<th>Water (aerated)</th>
<th>Water (non-aerated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank.</td>
<td>0.161</td>
<td>0.249</td>
</tr>
<tr>
<td>0</td>
<td>27.120</td>
<td>32.987</td>
</tr>
<tr>
<td>2</td>
<td>20.467</td>
<td>19.562</td>
</tr>
<tr>
<td>4</td>
<td>10.205</td>
<td>17.248</td>
</tr>
<tr>
<td>24</td>
<td>2.310</td>
<td>8.648</td>
</tr>
<tr>
<td>72</td>
<td>1.091</td>
<td>5.141</td>
</tr>
<tr>
<td>168</td>
<td>3.748</td>
<td>12.939</td>
</tr>
<tr>
<td>336</td>
<td>0.877</td>
<td>7.147</td>
</tr>
<tr>
<td>504</td>
<td>0.786</td>
<td>5.374</td>
</tr>
<tr>
<td>672</td>
<td>0.731</td>
<td>4.865</td>
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</tbody>
</table>

The data in Table 3.16 is represented graphically in fig 3.5.

Table 3.17: The loss of $^{14}$C-Chlorpyrifos in ng/g from aerated and non-aerated water.

<table>
<thead>
<tr>
<th>Time(hrs)</th>
<th>Water(aerated)</th>
<th>Water(non-aerated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank.</td>
<td>0.460</td>
<td>0.219</td>
</tr>
<tr>
<td>0</td>
<td>32.988</td>
<td>26.627</td>
</tr>
<tr>
<td>2</td>
<td>30.054</td>
<td>24.550</td>
</tr>
<tr>
<td>4</td>
<td>25.029</td>
<td>17.930</td>
</tr>
<tr>
<td>24</td>
<td>7.073</td>
<td>14.688</td>
</tr>
<tr>
<td>72</td>
<td>0.900</td>
<td>10.596</td>
</tr>
<tr>
<td>168</td>
<td>0.566</td>
<td>2.239</td>
</tr>
<tr>
<td>336</td>
<td>0.444</td>
<td>1.189</td>
</tr>
<tr>
<td>504</td>
<td>0.613</td>
<td>1.228</td>
</tr>
<tr>
<td>672</td>
<td>0.503</td>
<td>1.150</td>
</tr>
</tbody>
</table>

Data in Table 3.17 is plotted in Fig 3.6.
Table 3.18: Distribution of $^{14}$C-Chlorpyrifos residues in ng/g in water, sediment, oysters and fish. Pesticide injected after every 24 hours.

<table>
<thead>
<tr>
<th>Time</th>
<th>Water (LFF)</th>
<th>Sediment (LFC)</th>
<th>Oyster (BCF)</th>
<th>Fish (LFF)</th>
<th>Fish (LFC)</th>
<th>Oyster (BCF)</th>
<th>LFF (BCF)</th>
<th>LFC (BCF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
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<td>14.25</td>
<td>134.0</td>
<td>-</td>
<td>-</td>
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<tr>
<td>0</td>
<td>2.003</td>
<td>14.25</td>
<td>134.0</td>
<td>189.9</td>
<td>206.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>1.549</td>
<td>19.27</td>
<td>334.4</td>
<td>329.6</td>
<td>526.4</td>
<td>215.88</td>
<td>212.78</td>
<td>183.00</td>
</tr>
<tr>
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<td>1.470</td>
<td>17.73</td>
<td>208.4</td>
<td>349.1</td>
<td>690.0</td>
<td>141.77</td>
<td>237.48</td>
<td>469.39</td>
</tr>
<tr>
<td>8</td>
<td>2.435</td>
<td>20.17</td>
<td>216.5</td>
<td>274.2</td>
<td>508.4</td>
<td>88.91</td>
<td>112.61</td>
<td>208.83</td>
</tr>
<tr>
<td>24</td>
<td>1.521</td>
<td>19.20</td>
<td>277.8</td>
<td>234.2</td>
<td>372.4</td>
<td>182.64</td>
<td>153.98</td>
<td>244.84</td>
</tr>
<tr>
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<td>2.144</td>
<td>21.06</td>
<td>483.1</td>
<td>366.9</td>
<td>529.1</td>
<td>225.33</td>
<td>171.13</td>
<td>246.78</td>
</tr>
<tr>
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<td>2.397</td>
<td>23.11</td>
<td>314.7</td>
<td>390.4</td>
<td>636.5</td>
<td>131.29</td>
<td>162.87</td>
<td>265.54</td>
</tr>
<tr>
<td>120</td>
<td>3.653</td>
<td>19.74</td>
<td>364.4</td>
<td>345.6</td>
<td>200.8</td>
<td>99.75</td>
<td>94.61</td>
<td>54.97</td>
</tr>
<tr>
<td>168</td>
<td>4.073</td>
<td>20.00</td>
<td>449.9</td>
<td>420.8</td>
<td>584.3</td>
<td>110.46</td>
<td>103.31</td>
<td>143.46</td>
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</tbody>
</table>

LFF---- *Lutrinus fulviflamma* - Dory Snapper (Snapper fish) (18 fishes)

LFC---- *Siganus Stellatus* - Rabbit fish (18 fishes)

Data in Table 3.18 is represented graphically on Fig 3.7.

Table 3.19: Distribution of $^{14}$C-Chlorpyrifos residues in ng/g in water, sediment, oysters and fish for pesticide injected once and the treated water replaced after 24 hours with fresh sea water.

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>Water (MFH)</th>
<th>Sediment (MFC)</th>
<th>Oyster (BCF)</th>
<th>Fish (MFH)</th>
<th>Fish (MFC)</th>
<th>Oyster (BCF)</th>
<th>MFH (BCF)</th>
<th>MFC (BCF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank.</td>
<td>0.575</td>
<td>16.00</td>
<td>124.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>216.3</td>
<td>-</td>
</tr>
<tr>
<td>0</td>
<td>0.575</td>
<td>23.92</td>
<td>160.0</td>
<td>540.5</td>
<td>219.0</td>
<td>278.3</td>
<td>940.0</td>
<td>380.9</td>
</tr>
<tr>
<td>2</td>
<td>0.770</td>
<td>20.45</td>
<td>184.9</td>
<td>603.7</td>
<td>181.2</td>
<td>240.0</td>
<td>784.0</td>
<td>235.3</td>
</tr>
<tr>
<td>4</td>
<td>1.098</td>
<td>23.70</td>
<td>178.1</td>
<td>493.0</td>
<td>193.5</td>
<td>162.2</td>
<td>449.0</td>
<td>176.2</td>
</tr>
<tr>
<td>8</td>
<td>0.833</td>
<td>19.65</td>
<td>176.3</td>
<td>381.3</td>
<td>189.3</td>
<td>210.4</td>
<td>457.7</td>
<td>227.3</td>
</tr>
<tr>
<td>24</td>
<td>1.132</td>
<td>19.06</td>
<td>140.1</td>
<td>320.4</td>
<td>171.4</td>
<td>123.8</td>
<td>283.0</td>
<td>151.4</td>
</tr>
<tr>
<td>48</td>
<td>1.006</td>
<td>30.58</td>
<td>144.7</td>
<td>267.1</td>
<td>170.3</td>
<td>143.8</td>
<td>265.5</td>
<td>169.3</td>
</tr>
<tr>
<td>72</td>
<td>1.213</td>
<td>19.84</td>
<td>143.5</td>
<td>300.3</td>
<td>145.5</td>
<td>118.3</td>
<td>247.6</td>
<td>120.0</td>
</tr>
<tr>
<td>120</td>
<td>0.804</td>
<td>19.01</td>
<td>127.6</td>
<td>250.9</td>
<td>150.4</td>
<td>158.7</td>
<td>312.1</td>
<td>187.1</td>
</tr>
<tr>
<td>168</td>
<td>0.929</td>
<td>16.48</td>
<td>119.0</td>
<td>195.0</td>
<td>110.3</td>
<td>128.4</td>
<td>209.9</td>
<td>118.7</td>
</tr>
</tbody>
</table>

MFH---- *Lethrinus harak* - Blackspot emperor fish (Scavenger fish) (20 fishes)

MFC---- *Siganus Stellatus* - Rabbit fish (20 fishes)

Data in Table 3.19 is represented graphically on Fig 3.8.