DISSIPATION RATE OF CHLORPIRIFOS WITHIN MOSQUITO LARVAE HABITATS IN MASENO AND ITS ACTIVITY AGAINST THE LARVAE RELATIVE TO DICHLORODIPHENYLTRICHLOROETHANE

BY

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DECLARATIONS

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This thesis is my original work and has not been previously presented for award of a degree in Maseno University or in any other University. The work herein has all sources of information supported by relevant references.

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DEDICATION

This work is dedicated to my dear wife Judith, our lovely daughters Cyndy, Janevieve and son Muma.

ABSTRACT

Despite more than a century of efforts to eradicate and control malaria, it still remains a major risk to public health and economies of countries in the tropical and subtropical regions of the world. Control of malaria has been based on eradication of the vector, mosquito, by use of pesticides. In order to reduce malaria incidence, some African countries are re-introducing the banned dichlorodiphenyltrichloroethane (DDT), however, organophosphates are known to have far more immediate toxicity than organochlorines and other related products. Chlorpyrifos is a wide spectrum organophosphate used to control range and forage insect pests as well as sediment dwelling grubs, rootworms, borers and subterranean termites and could therefore be used to replace DDT use in mosquito larval control, however, its persistence in the mosquito larval habitats and toxicity towards the mosquito larvae, in comparison to DDT is unknown. The objective of this study was to determine the degradation rates of these two pesticides in the various mosquito larval habitats and their toxicity on mosquito larvae in Maseno, an area in western Kenya characterized by endemic malaria. Water, moist sediment and *Justicia flava* leaves, the selected common habitats, were spiked with DDT and chlorpyrifos and samples were thereafter collected after 0, 2, 4, 6 hours and then at longer intervals of time up to 2120 hours, and analyzed separately using Gas chromatography to determine their environmental persistence. The dissipation of the pesticides were rapid and half-lives of DDT in moist sediment, *Justicia flava* leaves and water were; 15.4 days, 3.1 days and 6.1 hours and 7.8 days, 2.0 days and 6.3 hours, respectively, for chlorpyrifos. Toxicity tests of the pesticides against *Anopheles gambiae* larvae from Maseno using WHO standard test method in the laboratory were done, based on the LC_{50} and LC_{90} in water. The LC_{50} after 24 hours exposure were; 0.0014 ppm and 0.0398 ppm for chlorpyrifos and DDT respectively, while the LC_{90} were; 0.0132 ppm and 0.19 ppm respectively. Chlorpyrifos had a shorter dissipation half-life than DDT. Chlorpyrifos was more toxic to *Anopheles gambiae* than DDT. Chloropyrifos can therefore be used effectively in controlling both mosquito larvae formerly controlled by DDT. The information obtained in this study is most useful to the ministry of public health and researchers involved in malaria vector control work for planning malaria control on lethal doses and their persistence in the environment.

TABLE OF CONTENTS

ACRONYMS/ABBREVIATIONS

AEA	Alternative electron acceptor
ANOVA	Analysis of variance
CEC	Commission for Environmental Cooperation
DBP	Dichloro-benzophenone
DCB	Dichlorobenzene
DCM	Dichloromethane
DDA	Bis(4'-Chlorophenyl)acetate
DDCN	2,2, bis(chlorophenyl)-acetonitrile
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethene
DDM	Bis(4'-Chlorophenyl)methane
DDMS	2,2, bis(chlorophenyl)-1-chloroethane
DDMU	2,2, bis(chlorophenyl)-1-chloroethylene
DDNU	Unsym-bis(4'-Chlorophenyl)ethylene
DDOH	2,2-bis(4'-Chlorophenyl)ethanol
DDT	Dichlorodiphenyltrichloroethane
DDTr	DDT and its metabolites
DNA	Deoxyribonucleic acid
DOC	Dissolved organic carbon
DOM	Dissolved Organic Matter
ECD	Electron capture detector
EIA	Environmental Impact Assessment

EPA	Environmental protection agency
GC	Gas chromatography
GC-FID	Gas chromatography – Flame ionization detector
GC-MS	Gas chromatography-Mass spectroscopy
h	hour(s)
Ha	Hectare
HPLC	High performance liquid chromatography
HPLC	High Pressure Liquid Chromatography
IAEA	International Atomic Energy Agency
IRS	Indoor residual spraying
ITNs	insecticide treated nets
Kdr	Knock down resistance
KH	Henry's constant
Kow	Octanol-water partition coefficient
Kp	Sorption coefficient
L	Liter
LD_{50}	Lethal Dose to kill half of the population concerned in a standard experiment
LD_{90}	Lethal Dose to kill 90% of the population concerned in a standard experiment
M	Molar/Molarity
mg	Milligrams
MW	Molecular weight

NERs	Non-extractable residues
o,p-DDD	1-chloro-4-[2,2-dichloro-1-(2-chlorophenyl)ethyl]benzene
o,p-DDE	1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethenyl]benzene
o,p-DDT	1-chloro-2-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]benzene
^{O}C	Degree centigrade
OCP(s)	Organochlorine pesticide(s)
OP	Organophosphates
p,p-DDD	1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene
p,p-DDE	1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethenyl]benzene
p,p-DDM	1,1'-methyle ne bis(4-chlorobenzene)

LIST OF TABLES

LIST OF FIGURES

APPENDICES

CHAPTER ONE

INTRODUCTION

1.1: Background

Despite more than a century of efforts to eradicate and control malaria, it still remains a major risk to public health and economies of countries in the tropical and subtropical regions of the world. Malaria kills over a million people each year, mostly young children under five years of age (Hetzel *et al*., 2007).Control of malaria has been based on eradication of the vector, mosquito, by use of pesticides. Pesticides are physical, chemical or biological agents intended to prevent, destroy, repel and mitigate undesirable plant and animal pest or disease caused by micro-organisms. Though they are often misunderstood to refer only to insecticides, the term pesticide also applies to herbicides, fungicides, and various other substances used to control pests (USEPA, 2005). They are known to remain for long periods of times in water, sediment, air, and food and can bioaccumulate (Goncalves and Alpendurada, 2005; Kumar and Philips, 2006).

In order to reduce malaria incidence, some African countries are re-introducing dichlorodiphenyltrichloroethane (DDT) (Figure 1.1), a controversial insecticide once widely used throughout the world for agricultural and public health. The limited use of DDTin indoor house spraying against mosquitoes (UNEP/GEF, 2010) to control malaria is done under the supervision of United Nations (UN) (Thangavadivel *et al*., 2009).Due to its low water solubility, it tends to remain adsorbed to sediment particles. Its resistance to biodegradation leads to its persistence in the sediment environment for long period of time. The re-introduction DDT is based on its proven effectiveness compared to other alternatives. However, the reintroduction is of concern on both national and international scales (Carter, 2004).

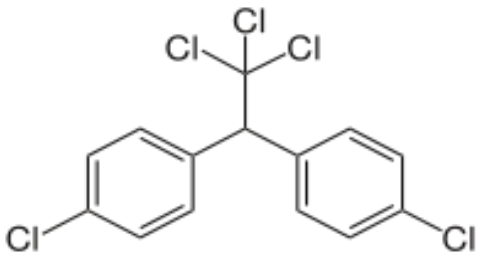


Figure 1.1: Chemical structure of DDT

Several studies have reported continued resistance to this pesticide with over 50 species of anopheles mosquitoes becoming resistant (Hemingway *et al*., 2006). Early studies attributed this resistance to its use in agriculture, since many vectors breed in such environments (Mouchet 1988) and get exposed to it over a long period of time due to its persistence in the environment. This is a major problem in malaria control since DDT is a major insecticides used for the control of mosquitoes leading to the campaign to substitute DDT with less persistant pesticides.

Organophosphorous pesticides (OPs) are commonly used in agricultural practices and disease vector control because of their rapid breakdown into environmentally safe products and they are less persistent in the environment than organochlorine pesticides(Kanekar *et al*., 2004). Organophosphates have far more immediate toxicity than organochlorines and other related products. They all produce toxicity by inhibiting acetyl cholinesterase (AChE) and cause a similar spectrum of symptoms (Rosenfeld and Sultatos, 2006). The OPs are effective insecticides and are very toxic to their target organisms (UNEP *et al.*,1991,Swati and Singh, 2002). Quinalphos, monocrotophos, chlorpyrifos, malathion, parathion are some of the widely used organophosphorous pesticides. Amongst these, chlorpyriphos (Figure 1.2) dominates. It is a synaptic poison having broadspectrum insecticidal activity and is used to control insects attacking corn, cotton, citrus, fruits, nut crops, potato, beets, pulses, *etc*. (Khanna and Vidyalakshmi, 2004). Chlorpyrifos is commonly sold as dursban and lorsban respectively and was first registered as a broad spectrum insecticide in 1965 (Hayes and Laws, 1990). Its persistence in the environment ranges from a few days to several months depending on application methods, formulation, and environmental conditions including microbial consortia and presence of organic matter (Grabusky, 2004).

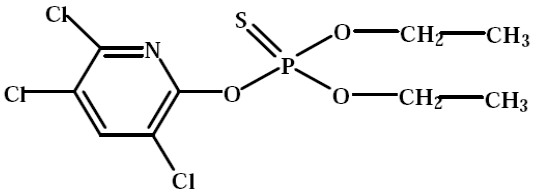


Figure 1.2: Chemical structure of chlorpyrifos

Chlorpyrifos shows a wide spectrum of biological activity and is used to control range and forage insect pests as well as sediment dwelling grubs, rootworms, borers and subterranean termites and could therefore be used to replace DDT use in mosquito larval control. However,

chorpyriphos activity against mosquito larvae has not been determined in comparison to DDT.

Malaria is a vector-borne infectious disease caused by protozoan parasites of thegenus Plasmodium. Malaria parasites are transmitted from person to person by thebite of an infected female Anopheles mosquito. Malaria is one of the most important causes of morbidity and mortality in developing countries in the sub-Saharan Africa (SSA) (WHO, 2003). For instance, in Kenya,approximately 20 million people are exposed to stable malaria transmission every year, including 3.5 million children below the age of 5 years (KMIS, 2010). Case fatality is very high among children with an estimated death toll of 26,000 per year(WHO, 2011). Malaria parasite transmission is often strongly associated with locality and Spatiotemporal distribution of vector species. This association focuses around specific mosquito breeding habitats (Carter et al., 2000, Wanjala et al., 2011). One of the most effective malaria control strategies isenhanced mortality of mosquito larvae in mosquito breeding habitats (Das *et al*., 1986). Persistence of pesticides in such habitats may, however,lead to resistence (Van den Berg, 2009). Mosquito habitats include plant leaves, sediment and water bodies.Maseno is situated in western Kenya, where malaria is repoted to be prevalent (Kuria *et al*., 2002).The most common broad leaf plant in Maseno is *Justicia flava* (Vahl) and provides a breedind habitat to mosquito, in addition to stagnant water and moist sediment.. Despite the potential of Chlorpyriphos in replacing DDT, its persistence on the mosquito breeding habitats in Maseno remain unknown. This needs to be determined to give foresight into its susceptibility to resistence by malaria vector, mosquito.

1.2: Statement of the problem

Malaria is the leading killer disease of infants (20% per annum) and also the leading disease in hospital admissions. Synthetic chemicals like DDT have been effectively used in the management of malaria vectors. The efficacy of DDT as a pesticide in the management of animal pests in the last 5 decades is appreciable. Recently, there have been environmental concerns that have been raised by environmentalists due to hazardous effects of DDT.. The development of environmental friendly organophosphates such as chlorpirifos as substitute to DDT has been achieved. The longevity of organophosphates and their metabolites in the mosquito habitats is documented, especially in the malaria prone Maseno area. Though data on lethal dosage of DDT against mosquito larva exists, little information on chlorpirifos is

available. Besides, there is limited information in Kenya pertaining to dissipation half-lives and lethal dosages of the two chemicalsin natural environments consequently, a comparative study on the activityand dissipation of DDT and chlorpirifos is necessary

1.3: Objectives

1.3.1: General objective

To compare activityof DDT and chlorpyrifos against mosquito larvae and their dissipation from mosquito larval habitats.

1.3.2:Specific objectives

1. To determine the dissipation of chlorpyrifos and DDT residues in stagnant water, moistsediment and *Justicia flava* (Vahl) leaf samples by calculating their dissipation half-life, DT_{50}in the three habitats.
2. To determine activityof chlorpyrifos and DDT as a mosquito larvicide by determining their toxicity on mosquito larvae.

1.4: Hypotheses

1. The dissipationhalf-lives of chlorpyrifos and DDT in various tropical mosquito vector habitats in Maseno i.e. stagnant water, moist sediment and *Justicia flava*leaves, are not different.
2. The activity of chlorpyrifos and DDT against mosquito larval are not different.

1.5: Justification and significance of the study

It is necessary to find out how pesticides degrade, dissipate and persist in the environment soas to predict their lethal dossing and effect to non-target organisms. Research shows that the persistence of pesticide behaves differently in different environments (Wandiga, 2001). Therefore research obtained by studies on temperate mosquito larval habitat cannot be used to predict its behavior in tropical environments(UNEP, 2009). An alternative effective pesticide such as chlorpyrifos can replace DDT in mosquito larval control. An ideal pesticide should persist long enough to control target organisms and then be degraded to inert or nontoxic products to avoid effects on non-target organisms and possibility of resistence. It is

therefore necessary to compare persistence and activity of DDT and chlorpyriphos to determine the environment-specific possibility of replacement of DDT with chlorpyriphos in malaria management.

CHAPTER TWO

LITERATURE REVIEW

2.1: Pesticide use

2.1.1: DDT use

Effectiveness of DDT on insects was discovered by Dr. Paul Müller (Carter, 2004). DDT is not used for malaria vector controlin Kenya due to its ban in 1986 (PCPB, 2008). Appendix III and IV shows the list of all the banned and the restricted pesticides in Kenya respectively. In developed countries, DDT was used extensively in agriculture as a general insecticide, and was also successfully used in exterminating insects that carried vector-borne diseases like typhus and malaria (EC, 1999). Although no longer used in the western world, many tropical countries still employ DDT to control malaria parasites and it remains one of the most effective and affordable insecticide available in the world (EC, 1999).

In order to reduce malaria incidence, some African countries are re-introducing dichlorodiphenyltrichloroethane (DDT) (Figure 1.1), a controversial insecticide once widely used throughout the world for agricultural and public health. The limited use of DDTin indoor house spraying against mosquitoes (UNEP/GEF, 2010) to control malaria is done under the supervision of United Nations (UN) (Thangavadivel *et al*., 2009).Due to its low water solubility, it tends to remain adsorbed to sediment particles. Its resistance to biodegradation leads to its persistence in the sediment environment for long period of time. The re-introduction DDT is based on its proven effectiveness compared to other alternatives. However, the reintroduction is of concern on both national and international scales (Carter, 2004).

Several studies have reported continued resistance to this pesticide with over 50 species of anopheles mosquitoes becoming resistant (Hemingway *et al*., 2006). Early studies attributed this resistance to its use in agriculture, since many vectors breed in such environments (Mouchet 1988) and get exposed to it over a long period of time due to its persistence in the environment. This is a major problem in malaria control since DDT is a major insecticides used for the control of mosquitoes leading to the campaign to substitute DDT with less persistant pesticides.

2.1.2: Chloropyrifos use

Organophosphorous pesticides (OPs) are commonly used in agricultural practices and disease vector control because of their rapid breakdown into environmentally safe products and they are less persistent in the environment than organochlorine pesticides(Kanekar *et al*., 2004). Organophosphates have far more immediate toxicity than organochlorines and other related products. They all produce toxicity by inhibiting acetyl cholinesterase (AChE) and cause a similar spectrum of symptoms (Rosenfeld and Sultatos, 2006). The OPs are effective insecticides and are very toxic to their target organisms (UNEP *et al.*,1991,Swati and Singh, 2002). Quinalphos, monocrotophos, chlorpyrifos, malathion, parathion are some of the widely used organophosphorous pesticides. Amongst these, chlorpyriphos (Figure 1.2) dominates. It is a synaptic poison having broadspectrum insecticidal activity and is used to control insects attacking corn, cotton, citrus, fruits, nut crops, potato, beets, pulses, *etc*. (Khanna and Vidyalakshmi, 2004). Chlorpyrifos is commonly sold as dursban and lorsban respectively and was first registered as a broad spectrum insecticide in 1965 (Hayes and Laws, 1990). Its persistence in the environment ranges from a few days to several months depending on application methods, formulation, and environmental conditions including microbial consortia and presence of organic matter (Grabusky, 2004).

Chlorpyrifos is used as an insecticide, acaricide, and nematicide to control Coleoptera, Diptera, Homoptera and Lepidoptera in sediment, on foliage, and on animals (WHO 2009a). Chlorpyrifos is neither restricted nor banned hence can be used in Kenya as a pesticide and it remains one of the most commonly used organophosphate insecticide active ingredients in the world (Grube, 2011). It is applied on nuts, fruit, vegetables, grain, seeds, fodder crops, and Christmas trees; in forestry, nurseries, greenhouses, food processing plants, industrial plants, warehouses, and ships; for disease vector control (mosquito larvicide and adulticide), household pests, fire ants, termites, and pests in animal houses; as a sheep dip for the control of lice, blowfly and ked; on golf courses and turf; as an anti-mildew agent in wood preservatives, and as ant baits; for treating poles, fence posts, railway ties, and railway box cars; in ear tags for cattle which may also contain other insecticides such as diazinon, cypermethrin or permethrin. Chlorpyrifos treated plastic bags are fixed over bunches of bananas to prevent insect damage in some Latin American countries (Bellamy 2012). It is also formulated into paint for controlling insects, in slow release microencapsulated form. Chlorpyrifos formulation, which also contains pyriproxifen, has been trialled as a painted

band around citrus trees to prevent ants from foraging in the trees (Juan-Blasco *et al*., 2011), and for preventing infestations of the red palm weevil, *Rhynchophorus ferrugineus,* in palms (Llácer *et al*.,2010).

2.2: Properties and chemistry of chlorpyrifos and DDT

2.2.1: Properties and chemistry of chlorpyrifos

Chlorpyrifos, [*O,O*-diethyl-*O*-(3,5,6-trichloro-2-pyridyl)-phosphorothionate] (Figure 1.1) is a sulfur-bearing organophosphate (OP) insecticide and one of the most widely used in the United States because it possesses a broad spectrum of activity against a wide range of arthropod and insect pests (USEPA, 2000).Chlorpyrifos is commonly known as dursban and lorsban. Direct toxicity results from metabolic activation to form chlorpyrifos oxon with inactivation of acetyl cholinesterase at the synapse (Barron and Woodburn, 1995).

Most organophosphates are insecticides although a few are listed as organophosphate pesticides. Organophosphates were developed during the early 19th century, but their effects on insects, which are similar to their effects on humans, were discovered in 1932. Some are very poisonous (used in World War II as nerve agents) but they are usually not persistent in the environment (Varfolomeyev *et al.,* 2002).

Organophosphates are esters, amides, or thiol derivatives of phosphoric, phosphonic, phosphorothioic or phosphonothioic acids. The true phosphates (triesters of phoshoric acid), where all four atoms surrounding the phosphorus are oxygen, are highly reactive and unstable substances, therefore not very appropriate for agricultural use. Generally, sulphur containing OP compounds, especially those with a P=S moiety (phosphorothionates; parathion -methyl and chlorpyrifos) and those with the P=S moiety and thioester bond (phosphorothionothiolates; Malathion) are most frequently used as insecticides (Wood, 2005). Their in *vitro* inhibitory potency toward AChE enzyme is relatively low in comparison to their oxo-analogs (P=O) double bond (Wood, 2005). The physical and chemical properties of chlorpyrifos are shown in Table 2.1.

Table 2.1: Physical and chemical properties of chlorpyrifos

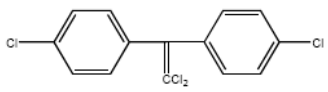
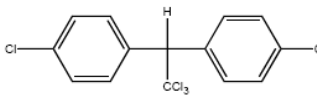
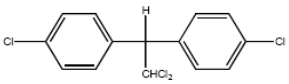
Property	Information	Reference
General pesticide name	Chlorpyrifos	Merck, 1989
Pesticide group	Insecticide	Merck, 1989
Chemical name (IUPAC)	o,o-diethyl-o-(3,5,6-trichloro-2-pyridyl) phosphorothioate	Merck, 1989
Molecular weight	350.57	Merck, 1989
Molecular formular	$C_9H_{11}Cl_3NO_3PS$	Merck, 1989
Molecular weight	350.5 g	Merck, 1989
Synonyms	Phosphorothioic acid O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl)ester, chlorpyrifos ethyl, chlorpyrifos	Merck, 1989
Color	Colourless/White granular crystals	Merck, 1989
Registered trade names	Dowco179, ENT2711; Dursban; Lorsban; Pyrinex; DMS-0971; coroban; piridan.	Merck, 1989
Physical state	Crystalline solid	EPA, 1988
Melting point	$41\text{-}42^0$C	Merck, 1989
Boiling point	Decomposses at approximately 1600C	Verschueren, 1983
Density at 43.5ºC	1.398 g/cm^3	Verschueren, 1983
Odor	Mild mercaptan	EPA, 1988
Solubility in water at at 20ºC	0.7 mg/L	Merck, 1989
Solubility in water at 25ºC	2 mg/L	Merck, 1989
Organic solvents	79% w/w in isooctane, 43% w/w in methanol and readily soluble in other organic solvents	Merck, 1989
Partition coeffi-cient	Log Koc is 3.73; Log Kow 4.82	McCall et al., 1980
Vapor pressure at 20ºC	1.87×10^{-5} mmHg	Verschueren, 1983
Vapor pressure 25ºC	1.87×10^{-5} mmHg	Merck, 1989
Conversion factors (25ºC)	1ppm=14.3mg/m^3	EPA, 1988
	1mg/m^3 =0.070ppm	
Major m/z signals in mass spectrum in GC	197, 242, 258, 286, 314, 351	Merck, 1989
Henry,s law costant at 25ºC	1.23×10^{-5} atm-m3/mol	HSDB, 1994
Sediment sorption coefficient	8498 mL/g	Merck, 1989

2.2.2: Properties and chemistry of the DDT

DDT was first synthesized in 1874 by a German chemist named Othmar Zeidler, but its insecticidal properties were not discovered until 1939 by a Swiss scientist named Paul Mueller who was the winner of 1948 Nobel Prize for his effort (Russel, 1955). Large scale industrial production was started in 1944 by Montrose Chemical Corporation in California (Singh, 1962).

Commercially available DDT is known as the technical grade DDT (TG-DDT) and is comprised of 4,4'-DDT (77.1%), 2,4-DDT (14.9%), 4,4'-DDE (4.0%), 2,4-DDE (0.1%), 4,4'-DDD (0.3%), 2,4-DDD (0.1%) and 3.5% unidentified products (WHO, 1989). TG-DDT is a non-flammable, odourless mixture that forms colorless crystals or a waxy solid at room temperature (Worthing and Hance, 1991). DDT and its related products are insoluble in water and strongly lipophilic. They are soluble in organic solvents such as acetone, xylene or other petroleum distillates (Budavari *et al*., 1989). Hydrophobic chemicals are classified by an octanol-water partition coefficient (log K_{ow}) greater than 3.5; therefore DDT with log K_{ow} of 6 is hydrophobic (Suntio *et al*., 1988). Both DDE and DDD are found in small amounts in commercial DDT samples, such as Anofex, Cezarex, Chlorophenothane, Clofenotane, Dicophane, Dinocide, Gesarol, Gyron, Ixodex, Neocid and Zerdane (WHO, 1979). The physical and chemical properties of DDT and its two major metabolites (Table 2.2).

Table 2.2: Chemical and physical properties of p, p'-DDT, p, p'-DDE and p, p'-DDD

Property	p,p'-DDE	p,p'-DDT	p,p'-DDD
Chemical structure			
Chemical Formula	$C_{14}H_8Cl_4$	$C_{14}H_9Cl_5$	$C_{14}H_{10}Cl_4$
Molecular Weight	318.0	354.5	320.4
Physical State	Crystalline solid	Crystalline solid	Crystalline solid
Melting Point	88 - 90°C	108.5°C	109 - 110°C
Density (at 20°C)	No data	0.98 – 0.99 g/cm3	No data
Henry's law constant	1.8 x 10^{-5} atm-m^3/mol	5.9 x 10^{-5} atm-m^3/mol	8.17 x 10^{-6} atm-m^3/mol
Vapor Pressure	6.0 x 10^{-6} mm Hg At 25°C	1.1 x 10^{-7} at 20°C , Torr	1.94 x 10^{-6} at 30°C, torr
Partition co-efficient Log K_{ow}	6.51	6.91	6.02
Partition co-efficient Log K_{oc}	4.70	5.18	5.18
Solubility in: Water at 25°C	0.12 mg/L (Range of publ. literature: 0.0011-0.12 mg/L)	0.025 mg/L	0.090 mg/L
Conversion Factors (at 25°C, 1 atm)	1 ppm= 13.01 mg/m^3 1 mg/m^3= 0.077 ppm	No data	No data

Sources: Swan *et al*. (1981); Suntio *et al*. (1988); Worthing and Hance (1991): Meylan*et al*. (1991); Howard and Meylan (1997); Lide (1998); USGS (2001b)

2.3: Transformation and Degradation of chloropyrifos and DDT

2.3.1: Breakdown in Sediment

2.3.1.1: Breakdown of chlorpyrifos in sediment

Chlorpyrifos is stable in sediments with reported half-lives ranging between 7 and 120 days. Studies have found chlorpyrifos residues in sediments for over one year following application. Sediment persistence may depend on the formulation, rate of application, sediment type, climate and other conditions. (Kamrin, 1997; Roberts and Hudson, 1999). Chlorpyrifos bound to sediment may be broken down by UV light, chemical hydrolysis, dechlorination, and sediment microbes. (Kamrin, 1999). Chlorpyrifos binds strongly to sediments, is relatively immobile, and has low water solubility. In contrast, its degradate 3,5,6-trichloro-2-pyridinol (TCP) adsorbs weakly to sediment particles and is moderately mobile and less persistent in sediments (Kamrin, 1997).

The major degradation products of chlorpyrifos found in sediments are similar to the metabolites created by plants and animals. The degradation products are formed by oxidative dealkylation or hydrolysis to diethyl phosphates and 3,5,6-trichloro-2-pyridinol (TCP). Chlorpyrifos was less persistent in the sediments with a higher pH (Racke, 1992). Sediment half-life was not affected by sediment texture or organic matter content. In anaerobic sediments, the half-life was 15 days in loamy sediment and 58 days in clay sediment (U.S. E.P.A, 1989). In a study of seven aerobic sediments ranging in texture from loamy sand to clay, with sediment pH values from 5.4 to 7.4, the sediment half-life for radiolabeled chlorpyrifos ranged from 11 to 141 days. After 360 days, researchers detected carbon dioxide (27-88%), 3,5, 6-trichloro-2-pyridinol (TCP) (up to 22%), and small amounts of 3,5,6-trichloro-2-methoxypyridine (TMP) (≤8%) in the sediment (Kamrin, 1997).

When applied to moist sediments, the volatility half-life of chlorpyrifos was 45 to 163 hours, with 62 to 89% of the applied chlorpyrifos remaining on the sediment after 36 hours (Racke, 1992). In another study, 2.6 and 9.3% of the chlorpyrifos applied to sand or silt loamy sediment, respectively remained after 30 days (Racke, 1992). Chlorpyrifos could be adsorbed strongly to sediment particles and it is not readily soluble in water (Racke, 1992). It is

therefore immobile in sediments and unlikely to leach or to contaminate groundwater (Racke, 1992).

In medium-textured sediments in field conditions in California, Illinois and Michigan, the half-lives reported for chlorpyrifos ranged from 33 to 56days. Chlorpyrifos is less persistent in sediments with a higher pH (Kamrin, 1997). Volatilization of chlorpyrifos from sediment is not likely. According to a laboratory volatility study, carbon dioxide appears to be the major volatization product of chlorpyrifos. In this study, less than 10% of chlorpyrifos applied to sediment volatilized within 30days after application. (US-EPA, 2000).

2.3.1.2: Breakdown of DDT in sediment

DDT (1, 1, 1-trichloro-2, 2-bis (4-chlorophenyl ethane) is a persistent environmentally toxic organochlorine insecticide. It has been used extensively since the 1940s for control of agricultural pests, and is still used in many tropical countries for mosquito control (US-EPA, 2007). Processes such as volatilization, adsorption, run off and plant uptake contribute to the dissipation of DDT residues (DDTr) in sediments, often without substantial alteration of the chemical structure (Foght *et. al.*, 2001). In contrast, biodegradation has the potential to degrade DDTr significantly and reduce sediment concentrations in a cost-effective manner (Foght *et. al.*, 2001). Biodegradation may occur under both aerobic and anaerobic conditions due to sediment microorganisms including bacteria, fungi, and algae (Aislabie *et al*., 1997). During biodegradation of DDT, both DDE and DDD are formed in sediments. Both metabolites may undergo further transformation but the extent and rate are dependent on sediment conditions and, possibly, microbial populations present in sediment. DDE is often resistant to biodegradation under aerobic and anaerobic conditions (Strompl and Thiele, 1997). The degradation pathways of DDT in sediment under aerobic and anaerobic conditions have been reviewed by Aislabie *et al*., (1997). DDT biodegradation is typically co-metabolic and includes dechlorination and ring cleavage mechanisms.

2.3.2: Breakdown in water

2.3.2.1: Breakdown of chlorpyrifos in water

Chlorpyrifos does not partition easily from sediment to water. Therefore, chlorpyrifos found in runoff water is likely a result of sediment-bound chlorpyrifos from eroding sediment, rather than from dissolved chlorpyrifos. (US-EPA, 2005). Volatilization of chlorpyrifos from water is the most likely route of loss for chlorpyrifos, with volatilization half-lives of 3.5 and 20 days estimated for pond water (Kamrin, 1997). During midsummer, the photolysis half-life of chlorpyrifos in water is between three and four weeks (Kamrin, 1997).The rate of hydrolysis for chlorpyrifos increases with temperature and alkalinity. Half-lives ranging from 35 to 78 days have been reported in water with a pH of 7 and a temperature of 25 ºC (Howard, 1991; Kamrin, 1997).

2.3.2.2: Breakdown of DDT in water

DDT, DDE, and DDD present in water may be transformed by both photo degradation andbiodegradation. Since the shorter wavelength radiation does not penetrate far into a body of water, photolysisprimarily occurs in surface water and is dependent on the clarity of the water. Direct photolysis of DDTand DDD are very slow in aquatic systems, with estimated half-lives greater than 50 years (EPA, 1979c). Direct photolysis of DDE will vary as a function of photoperiod and brightness, resulting in different half-lives depending on the season and latitude. Over the United States, the direct photolysis of DDE results in a half-life of about 1 day in summer and 6 days in winter. DDE also undergoes photoisomerization when exposed to sunlight. Photolysis of DDE photoisomers is slower by at least one order of magnitude compared to DDE. Studies with DDT at shorter wavelengths suggest that the initial reaction results in thedissociation of the $Cl_2C–Cl$ bond. Some information exists on the indirect photolysis of DDT; no information on the indirect photolysis of DDE or DDD was located (EPA, 1979c; Coulston, 1985). Photo induced 1,2 addition of DDT to a model lipid, methyloleate, indicates that light-induced additionsof DDT to unsaturated fatty acids of plant waxes and cutins may occur on a large scale (Schwack, 1988). DDT undergoes hydrolysis by a base-catalyzed reaction resulting in a half-life of 81 days at pH 9. Theproduct formed in the hydrolysis is DDE. Hydrolysis of DDE and DDD is not a significant fate process (EPA, 1979c). Biodegradation of DDT in water is reported to be a

minor mechanism of transformation (Johnsen, 1976).Biodegradation of DDE and DDD in the aquatic environment is slower than that of DDT (EPA , 1979c).

2.3.3: Breakdown in vegetation:

2.3.3.1: Breakdown of chlorpyrifos in vegetation

Chlorpyrifos may be toxic to some plants, such as lettuce (McEwen and Stephenson, 1979). Residues remain on plant surfaces for approximately 10 to 14 days. Data indicate that this insecticide and its sediment metabolites can accumulate in certain crops (U.S.P.H.S.,2005).Chlorpyrifos is not expected to be taken up from sediment through the roots of plants (Tomlin, 2006). Chlorpyrifos was applied to the leaves and fruit of orange and grapefruit trees, and residues and dissipation on the rinds were measured using gas chromatography. Chlorpyrifos residues on fruit rinds were found to dissipate quickly, with initial mean half-lives of 2.8 days in oranges and 3.7 to 6.7 days in grapefruit, at which point residues were at or below 2ppm. Chlorpyrifos residues were not found above levels of detection (0.03 ppm) in the edible portion (pulp) of citrus fruits tested (Iwata *et al*., 1983).

Though some chlorpyrifos may be taken up by plants through leaf surfaces, much of the applied chlorpyrifos is usually lost through volatilization, and very little is translocated throughout the plant. Chlorpyrifos taken up by plant tissues is primarily metabolized to TCP, which is then stored as glycoside conjugates. Foliar applied chlorpyrifos on leaf surfaces is lost primarily by volatilization. Studies report chlorpyrifos residues remain on plant surfaces for 10 to 14 days after application (Kamrin, 1997).

Although most of the chlorpyrifos applied to plants is lost through volatilization or converted to TCP and sequestered, desulfuration to the chlorpyrifos oxon on plant surfaces has been reported (Roberts and Hudson, 1999).

2.3.3.2: Breakdown of DDT in vegetation

Plants can act as significant pathways for DDT exposure to receptors in the ecosystem. Uptake into plants is the first step towards the bioaccumulation of DDT in the terrestrial food web (Trapp, 1993). Three main pathways for chemical movement from sediments to plants exist: root uptake into conduction charnels and subsequent translocation, uptake from vapor

in the surrounding air. And uptake by external contamination of shoots by sediment and dust, followed by retention in the cuticle or penetration through it (Bell andFailey, 1991).

Despite being strongly bound to sediment, DDT, DDE, and DDD can be bioavailable to plants (ASTDR, 1994). Verma and Pillai (1991) reported that grain, maize, and rice plants can accumulate sediment-bound residues of DDT. The majority of residues were found in roots of plants, and the lowest concentration of DDT residues was found in shoots, indicating low translocation of DDT. Ware*et al*., (1980) found that the epidermal layer of alfalfa roots contained five times the amount of DDT in whole roots and six times that found in the cortex which suggests that DDT and (or) its degradation products become bound to the root epidermis and thus cannot move inward.

Soi1 characteristics can influence the behavior and fate of ∑DDT compounds in plants. Fuhremam and Lichtenstein (1980) applied '"c-labeled *p,p'-DDT* to loam or sandy sediment and grew oat plants on treated sediments for 13 days. Very little DDT (0.2% of the total DDT applied) and none of its metabolites, were detected in oat roots grown in loam. Uptake was greater (4.6%) in roots of oats grown on sand, but uptake of labeled carbon into plant tops, from both sediments, was below detection limit. The low uptake of DDT by plants is consistent across taxa. Experimentally-derived bioaccumulation factors (BAFs) for DDT residues in plants are generally below 1.00and often below 0.50 (Jongbloed *et al*., 1996).

2.4: Factors influencingdissipation of chlorpyrifos and DDT

Fate of pesticides in the environment will differ among pesticides, field and season as processes governing fate such as volatilization, photolysis, sorption, hydrolysis and biodegradation are influence by physico-chemical properties of the pesticide and environmental conditions.

2.4.1: Pesticide volatilization

Volatilization is the process whereby pesticides evaporate from sediment, foliage or surface waters. Fumes and vapors in the air can move away from the site of application and reach non-target vegetation or sediment. It is the main dissipation route for many pesticides and it has been confirmed that up to 90% of applied pesticides volatilize from the sediment and

surface waters within a few days after application (Majewski and Capel, 1995). Pesticides dosed in the sediment volatilize as a result of interaction between adsorption-desorption of the chemicals from sediment particles and organic matter into the solution phase, as well as convection and diffusion at the sediment atmosphere interface (Lalah et al., 2001).

2.4.1.1: Chlorpyrifos volatilization

Chlorpyrifos has a moderately high vapor pressure of 1.8-2.0 x 10^{-5}mm Hg at 25^{0}C, (Racke, 1993). Volatilization from sediment depends on a number of environmental factors such as temperature, formulation, and sediment properties. In other countries, the rate of chlorpyrifos volatilization from sand and silt loam sediment is higher in the first 8 days after application, where 2.6 and 9.3% of applied chlorpyrifos are volatilized one month after application. Whang et al. (1993) investigated a number of pesticides including chlorpyrifos, fonofos, and atrazine from conventional and no-till surface sediments in the field. They found that as much as one-half of the chlorpyrifos and fonofos respectively were volatilized from no-till surface sediments within 26 days.

2.4.1.2: Volatization of DDT

Volatilization of DDT, DDE, and DDD are known to account for a considerable loss of these compounds from sediment surfaces. Volatile loss is most pronounced immediately following DDT application, and with certain land practices. Despite their low vapor pressure and solubility, chemicals such as DDT are subject to evaporative loss (Sunito *et al*., 1988). Of all ∑DDT (∑ is used to mean sum of) compounds, p,p'-DDE has a volatility tenfold greater than p,p'-DDD and fifty-fold greater than p,p'-DDT. A field test of the rate of disappearance of DDT from sediment near Lake Nakuru, Kenya, found that DDT sublimed directly without prior degradation to DDE (Sleicher and Hopcraft, 1984). In India, high sediment temperature, intense sunlight and humidity were identified as the major factors responsible for dissipation by volatilization (Samuel and Pillai, 1989). In tropical climates, volatilization is the predominant fate for DDT. In sandy loam sediment, loss through volatilization has been found to increase five-fold when the temperature increased from 15 to 45°C (Samuel and Pillai, 1989). Loss due to volatilization differs among climates on the basis of such temperature differences. Correction for climatic temperature has been applied to a northern environment to explain the partitioning of ∑DDT in air, Water, sediments, and sediments in Lake Baikal, Russia. Following correction from standard conditions to an average

temperature of 2°C, volatile loss for p,p'-DDT reduced by a factor of five and p,p'-DDE reduced by a factor of ten (Iwata *et al*., 1995).

2.4.2: Photodegradation of pesticides

Once chlorpyrifos is applied, it may be exposed to photodegradative conditions either directly or indirectly. Direct photodegradation can occur from direct absorption of sunlight by chlorpyrifos itself, mostly in the ultraviolet region of the spectrum. Indirect photodegradation can occur when sunlight is absorbed by secondary reagents/substrates such as sediment humic and inorganic substances. These activated reagents, in turn, are capable of reacting with chlorpyrifos (vanLoon and Duffy, 2005).

2.4.2.1: Chlorpyrifos photodegradation

Photodegradation rates depend on a number of factors including the wavelength and intensity of light, the transparency of the medium, and the properties of the environment itself. Klisenko and Pis'mennaya (1979) reported a half-life of 136 minutes for chlorpyrifos exposed to an artificial light source. A half-life of 2.2 days for chlorpyrifos on glass plate surfaces exposed to artificial sunlamps has been reported (Chen *et al*., 1984; Chen, 1985). However, on a dry sediment surface, chlorpyrifos is quite resistant to photodegradation (Getzin, 1981b). Walia *et al*. (1988a) reported a half-life of 13.7 days on glass, 17.2 days on moist sediment, and 52.6 days on the surface of Polystichum setiferum (one species of plant) upon exposure to 254 nm irradiation. In aqueous solution, Meikle *et al*. (1983) investigated the photolysis-hydrolysis rate of chlorpyrifos at pH 5.0, 6.9, and 8.0 aqueous buffers exposed to ultraviolet radiation. In these buffers, the half-lives after combined photolysis-hydrolysis were 11.0, 12.2, and 7.8 days, respectively, and the corresponding calculated photolysis half-lives were 13.9, 21.7 and 13.1 days, respectively. Photodegradation products of chlorpyrifos are show in Figure 2.1.

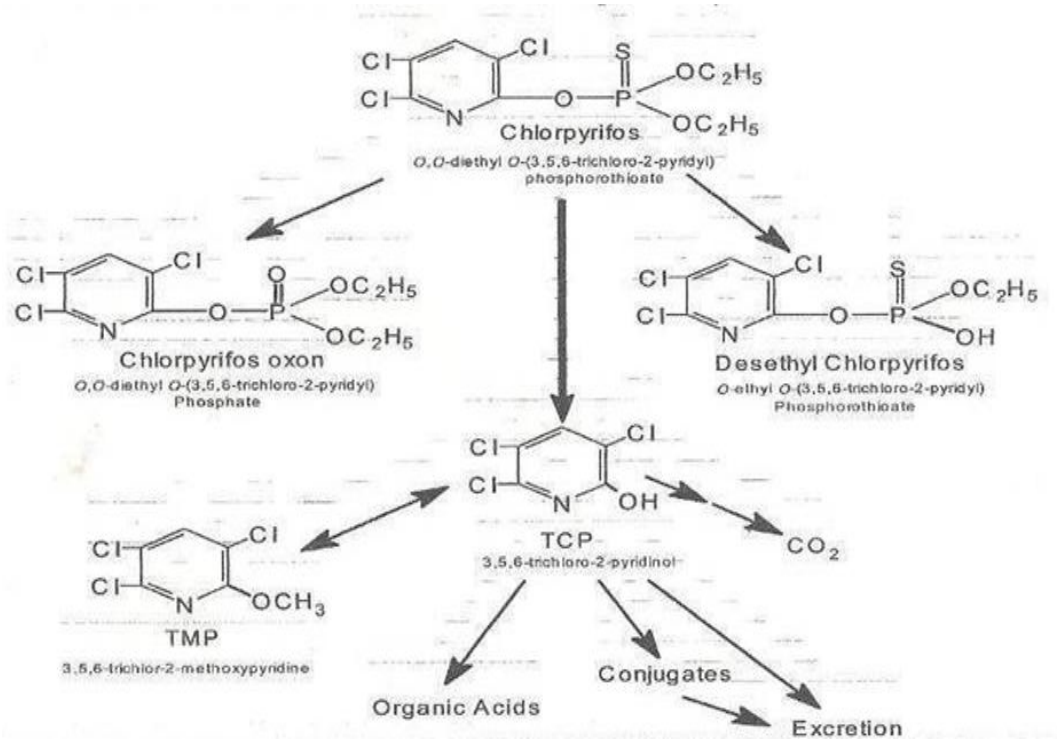


Figure 2.1: Generalised pathways of photo-transformation of chlorpyrifos (Racke, 1993)

The pathways of chlorpyrifos photodegradation are not clearly understood, but partial photodegradation products have been isolated and identified (Meikle *et al.*, 1983; Walia *et al*., 1988b). A metabolite, 3,5,6-trichloro-2-pyridinol (TCP) has been identified as a photolysis metabolite on glass, sediment, and leaf surfaces (Walia *et al*., 1988b). The researchers determined nearly 14.5% of chlorpyrifos was converted to TCP in light, while only approximately 0.5% was converted to TCP under dark conditions. However, TCP is not considered to be a major photolysis product in aqueous solutions (Meikle *et al*., 1983) because of the photo instability of TCP. Smith, (1966) reported 100% photodegradation of TCP at pH 8 aqueous buffer within 24 hours. Approximately 17% of applied TCP is converted to carbon dioxide, while the rest is postulated to be a series of partially dechlorinated pyridine-based diols and triols. Walia *et al.* (1988b) investigated the photodegradation of chlorpyrifos in hexane, methanol, and on glass, leaf surfaces and sediment. They isolated and identified a range of photodegradation products that are soluble either in hexane or methanol. Those found in hexane included: O,O-diethyl-O-(3,5-dichloro-2-pyridyl) phosphorothioate, O,O-diethyl-O-(3,6-dichloro-2-pyridyl) phosphorothioate, O,O-diethyl-O-(5,6-dichloro-2-pyridyl) phosphorothioate and O,O-diethyl-O-(monochloro-2-

pyridyl) phosphorothioate. Photolysis products found in methanol extracts included chlorpyrifos oxon, andtwo methylated products O,O-diethyl-O-(3, 6-dichloro-5-methoxy-2-pyridyl) phosphorothioate andO,O-diethyl-O-methyl phosphorothioate. They also proposed a photodegradation pathway for the various experimental conditions.

2.4.2.2: DDT photodegradation

2,2-bis(4-chlorobiphenyl)-1,1,1-trichloroethane (p,p'-DDT), which is one of the most prevalent chloro organic pollutants, provides upon UV irradiation a variety of photoproducts depending on the environment (Hong *et al*., 1997), such as p,p'-DDE or p,p'-DDD (Figure 2.2). Reductive dechlorination and hydrolysis are the main degradation pathways of DDT photolysis on the wet silica gel surface (Llompart *et al*., 2003), whereas photodegradation on aquatic plant Elodea undergoes via reductive dechlorination only (Garrison, 2000) UV-irradiation of ^{14}C-p,p' DDT on sediment for 10 hours mineralized less than 0.1% of the initial amount (Vollner and Klotz, 1994). Figures 2.2shows photodegradation products of DDT.

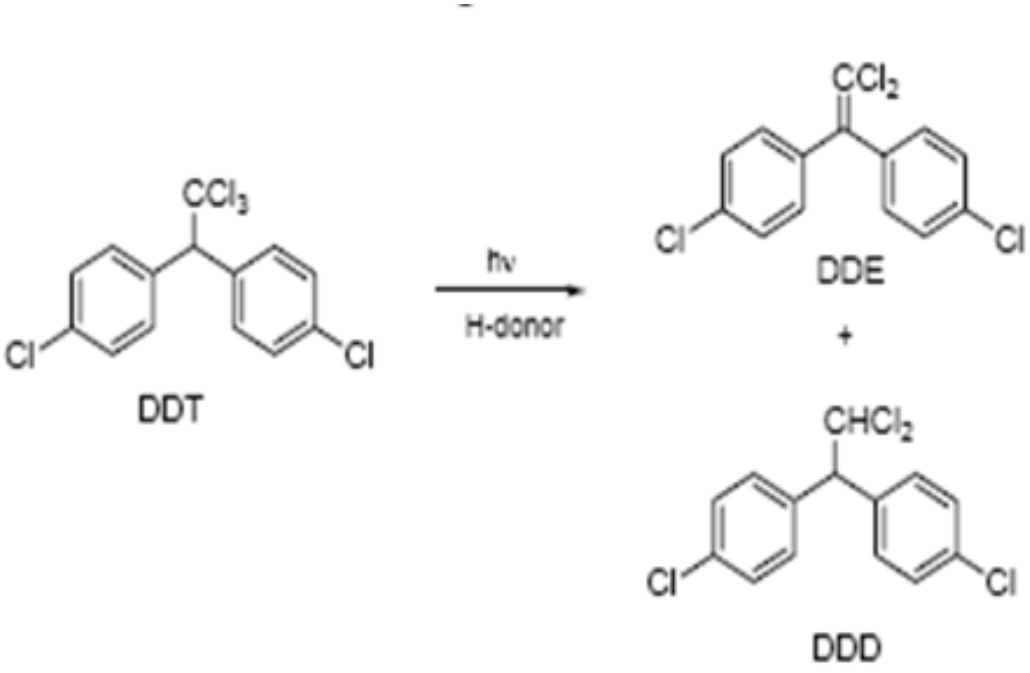


Figure 2.2: Photodegradation of DDT (Vollner and Klotz, 1994)

2.4.3. Hydrolysisof pesticides

Hydrolysis is a chemical process where molecules react with water causing replacement by the hydroxyl group of water (OH-) in an interacting molecule. Hydrolysis reactions can occur either by purely chemical or microbiological mechanisms (Connell, 2005; vanLoon and Duffy, 2005).

$R\text{-}X + H_20 \rightarrow R\text{-}OH + HX$.....eq 1

where "R" represents a hydrocarbon group and "X" represents a halogen atom or ester group or analogue of an ester group (amide, thioester).

Most chemical degradation reactions in sediment are mediated through water which act as a reactant or provides a reaction medium (Lalah *et al.,* 2001). Some of the chemical reactions involved in pesticide degradation in sediment include hydrolysis, oxidation/reduction, isomerization and nucleophilic substitution reactions with reactive groups of sediment organic matter, and free radical mechanisms (Lalah *et al.,* 2001). Some of these reactions are catalyzed by clay surfaces, metal oxides and metal ions in sediment. The degradation of organophosphorous pesticide such as chlorpyrifos proceeds by hydrolysis of the P-XA bond, where X=O or S atom and A is the electron attacking moiety of the pesticide molecule (Figure 2.3).

2.4.3.1: Hydrolysisof chlorpyrifos

The hydrolysis of chlorpyrifos is an important process in the degradation of organophosphorous insecticides, usually resulting in an increase in the number of polar metabolites and a reduction in acute toxicity(Connell, 2005; vanLoon and Duffy, 2005).Two mechanisms of chlorpyrifos hydrolysis may occur due to pH effects: neutral hydrolysis and alkaline hydrolysis. Neutral hydrolysis involves nucleophilic attack of water at the ethoxy carbon, hydrolyzing chlorpyrifos to deethylchlorpyrifos and ethanol. Alkaline hydrolysis of chlorpyrifos involves in the phosphorus atom which is attacked by the nucleophilic hydroxide ion.

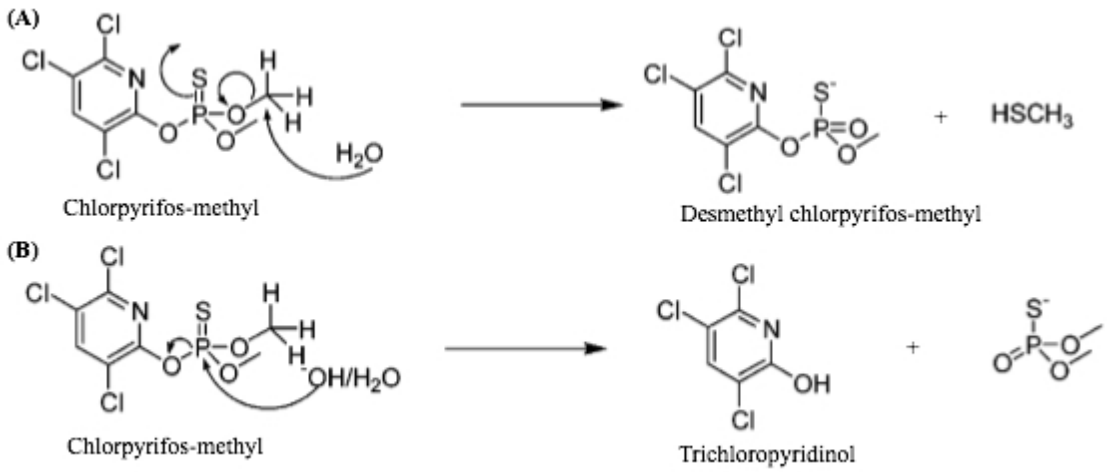


Figure 2.3. Schematic illustrating hydrolysis mechanisms for chlorpyrifos
(A) neutral and (B) alkaline hydrolysis . Adapted from Wu (2006b).

Two possible mechanisms are responsible for the hydrolysis of chlorpyrifosmethyl: neutral hydrolysis involving the nucleophilic attack of H_2O at the carbon atom of the methoxy group (Liu, 2001; Jans, 2003), and the more common nucleophilic substitution of OH- and H_2O at the phosphorus atom, cleaving the P-O bond (Pehkonen, 2002; Jans, 2003;) (Figure 2.4). Both desmethyl chlorpyrifos-methyl and trichloro-2-pyridinol (TCP) are formed due to hydrolysis depending on the reaction pathway (Figure 2.4). Alkaline-catalyzed hydrolysis occurs via an S_N2 mechanism and should therefore be pH dependent. Although studies have shown increased rates of hydrolysis in alkaline solutions (Jans, 2003) the effect is not significant at environmentally relevant (5.4 to 8.6) pH values, as demonstrated by this study and others (Liu, 2001; Jans, 2003).

The hydrolysis rate of chlorpyrifos is pH dependent. The pH plays an important role both in the characteristics of pesticide and generation of hydroxyl radicals. With increased pH the degradation also increases but only up to certain level. At pH greater than 7.5-8.0, hydrolysis rates increase rapidly (Meikle and Youngson, 1978; Macalady and Wolfe, 1983). Hydrolysis increases fairly consistently with increased pH. Meikle and Youngson (1978) reported that chlorpyrifos degrades in distilled water with half-lives of 22.8, 35.3, and 62.7 days at pH 8.1, 6.9, and 4.7, respectively. Freed *et al*. (1979) reported that the half-lives of chlorpyrifos degradation in water were 120 and 53 days at pH 6.1 and 7.4 at 20^0C, respectively. Chapman and Cole (1982) have reported on chlorpyrifos hydrolysis in sterile 1% ethanol phosphate buffer at pH 4.5 to 8.0. They found that the half-life of chlorpyrifos in this medium was 77

days in highly acidic conditions (pH 4.5 and 5.0), but the half-life consistently decreased from 77 days to 19 days at pH 5.0 to 8.0. The second hypothesis suggests that the rate hydrolysis rate of chlorpyrifos is fixed from acidic to neutral conditions, while it proceeds at an increased rate under alkaline conditions. Macalady and Wolfe (1983) investigated the hydrolysis of chlorpyrifos over the pH range of 1-13 in distilled water, aqueous buffer, 50% methanol-water, and 50% acetonitrile-water solutions. They reported a constant rate of hydrolysis from pH 1 to pH 7 at 25^0C. The rate of hydrolysis increased above pH 7.5 with half-lives reduced from an average of 77.4 days over pH range 1-7.5, to 10.2 and 0.5 days at pH 9.8 and 11.1, respectively.

Increased rates of chlorpyrifos degradation occur at higher temperatures. A 3.5-fold increase in hydrolysis rate has been reported for each 10^0C rise in temperature (Meikle and Youngson, 1978). The half-lives of chlorpyrifos hydrolysis at pH 7.4 were 53 and 13 days at 20^0C and 37.5^0C,respectively (Freed *et al*., 1979). Increased temperature elevates the energy of nucleophilic attack on chlorpyrifos molecules, which has an average 21.1 kcal M^{-1} activation energy for the hydrolytic reaction (Meikle and Youngson, 1978).

There are three bonds in the chlorpyrifos molecule that are subject to cleavage during the hydrolytic processes: two tertiary alkyl ester bonds and one phosphate ester bond. Macalady and Wolfe (1983) reported that TCP and O,O-diethyl phosphorothioic acid were the major metabolites of chlorpyrifos hydrolysis in aqueous buffers and aqueous/polar solvent mixtures over a pH range of 9-13. When temperatures were elevated to $70\text{-}80^0$C, the major products were ethanol and desethyl chlorpyrifos, with a smaller amount of TCP and diethylthiophosphate at pH 7.68. The metabolite species formed during hydrolysis are also affected by the pH of the medium. McCall (1986) reported a relatively constant amount of TCP (13.2-14.35%) and desethyl chlorpyrifos (16.4-17.7%) formed over the pH range of 5 to 7 in buffered distilled water. However, the percentage of TCP increased up to 47.9% while that of desethyl chlorpyrifos remained at a level of 12.5% at pH 9. Thus, the alkaline pH favored TCP production, and the hydrolysis rate increased when compared with neutral or acidic conditions (Figure 2.4).

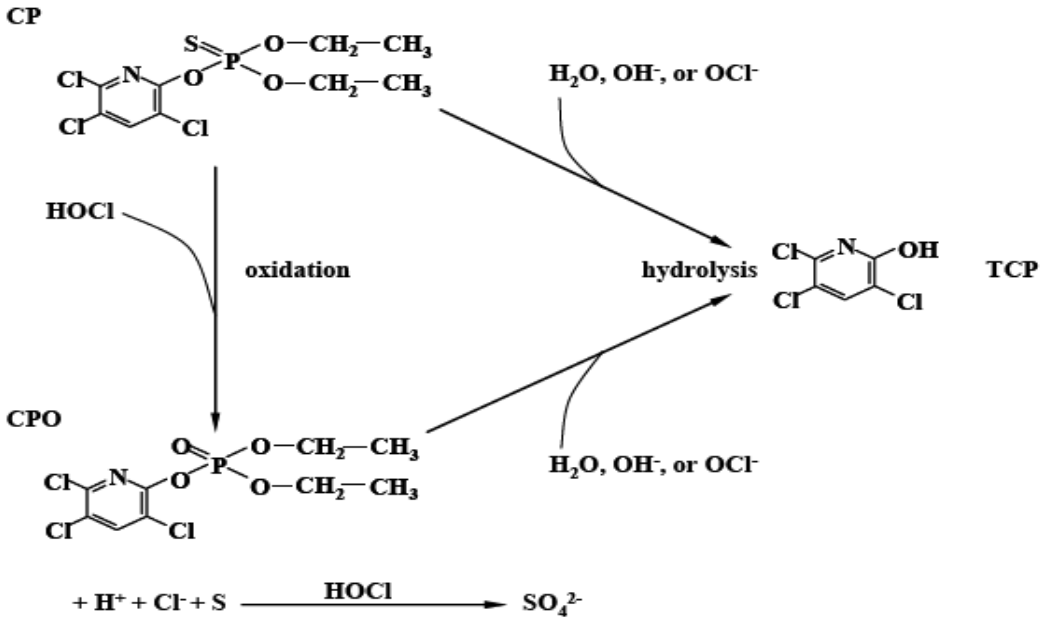


Figure 2.4: Simplified schematic diagram of chlorpyrifos degradation pathways in the presence of chlorine at near neutral and alkaline pH conditions

(Macalady and Wolfe, 1983)

CP-chlorpyrifos, CPO-chlorpyrifos oxon and TCP-3,5,6-trichloro-2- pyridinol

Hydrolytic breakdown contributes to the loss of chlorpyrifos that is dissolved in water and adsorbed on the suspended organic matter in the water. Chlorpyrifos typically breaks down by cleavage of the phosphate ester group to form its primary metabolite 3,5,6-trichloro-2-pyridinol (TCP).

Several studies have shown rapid degradation of chlorpyrifos in natural waters compared to distilled or tap waters (Thomas and Mansigh, 2002). However, in natural waters with high organic concentrations, the loss of chlorpyrifos can be slower because of the presence of organic matter that binds chlorpyrifos and slows its decay (Wu and Laird, 2002). It may be possible however, for turbid water to have a high rate of chlorpyrifos loss because of close positive relationship between microorganism population size and the amount of dissolved or particulate organic matter in water (Rao *et al.,* 1991). The presense of organic matter may thus permit increased microbiol degradation. Indeed Bondarenko *et al*., (2004) showed that microbiol transformations could be responsible for between 50% and 80% of the breakdown of chlorpyrifos in natural waters. Figure 2.5bellow showsdegradation pathway of chlorpyrifos in water.

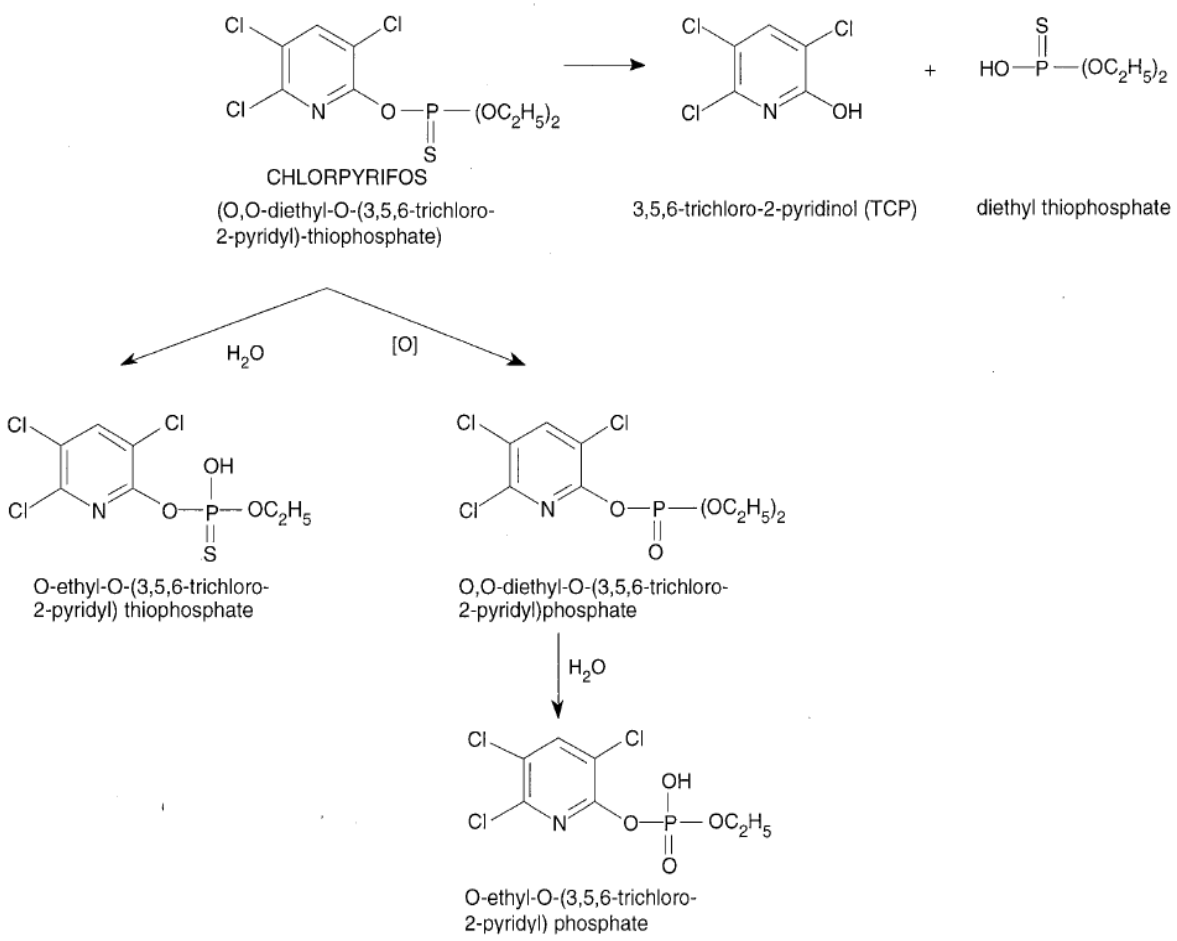


Figure 2.5: Degradation pathway of chlorpyrifos in water (Racke, 1993)

2.4.3.2: Hydrolysis of DDT

There are several different factors which influence the process in a positive way. The main and most important attributes as mentioned in the previous paragraph are the amount of oxygen added to the system, the appropriate temperature and the neutral pH of the sediment-water solution, and sufficient moisture, just as sufficient and appropriate nutrients (Vidali, 2001). According to Aislabie *et al*. (1997), flooding of sediment and additional organic matter can improve the degradation of DDT (Aislabie *et al*., 1997).

Several studies have shown that the pH of the sediment affects degradation rate of DDT. Most studies suggest that the sediment pH most competent for the best grade of degradation is around pH 7 (or neutral pH) (Marve and Dupont, 2001) and usually below this range the breakdown is slowed down (Andrea *et al*., 1994). pH can be regulated using buffer solutions. Buffer M8 stock solution was used to guarantee the right pH (Muter *et al*., 2008).

The rate of the degradation increases with rise in temperature, as proven in studies completed under tropical circumstances. At these climates most of the DDT dissipate through volatilization (Hussain *et al*., 1994). The role of temperature is important as well in the bioreactor experiments; mostly the aim is keeping the temperature on approximately room temperature, for the best results not below 10°C (Marve and Dupont, 2001) but between 15-45°C (Vidali, 2001). Some studies suggest temperatures 40°C, or higher, as the desirable temperature for the best degradation rate of DDT (Guenzi and Beard, 1976). The rate of the biochemical reactions rise with temperature rise, although above a certain temperature, the microbial cells decrease (Vidali, 2001).

In microcosm experiments, Boul (1995) found that increasing sediment water content enhanced DDT loss from generally aerobic sediment. His results suggested that increased biodegradation contributed to the enhanced DDT dissipation. In laboratory experiments with marine sediments, DDT has been shown to degrade to DDE and DDD under aerobic and anaerobic conditions, respectively (Kale *et al*., 1999) and that DDE is dechlorinated to DDMU under methanogenic or sulfidogenic conditions (Quensen *et al*., 1998). The rate of DDE dechlorination to DDMU was found to be dependent on the presence of sulfate and temperature (Quensen *et al*., 1998). DDD is also converted to DDMU, but at a much slower rate. DDMU degrades further under anaerobic conditions to DDNU and other subsequent degradation species, such as DDOH and DDA, through chemical action (Ware *et al*., 1980; Heberer and Dunnbier, 1999) (Figure 2.6).

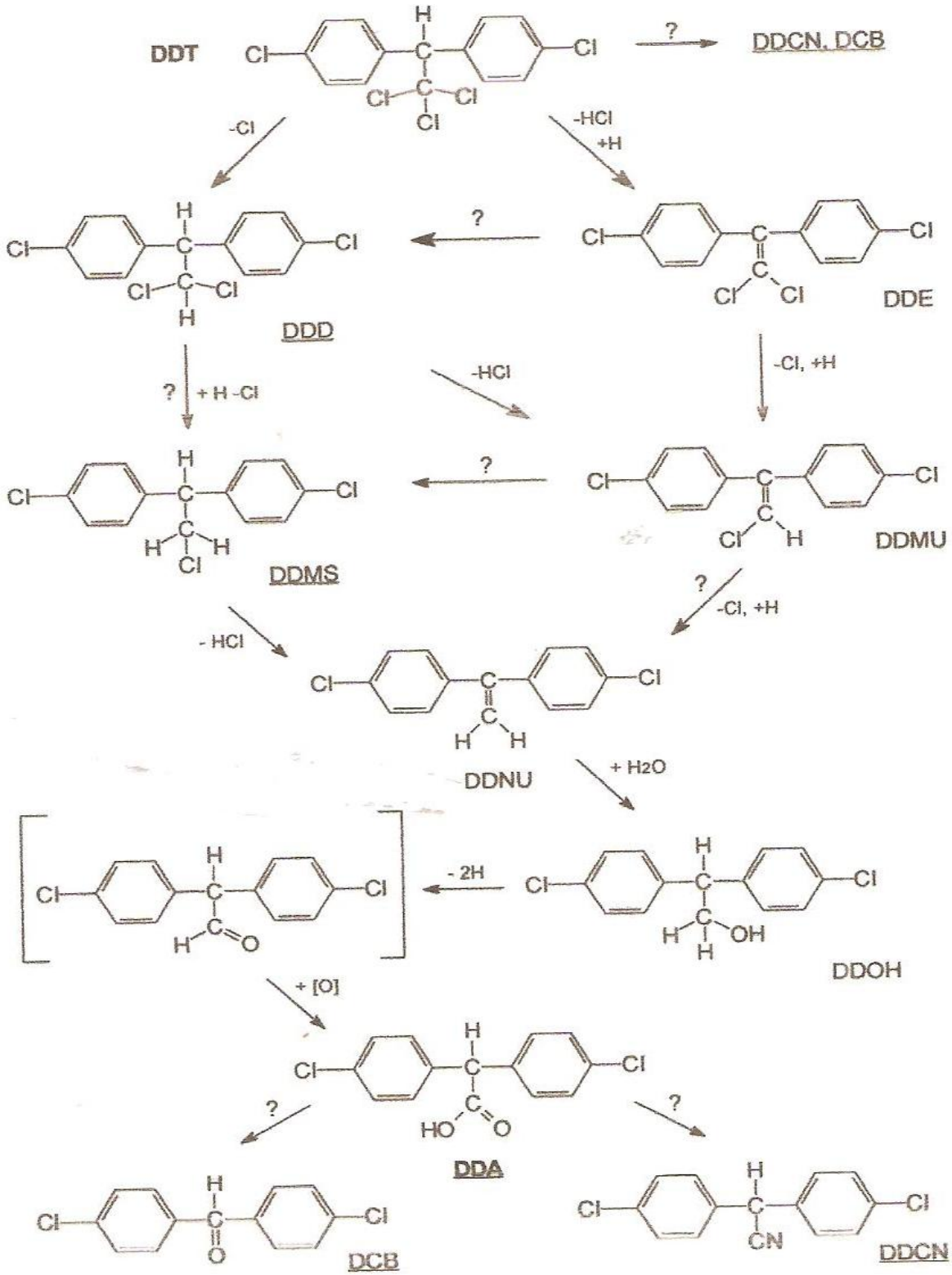


Figure 2.6: Proposed metabolic pathway of DDT in water (Heberer and Dunnbier, 1999).

Derivatives undelined are key metabolites found in aqueous phase.

Oxygen is inevitable for the aerobic degradation of the contaminants, as it works as electron acceptor in the process. Typically solubility of oxygen cannot exceed 1 mg/L in aqueous solution, considering that above this amount the microbial reactions are limited. This means aerobic, minimum air-filled pore space of 10%. The amount of the accessible oxygen will determine whether the system is aerobic or anaerobic. Air can be sprayed in the system, if the oxygen supply is not sufficient. The advantages of using oxygen as electron acceptor is that oxygen accelerates degradation, oxidized end products at the end of the process are non toxic, Oxygen increases system stability and lastly Oxygen causes efficient system performance (Marve and Dupont, 2001).

The nutrients and oxygen will stimulate the microorganism growth, which is essential to reaching the right degradation rate. The nutrients will let the microbes produce the necessary enzymes, which will degrade the contaminants. Carbon is the most needed nutrient, followed by nitrogen, oxygen, hydrogen and phosphorus. The most desirable ratios are carbon to nitrogen 10:1, and carbon to phosphorus 30:1. Sulfur, potassium, sodium, calcium, magnesium, chloride, iron are other important elements building up the microbial cells (Vidal, 2001).

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2.5: Degradation kinetics of pesticides

2.5.1: Rates of biotransformation reactions

A number of rate equations, including first-order and second-order as well as hyperbolic functions have been used to describe pesticide transformation in the environment (Wolfe *et al.*, 1990). First order rate equations, where the dissipation of a pesticide is proportional to the amount remaining in the sediment have been found adequate to describe the fate of some pesticides (Scheunert, 1992a). There are, however, limitations to use of first-order equations in cases where several reaction mechanisms and several populations of organisms may be responsible for degradation process. In the case of biotic reaction, for example, first-order kinetics requires, among other things, that population of degrading organisms remain stable, that pesticide concentration be very low and that the pesticide is the sole carbon source for the degrading organism. In sediment, many of these conditions may not be met in that; pesticide may be toxic to sensitive microbial population particularly at high concentration or

most sediment microbes are capable of using both pesticide and sediment carbon or sediment carbon alone as energy source (Scheunert, 1992a).

In addition, as was previously mentioned, many pesticide degradation reactions do not follow first-order kinetics in that there are often two distinct phases, a lag phase and a phase of rapid dissipation (Saltzman and Yaron, 1986). The opposite pattern has also been observed where a pesticide may dissipate rapidly immediately following application after which dissipation may proceed at a slower pace. In this case the initial rapid phase may be attributed to physical processes, such as volatization and transport to deeper sediment layers, whereas the second phase may be due to slower metabolic processes (Scheunert, 1992a). Hyperbolic and high-order rate expressions, as well as a number of empirically derived equations, have been formulated to better describe the degradation kinetics of various pesticides in cases where first order kinetics are not observed (Scheunert, 1992a).

2.5.2: Calculation of degradation rate and half life of dissipation of pesticides

The entire discussion will be based on n^{th} order reaction represented by

$$a\,A \longrightarrow \text{Products} \quad \ldots\ldots(2)$$

Where A=Single reactants and a=Stoichiometric coefficient

Then rate law of the reaction can therefore be written as:

$$Rate = -\frac{d[A]}{adt} = k\,[A]^n \quad \ldots\ldots(3)$$

Hence

$$\frac{d[A]}{[A]^n} = -akdt \quad \ldots\ldots(4)$$

Where

[A] – concentration of species A, dt – change in time and k – rate constant

When n≠1, then integration of equation (3) gives;

$$t_{\frac{1}{2}} = \frac{2^{n-1}-1}{(n-1)[A]_0^{n-1}ak} \quad \ldots\ldots(5)$$

for n≠1 $\log t_{\frac{1}{2}} = \log\frac{2^{n-1}-1}{(n-1)k} - (n-1)\log[A]_0$

Plot of log $t_{\frac{1}{2}}$ verses log $\left[A\right]_0$ gives a straight line of slope (1-n) and thus the reaction order can be obtained from the slope. When *n* = 1 the slope is zero.

Assuming that in equation **(3)** a=1

For Zeroth order n=0 then equation 2becomes;

$$\left[A\right]-\left[A\right]_0 = -kt \quad \text{……(6)}$$

At $t_{1/2}$, $[A] = \frac{[A]_o}{2}$ and $t = t_{1/2}$, thus (5) becomes;

$$\frac{\left[A\right]_0}{2} = \left[A\right]_0 - kt_{\frac{1}{2}} \rightarrow t_{\frac{1}{2}} = \frac{\left[A\right]_0}{2k} \quad \text{For n=0……(7)}$$

Since, according to Yang *et al*. (2006), pesticide dissipations are first order reactions,

When (n = 1), then equation (3) reduces to:

$$\frac{-d[A]}{[A]} = kdt \quad \text{……(8)}$$

If $[A]_o$ is the initial concentration of the reactant at time t = 0 when the reaction commences and [A] the concentration at any subsequent time t, equation (8) when integrated;

$$-\int_{[A]_o}^{[A]} \frac{d[A]}{[A]} = k\int_0^t dt \longrightarrow In\frac{[A]_o}{[A]} = kt \longrightarrow In[A] = In[A]_o - kt \quad \text{……(9)}$$

When compared to the equation of a straight line

$y = b + mx$

Then $In[A]$ = y, $In[A]_o$ = b and $-k$ = gradient

Thus a plot of $In[A]$ against time (t) will give a straight line that can satisfy the above condition.

The application of equation (9) in expressing half life is as follows:

At $t_{1/2}$, $[A] = \frac{[A]_o}{2}$ and $t = t_{1/2}$, thus (9) becomes;

$$In\frac{[A]_o}{2} = In[A]_o - kt_{1/2} \rightarrow kt_{\frac{1}{2}} = \ln 2 = 0.693 \rightarrow t_{1/2} = \frac{0.693}{k} \quad \text{..... (10)}$$

(SAS Institute, 2001)

For second order, n=2 then;

$$\text{Rate} = k[A]^2 = -d\frac{[A]}{dt} \rightarrow -\frac{d[A]}{[A]^2} = kdt \quad \text{..... (11)}$$

Integrating equation (10) within the limits of concentrations and time $\lim_{0 \rightarrow t}$ *and* $\lim_{[A]_0 \rightarrow [A]}$

$$\int_{[A]_0}^{[A]} [A]^2 d[A] = -k\int_0^t dt \rightarrow \frac{1}{[A]} = kt + \frac{1}{[A]_o} \quad \text{..... (12)}$$

From equation (12) half life for third order (n=3) will be;

$$\frac{2}{[A]_0} - \frac{1}{[A]_0} = kt_{\frac{1}{2}} \rightarrow t_{\frac{1}{2}} = k\frac{1}{[A]_0} \quad \text{..... (13)}$$

For third order, n=3 then;

$$\frac{-d[A]}{dt} = k[A]^3 \rightarrow \frac{1}{[A]^2} - \frac{1}{[A]_0^2} = 2kt \longrightarrow t_{\frac{1}{2}} = \frac{3}{2k[A]_0^2} \quad \text{..... (14)}$$

It should be noted that equation (3) could be used to solve half-lives for n≠1 directly.

Half life is the time required for 50% of the compound to disappear, which is being determined by first-order or pseudo first- order reaction (Drossman *et al*., 1988). The residue data were subjected to regression analysis and the fit of the data to first order kinetics was confirmed by testing the statistical significance of correlation coefficient. The half-life values were calculated from dissipation constant calculated from regression analysis.

2.6: Gas-chromatography and analysis of pesticide residues

Gas chromatography (GC) is one of the most versatile and ubiquitous analytical techniques and several workers have used it for determination of organic/pesticides (Luthje *et al.*, 2005). In this technique, over 40 detectors have been developed according to selectivity and sensitivity of the particular type of substances for their analysis (Christian, 2004). For example Nitrogen phosphorus detector (NPD) for nitrogen and phosphorous containing compounds (Choudhury *et al*., 1996) and electron capture detector (ECD) which is extremely sensitive for halogenated compounds (Ueno *et al*., 2004a). The two detectors were used for chlorpyrifos and DDT, respectively. Dual detectors are also being are also being used for accuracy of the results (Ueno *et al*., 2004a).

Gas chromatography–mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. The use of a mass spectrometer as the detector in gas chromatography was developed during the 1950s after being discovered by James and Martin in 1952 (Robert and Adams, 2007). The development of affordable and miniaturized computers has helped in the simplification of the use of this instrument, as well as allowed great improvements in the amount of time it takes to analyze a sample.

2.7:Activitystudy

2.7.1: General statistics on malaria in Kenya

Malaria is a major contributor to global burden of disease and a significant impediment to socioeconomic development in poor countries. It is estimated that 300 to 660 million clinical attacks of malaria occur globally (Geissbűhler et al., 2007) and result in over a million deaths (Hetzel et al., 2007), over 80% of these deaths occurring in Africa (Geissbűhler et al., 2007). In Kenya, the disease accounts for 30% of all outpatient cases and 19% of all admissions, 5.1% of these patients die, and 72 children die daily before the age of 5 years (Kuria *et al*., 2002). The statistics showing malaria cases in Kenya can be found in Appendix III.

2.7.2: Mosquito larval habitat and its attributes

Mosquito larvae occupy a wide range of habitats in diverse environmental conditions. Aquatic environments differ chiefly in the chemistry of the water (acid or alkaline; fresh, salt

or brackish). These environments may be natural or man-made and may also differ in the amount or type of vegetation present and the amount of sun or shade. Abundance of different mosquito species in a water body may differ depending on the geographic location, water level fluctuation as well as perpetual presence of water, size of water body, vegetation, predator abundance and organic composition of the water (Russel, 1999). Standing water habitats like freshwater marshes, lakes, ponds and drainage ditches are exploited mainly by *Anopheles* (Trape and Zoudani, 1987). Usually, mosquitoes exploit small shallow water bodies which are high in nutrients and salinity and low in dissolved oxygen content (Tenessen, 1993). In such habitats mosquitoes have higher rates of survival due to abundant food source and low predator populations (Tenessen, 1993, Sarneckis, 2002). Malaria is predominantly a rural disease because the larvae of its *Anopheles* vectors cannot tolerate polluted water. However, in the suburbs around many African cities *Anopheles gambiae* penetrates (Trape and Zoudani, 1987) especially where there are marshy patches or cultivation with irrigation. Cities located near rivers, lakes, dams and other water bodies such as Kisumu City also have high cases of malaria.

Among the abiotic factors, mosquitoes usually prefer high air humidity. Rainfall can be both a limiting factor as it may flush out breeding places and a positive factor as it fills up water bodies and hence providing more potential habitats. Effect of sunlight or shade varies depending on the species (Fritsch, 1997). Physico-chemical water quality factors are difficult to quantify with respect to mosquito abundance. Studies have shown that mosquitoes are present in highest density when the average water temperature is between 23 °C and 33 °C (Fritsch, 1997). Among the chemical factors, orthophosphate, ammonia nitrogen, and dissolved solids are positively correlated with overall mosquito abundance, while chloride and dissolved oxygen appear to be inversely correlated (Muturi *et al*., 2008). *Anopheles*prefer clean water (Pathak *et al*., 2002). Mosquito larvae in natural waters are usually inhibited by extremes of pH conditions and occur mostly between the pH ranges 5.8 and 8.6 with *Anopheles* having higher range than Culicines.

Biotic factors like vegetation type and proportion of coverage are implicated as being better predictors of larval abundance than the physicochemical factors (Walton *et al*., 1990). The presence of vegetation and floating plants provide optimal breeding conditions by acting as food sources as well as shelter from predators. Vegetation also creates stagnant conditions by

decreasing water movement. The abundance of a number of mosquito species is linked to the presence of specific plants (Fritsch, 1997). *Justicia flava* (Forssk.) Vahl of the family Acanthaceae is found growing in disturbed habitat, on a wide range of sediment types and in full sun or semishady areas. It is widespread in tropical and southern Africa. It is widespread in tropical and southern Africa. It tolerates moderate frost. It is able to withstand dry conditions. Its species are widespread in tropical regions of the world (Wasshausen & Wood, 2004) and are poorly represented in temperate regions (Mabberley, 1997). *Justicia* is the largest genus of Acanthaceae, with approximately 600 species that are found in pantropical and tropical regions (Durkee, 1986). That the reason as to why *Justicia flava* was used.

CHAPTER THREE

MATERIALS AND METHODS

3.1: Materials

3.1.1: Chemicals

The solvents: n-hexane, dichloromethane and diethyl ether used were supplied by Kobian Kenya Ltd.Nairobi-Kenya., and were all AR grade. Analytical grade anhydrous Na_2SO_4, and NaCl (both 99% pure), Florisil PR grade, activated charcoal PR grade and Whatman No. 1 filter papers were also obtained from Kobian Kenya Ltd.Nairobi-Kenya and were manufactured by Delhi-b110 020 India, p,p' DDT standard, chlopyrifos standard were obtained from Dr. Erhenstoffer's laboratory in Germany.

3.1.2: Instruments

Instruments used included; Vulcan oven (model A-550, Dentsply International, USA), analytical balance (Sartorius BP 210S, Germany), suction pump (model 7049-05, Chicago, USA), rotary evaporator (Eyela N-100, Japan), Gas Chromatograph (Varian chrompack, CP-3400 and 17A, Japan).

3.2: Study area

The study was carried out in Maseno University Farm, in the field plots which simulated field conditions encountered in actual mosquito larval habitats. The site lies along Kisumu-Busia highway in Maseno Division, Nyanza Province, Western Kenya within the upper Midland 1 agro-ecological zone (Jaetzold and Schimidt, 1982). The first rainy season falls between March and July and the second between September and early December. No month, however, is completely dry making it a suitable site for mosquitoes to breed in (Jaetzold and Schmidt, 1982). It is approximately 1500 m above sea level and lies betweenlatitude 0^0 1' N - 0' 12' S and longitude 34^0 25' E - 47'E. There is significant rainfall throughout the year in Maseno. The average temperature is 20.6 °C and average annual rainfall is 1792 mm.

3.3: Sampling and sample preparation

3.3.1:Sedimentsamples

The sediment samplesused in this experiment were collected in black polythene bags from fields with no pesticide application history. The sediment samples were scooped in triplicates using stainless steel, randomly in a pond near Maseno University Dairy farm located at latitude 00º 00.303'S and longitude 034º 35.38'E;1,508.7m above the sea level. The sedimentwas obtained by scraping the upper ca. 10 cm of the surface sediment at the bottom of a pond using a shovel. Prior to sampling, metals scoops were heatedto 300°C for eight hours and wrapped in aluminium foil to ensure scoops were free of organic material.

The moist sedimentsample was carried in black polyethylene bags. All the stones, pebbles and plant debris were removed from the sample collected. Samples were dried in the oven at 60°C overnight, well mixed and sieved. Approximately 4kilogram was collected as a laboratory sample and the remaining portion was stored. The laboratory sample (4000g) was ground and homogenized, in a mortar and pestle and passed through a 2 mm pore sieve. The sediment(1000 g) was then spiked with 2 mL of a pesticide stock solution containing 1000 mgL^{-1} chlorpyrifos and DDT to give a final concentration of 2 mg kg^{-1} of each pesticide. Sedimentspiked with 2 mL acetone alone was set up as a control and sedimentwithout spiked pesticide mixture was blank sample. Samples for analysis were collected after 0, 3, 7, 14 days and then at longer intervalsof time up to 90 days. At day zero, sediment was collected from a blank and control set to confirm dose levels. Blanks and controls were also sample at the end of the experiment. Distilled water was added to maintain 60-80% field moisture suitable for mosquito larvae.

3.3.2: Water samples

Stagnant water (5 liters) samples were collected in triplicates randomly from the pond in pre cleaned amber bottles with Teflon-lined caps. The samples were transported to the laboratory within 24 hours in cool-boxes. Approximately 4liters was collected as a laboratory sample and the remaining portion was stored. The laboratory sample (4 L) was spiked with 2 mL of a pesticide stock solution containing 1000 mg L^{-1} chlorpyrifos and DDT to give a final concentration of 2 mg L^{-1} of each pesticide in overlying water. Water spiked with 2 mL

acetone alone was set up as a control and water without spiked pesticide mixture was blank sample. Samples were collected after 0, 2, 4, 6 hours and then at longer intervalsof time up to 504 hours. At day zero, sediment was collected from a blank and control set to confirm dose levels. Blanks and controls were also sample at the end of the experiment. Distilled water was added to maintain 60-80% field moisture suitable for mosquito larvae.

The set ups of sediment and water samples were put in glass jars and placed in a green house simulating field conditions at a latitude 00º 00.125'S and longitude E034º 35.592'.1491 m above the sea level. The sample glass jars were checked for water level on a weekly basis and deionised water was added to make up to the mark to compensate for water loss.

3.3.3: Leaf samples

Leaves of *Justicia flava* (Vahl) were treated with DDT and chlorpyrifos, respectively in the field in Maseno University farm .The experimental fields for both chlorpyrifos andDDTmeasured 25 m x 25 m (625 m^2). Three plants in each of the three plots had their leaves spotted with pesticides using a micro pipette and the spotted leaves were marked. Each leaf received 100 µl of the pesticide solution in acetone on a spot having diameter of ca. 15 mm. Sampling was done by cutting the spotted leaf at the petiole with a pair of scissors and wrapped in a polythene bag. All treated leaves in three plots were sampled randomly at every sampling interval. Subsequent sampling was done at the following hours after application of pesticide; 2 h, 4 h, 6 h, 12 h and then at longer intervalsof time upto 672 hours. The pesticides were applied as a single application only. The concencentration of the dossed pesticide was 2ppm. Triplicate samples from each plot were analyzed immediately and where not possible they were frozen at 5^0C for analysis later. A sample of 50 g of *Justicia flava* (Vahl) leaves was usedin theanalysis of pesticide residues according to the method described by DGCCRF (1998). For control, dosing was done using water only.

3.4: Spiking sediment and water samples

The sediment and water samples were treated with solvent acetone containing the two pesticides separately (chlorpyrifos and DDT). In the treatment procedure, 25 mLacetone containing the pesticide was added to 25% of the sedimentsample (250 mg); the flask was closed for 20 minutes to let the solvent disperse. Thereafter the solvent was evaporated for 5 minutes at room temperature, and the samples were mixed with the remaining 75% (750 mg)

of the sedimentsample. All samples were mixed thoroughly with a metal spatula (Brinch *et al*., 2002). The sedimentand water samples were spiked to reach 2 ppm each. Distilled water was added to sediment samplesto maintain 60-80% field moisture capacity and to water samples to compensate for water loss due to evaporation. Following evaporation of the solvent from spiked sediment, the sediment samples were extracted to determine the initial (zero time) of chlorpyrifos and DDT in the sediment, respectively. This served as zero time chlorpyrifos and DDT extraction from the sediment.

3.5: Determination of physicochemical parameters

3.5.1: Determination of physicochemical parametersof sediment samples

3.5.1.1: Determination of the Dry Weight (DW)

The dry weight was determined as follows; three beakers were weighed (and marked as I., II. and III.) (Table 3.1). Three sets of 10 g of wet sedimentsamples were weighed in the beakers. The weights of the glass dishes containing the sediment were measured and the values were recorded. The dishes were placed in the oven (set to 105 °C) overnight. After the sediment was taken out from the oven, the samples were placed in a desiccator (equipped with a vacuum pump) for 1 hour and 20 minutes. This way the rest of the moisture was removed from the sediment samples. The sediment weight was measured, and the amount of the water volatilized from the samples was calculated and from this data the dry weight of sediment was calculated (UNEP, 1984).

Table 3.1:Determination of dry weight of experimental sediment

	Beaker I (g)	**Beaker II (g)**	**Beaker III (g)**
Weight of the beaker (g)	45.495	46.300	54.203
Weight of the sediment and the Beaker (g)	55.515	56.501	64.206
Weight of the sediment (g)	10.020	10.201	10.003
Weight of the sediment after drying(with dish) (g)	49.060	49.911	47.825
Amount of water volatilized from the sample (g)	6.455	6.510	6.378
Dry weight (g)	3.565	3.620	3.625
Mean dry weight (g)	3.603		

3.5.1.2: Particle size analysis

Particle size analysis was accomplished using the hydrometer method as outlined by Lavkulich (1978). The sediment sample (1000 g) was ground and homogenized, in a mortar and pestle and passed through a 2 mm pore sieve. Two sets of samples were taken from the sediment, one for moisture content determination by oven drying and one for hydrometer analysis. The set of samples for hydrometer analysis consisted of three replicates. Following organic matter removal and dispersion, the hydrometer samples were transferred to sedimentation cylinders and made up to 1 liter. The sand fraction of the samples was not removed prior to hydrometer analysis. An ASTM type 152H hydrometer with graduations in g/L was used and readings were taken at 30 seconds, at 1, 2, 3, 4, 10 and 30 minutes and at 1,2,3,6, and 24 hours. The temperature in the room and the blank remained relatively constant throughout the course of analysis. Following hydrometer analysis, all sand fractions were removed from sediment samples by wet sieving. Wet sieving was accomplished by passing water continuously through a 50 µm sieve until all of the clay and silt fractions were removed. The resulting sand fraction was then oven dried and weighed. The percentage clay in sediment was then calculated based on the hydrometer readings and the sand fraction was determine based on the oven dry weight of the sieved sample. The fraction remaining, which corresponded to the silt fraction, was then calculated. The gravel content of the sediment was determined by passing sediment samples through a 2 mm sieve and weighing the resulting

fraction after oven drying. The percent gravel content then was expressed based upon the weight of gravel in any sample relative to the total weight of the sample (Table 3.2).

Table 3.2: Chemical and physical properties of sediment used in this study

Texture	Sand %	Silt %	Clay %	Sediment Organic Carbon %	pH
Loamy	35.8	30.5	33.7	1.21	5.7

3.5.1.3: Measurement of pH and electrical conductivity of sediment samples

Sediment pH was determined using a method adopted from Rhodes (1982); where 50 mL of deionised water was added to 20 g of crushed sediment, stirred well for 10 minutes; then pH measured using a pH meter (3071 Jenway). To confirm the result, an alternatively method was used whereby 5 g sediment (oven- dry weight equivalent) was weighed into 50 mL falcon tubes and double distilled water added to give a final sediment: water ratio of 1:2. The tubes were shaken on an overhead shaker for 1 hour. They were then allowed to stand for 1 hour after which they were shaken vigorously for 1 minute, followed by pH measurement on 3071 Jenway pH meter. The pH meter was calibrated using buffers of pH 10.0, 7.0, and 4.0 before use.

3.5.2 Physicochemical parameters of water sample

3.5.2.1: Chemical Oxygen Demand (COD)

Closed reflux colorimetric method described fully in standard methods of Franson (1995) was adopted. In this method, to 500 mL distilled water, 10.216 g of $K_2Cr_2O_7$, primary standard grade, previously dried at 103^oC for 2 hours was added, then 167 mL of conc. H_2SO_4 and 33.3 g of $HgSO_4$ added and dissolved before cooling to room temperature and diluting to 1000 mL in order to make a digestion solution. Powdered Ag_2SO_4, technical grade, was added to conc. H_2SO_4 at the rate of 5.5 g Ag_2SO_4/kg H_2SO_4 and the mixture was left to stand for a day in order to dissolve so as to make a sulfuric acid reagent. Potassium hydrogen phthalate ($HOOCC_6H_4COOK$) was used as a standard.The samples were treated by taking 5.0 mL of water sample into a digestion vessel and 3.0 mL of digestion solution added followed

by careful addition of 7.0 mL sulfuric acid so that an acid layer was formed under the sample and the digestion solution layer and mixed thoroughly. Ampule tubes were placed in a block digester preheated to 150^{o}C and refluxed for two hours. After two hours the samples were cooled and put in 50 mL flasks ready for analysis. A blank and four standards were prepared in the same manner. Cooled samples, blank, and standards were inverted several times and solids allowed to settle before measuring absorbance. Solids that adhered to the container walls were dislodged by gentle tapping and settling.

Absorbance was measured using a spectrophotometer (UV-1650 PC UV-Vis spectrophotometer, Shimadzu, Japan) set at 600 nm. Absorbance was compared to a calibration curve by the machine and concentrations of unknown samples read directly. In preparing the calibration curve, at least eight standards of potassium hydrogen phthalate solution with COD equivalents of 20, 100, 300, 500, 700 and 900 mg O_2/L were prepared. Volume was made to the mark with distilled water; same reagent volumes were used, tube, or ampule size, and digestion procedure followed as for the samples and COD calculated as mg O_2/L = (mg O_2 in final volume x 1000)/mL sample. Results are shown in Table 3.3

Table 3.3: Physico-chemical parameters of the water used for the experiment

Temperature ^{0}c	pH	Turbidity (NTU)	DO mg/g	COD mg/g	Conductivity (μscm^{-1})
24.598 $\pm$2.378	6.014 $\pm$0.265	394.934 $\pm$26.431	4.393 $\pm$0.025	604.49 $\pm$25.856	150.27 $\pm$1.528

3.5.2.2: Dissolved Oxygen (DO)

The Winkler method as outlined by Anil (1994) was used to analyze dissolved oxygen, in which DO was allowed to react with I^- to form I_2, which was then titrated with standard $Na_2S_2O_3$ solution. A fast quantitative reaction was ensured by addition of Mn (II) salts in strongly alkaline medium:

$2Mn^{2+} + 2\ O_2 \rightarrow 2\ MnO_2$.....eq 15 (the oxygen in the equaton is the one dissolved in water)

$MnO_2 + 2I^- + 4 H^+ \rightarrow Mn^{2+} + I_2 + 2H_2O$.....eq 16

$I_2 + 2S_2O_3^{2-} \rightarrow 2I^- + S_4O_6^{2-}$ .....eq 17

5 ml of 0.025 M $Na_2S_20_3 \equiv 1$ mg L^{-1} DO

A volume of 50 mL of sample was put in a 250 mL bottle, 2 mL of 40% potassium fluoride (KF) (to mask Fe^{3+}) was added, and 2 mL of 36% $MnSO_4$ plus 2 mL of alkaline iodide-azide solution (50 g NaOH + 13.5 g NaI + 1.0 g NaN_3 diluted to 1 L.) was also added according to equation 14. The mixture was shaken well and the precipitate allowed to settle then 6 mL of 12 N H_2SO_4 added (eq 2). The mixture was shaken well until the precipitate dissolved. Interference due to oxidizing agents such as NO_2^- and SO_3^{2-} present in waste water ware eliminated by addition of NaN_3 to alkaline solution. The liberated iodine was titrated with 0.025 M $Na_2S_2O_3$ solutions (eqn 3) and the results calculated: 5 ml of 0.025 M $Na_2S_2O_3 \equiv 1$ mg L^{-1} DO (Anil, 1994) (Table 3.3).

3.5.2.3: Measurement of pH, temperature, turbidity and electrical conductivity of water samples

Water pH and temperature were measured directly using pH meter (3071 Jenway) and a mercury thermometer, respectively. The pH meter was calibrated using buffers of pH 10.0, 7.0, and 4.0 before use. Turbidity and electrical conductivity of the water samples were measured using a turbidity meter (Hanna instrument Hi93703 microprocessor turbidity meter) and an electrical conductivity meter (Kondaktomer CG857) respectively.The calibration was done by dipping the electrode in 20 ml buffer solutions of 84 μS/cm, 1,413 μS/cm and 12,880 μS/cm (Table 3.3).

3.6: Sample extraction for pesticide analysis

3.6.1: Extraction of chlorpyrifos and DDT from sediment samples

Extraction of p,p'-DDT and chlorpyrifos in sediment was performed according to the method described by Songlai (1997). Twenty grams of wet sediment samples were vigorously shaken with 60 mLacetone for 1 h. The organic phase was decanted into 250 mL flask and sediment slurry was mixed with acetone: hexane (1:1) mixture. Samples were again vigorously shaken for 1 h. The organic phase was combined and further extracted with a mixture of 20 mL hexane and 50 mL distilled water in a 250 mL seperatory funnel. The water phase was

discarded and anhydrous Na_2SO_4 was added to organic phase to remove remaining moisture. The extract was evaporated in a rotary evaporator and then taken up in hexane to a volume of ca. 5 mL.

3.6.2: Extraction of chloropyrifos and DDT from water samples

A liquid-liquid extraction procedure was adopted for extraction of pesticides from water samples. Volumes of 40 mL portions of each water sample (treated with chlorpyrifos and DDT, respectively) were taken in a conical flask and approximately 4 g NaCl (sodium chloride) was added to salt out pesticide from aqueous phase. Each sample was then extracted with 20 mL dichloromethane (CH_2Cl_2) by shaking in a separatory funnel for five minutes while releasing pressure and allowed to settle for 30 minutes for better separation of phases. Thereafter the organic layer was collected in a dry 100 mL conical flask and kept at 4^0C in refrigerator. The extraction was repeated twice using 20 mL portions of CH_2Cl_2. The CH_2Cl_2 extracts were combined, dried with anhydrous Na_2SO_4 and concentrated to ca. 5 mL using a rotary evaporator at 37°C under vacuum pressure prior to clean up (UNEP, 2003).

3.6.3: Extraction of chlorpyrifos and DDT from leaf samples

The method used for the extraction of chlorpyrifos and DDT on *Justicia flava* (Vahl) was adopted from Charles and Raymond (1991). For each 50 g of the sample ground using a food processor, 100 mL of acetone was added and the mixture was stirred for 2 hours. The extraction was carried out with 100 mL and 50 mL of acetone, respectively. After filtration, the residues in acetone were partitioned with saturated aqueous 1 molar sodium chloride (30 mL) and dichloromethane (70 mL) in a separating funnel. The dichloromethane fractions were collected, combined and dried over anhydrous sodium sulphate. The solvent was removed under reduced rotary vapor pressure at 37°C and the residues were dissolved in an acetone-hexane (1:9) mixture (10 mL) for clean up.

3.6.4:Concentration and clean-up of DDT and chloropyrifos extracts

To remove interferences the separation and clean up of sample extracts was performed using florisil in small glass column (Lalah *et al*., 1996). The glass columns (2 cm i.d) were plugged with glass wool at the bottom end, 4 grams of pre-extracted florisil (magnesium silicate 60-100 mesh) was added then 2 grams anhydrous sodium sulphate placed at the top in order to dry the solvent and to avoid ressuspension of the top layer when pouring solvents into

column. For plants and coloured sample extracts, 2 grams of activated charcoal was added at the top of the column for decolorizing the pigments (Lalah *et al*., 2003). In the extraction column, 10 mL of the dichloromethane was added to condition it. The extract (5 mL) was poured on top of the column and eluted with 10 ml dichloromethane, then 10 mL dichloromethane/acetone (95:5, volume) and finally with 10 mL dichloromethane/acetone (10:90 volume) (Vyas *et al.*, 2005). To avoid loss of sample, the collection of the elute was started at the same time as the sample was applied to the column.The elutes were pooled and evaporated to dryness using rotary evaporator before being dissolved in 4 ml methanol for Analysis by GC-MS. This separation and clean-up was done according to Lalah *et al*. (1996). For chlorpyrifos samples diethyl ether was used instead of dichloromethane.

3.7: Sample analysis

All glassware used in the sampling and analysis were washed with detergent and water, and rinsed several times with water then distilled water. Next they were rinsed with polar and non polar solvents (acetone, hexane) and dried in oven at 110ºC for 12 hours. The openings of the glassware were covered with aluminum foil as soon as they were removed from the oven. Prior to use, glassware was rinsed with dichloromethane.

3.7.1: Preparation of external standards

External standards for DDT and chlorpyrifos was prepared from stock standard solution of each using isooctane. Accurately measured 10 ppm of each standard was prepared.

3.7.2: Gas chromatographic analysis of the samples

Into a Varian chrompack, Japan CP-3400 and 17A GC models, 5µl ofStandard solutions of DDT was run to determine the retention time of DDT and the metabolites and their quantities at each sampling time. This was then followed by injection of 5µlcleaned samples spiked by DDT. The same procedure was followed for chlorpyrifos. The GC operational conditions are shown in Table 3.4.. Pesticides in the samples were identified on the basis of their retention times, quantified on the basis of peak areas relative to standards, and reported on the basis of sample volume or weight expressed in µg/g.

Table 3.4:GC operation conditions

Condition	**Chlorpyrifos**	**DDT**
Carrier gas	Nitrogen	Helium
Detector	NPD	ECD
Column type	DB-17	DB-17
Column length and i.d	30.0 m, 0.25 mm	30.0 m, 0.25 mm
Column oven temperature	100^{o}C to 200^{o}C with a hold time of 2.0 min.	140^{o}C to 280^{o}C min^{-1} with a hold time of 2.0 min.
Detector temperature	300^{o}C	290^{o}C
Flow pressure	77.0 kPa	77.0 kPa
Total flow	3 mL/min.	3 mL/min.
Column flow	1 mL/min.	1 mL/min.
Injection mode	Splitless.	Splitless
Injector temperature	250^{o}C	240^{o}C

3.7.3: Identification and recoveryof pesticides

Identification of the peaks was based on peak on retention times observed in the authentic external standards whilequantification was done by comparing the peak areas of analytes with those of the authentic external standards.

The control and blank samples were used to calculate the recovery percentage after extraction and clean up processes as shown below.

$$\%\ \text{recovery} = \frac{\text{Amount determined-Amount spiked in sample}}{\text{Amount spiked in sample}} \times 100\%$$

Where: Amount found is the calculated concentration from the response of the spiked sample.

Amount from unspiked sample is the original concentration of the blank.

3.7.4: Data Analysis

Reaction rate constants and statistical analyses were conducted using Microsoft Excel.

3.8: Activity studiesof chlorpyrifos and DDT to mosquito larvae

3.8.1: Specimen collection, identification and rearing

Larvae Specimens were collected both from ponds using standard dippers .Collections were made on 18th and 19th May 2011 during the rainy season, which coincided with the main farming season when most ponds were flooded. Specimens were identified as *An. gambiae s.s*. (sensu stricto) based on morphological characteristics (Gillies and DeMeillon, 1968). Larvae from the different ponds were preserved live in separate bottles and transported overnight to the Centre for Biotechnology Research and Development at KEMRI, Nairobi.

3.8.2: Insecticide susceptibility bioassays

Test procedures were done according to WHO (1981). Late 2nd larval instars and early 3rd larval instars of *An. gambie* were exposed to technical grade DDT (98.5%) chlorpyrifos (98.5%) at different concentrations, Each concentration of the tested pesticide together with an untreated control group were replicated four times, with 25 larvae per replicate was transferred to 120 mL beakers containing 100 mL of dechlorinated water. The larvae, standardized for size and age, were removed from culture bowls, washed twice in tap-water, pipetted individually onto a muslin net and then transferred to the beakers in order to minimize the amount of water carried with them. The pesticides were dissolved in acetone 1% stock solution (w/v). Untreated controls received only 1ml of acetone. After starting the experiments, the larval mortality counts were determined daily until 100% of the pupations were knocked down. Accordingly, larvae were continuously exposed to the pesticides fot 24 hours in order to determine the LC_{50} and LC_{90} values, respectively. In order to determine the latent outcome of the used pesticide on some biological aspects, the number of developed pupae, for each concentration, was counted and the pupae were placed in a separate cage until the emergence of adults. Consequently, the developmental periods, pupation rates, and adult emergences were determined. During that time, the morphological abnormalities of larvae, pupae, and adults were recorded.

3.8.3: Statistical analysis for toxicity test

Mean mortality was determined across all batches of larvae tested for a particular insecticide and the WHO (1992) criteria used to evaluate the resistance/susceptibility status of the larvae.

Resistance wasindicated by mortality rates of less than 80%, 24 h after exposure to insecticide while mortality rates greater than 98% wereindicative of susceptibility.

Mortality rates between 80-90% suggest the possibility of resistance that needs to be clarified.

Table 3.5: Percentage mortalities laboratory reared late second instars and early third instars of *A. gambiae* when exposed to different concentration of chlorpyfos, dursban and DDT

Chlorpyrifos		DDT	
Concentration in ppm	Percentage mortality	Concentration in ppm	Percentage mortality
0.0015	30	0.040	45
0.002	35	0.045	50
0.003	45	0.050	60
0.004	50	0.053	60
0.005	60	0.055	60
0.0055	78	0.160	63
0.006	80	0.165	66
0.007	90	0.180	90
0.0075	91	0.185	94
0.120	95	0.190	96
0.125	95	0.20	96
0.140	100	0.21	96
0.160	100	0.22	96

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1: Dissipation of DDT and chlorpyrifos

4.1.1: Dissipation of DDT and chlorpyrifos from moist sediment after treatment

In the field experiment, half-lives of dissipation for DDT and chlorpyrifos from moist sediment were obtained (Table 4.1). These half –lives (DT_{50}) were calculated from equation $DT_{50} = 0.693/k$ where k is the gradient of total recovered residues after time t, versus time t, based on first order reaction kinetics (Equation 9 on page 42). Chlorpyrifos and DDT dosed in the moist sediment dissipated rapidly. The concentration in the sediment dropped by 35% and 30.5% for chlorpyrifos and DDT respectively, after three days. After 66 days, 90% and 74.5% of chlorpyrifos and DDT respectively, had dissipated. The precision percentages given in the Table 4.2 were calculated based on total DDT and chlorpyrifos added at the start of the experiment. The half-lives for dissipation obtained for chlorpyrifos (DT_{50}=7.77 days) and DDT (DT_{50}=15.4 days) were also lower than the highest values reported in temperate sediments. The percentage of recovered residues at 0 day was less than 100% because of the dissipation which may have occurred during spiking, extraction and analysis processs.

The half-lives of dissipation obtained for DDT and chlorpyrifos were comparable to those reported from other tropical countries where similar procedure and methods were used.However, these half-lives are much lower compared to those reported from temperate studies. Data reported from temperate sediments gave half-lives of 23 years in California and 45 years in New Zealand respectively, for DDT (Lalah *et al.*, 2001). However, workers in Nigeria and Taiwan also reported very low half-lives of disappearance of DDT in tropical sediments. Lalah *et al*. (2001) reported that only 2% of total sediment applied DDT was left in the sediment after 4 years in field studies in Nigeria, while a 20% decline in DDT residue concentration levels six weeks after the pesticides was incorporated into the sedimentwas reported in Taiwan sediments.

Table 4.1: Dissipation of chlorpyrifos and DDT from sedimentafter treatment

Residue recovered(ppm), mean± S.D, Days after treatment				
No. days `	Sediment (DDT)	Percentage residues recovered (%)	Sediment (chlorpyrifos)	Percentage residues recovered (%)
0	I.47±0.03	73.5	1.50±0.02	75
3	1.39±0.05	69.5	1.30±0.03	65
7	1.26±0.03	64	1.11±0.05	55.5
14	1.10±0.02	55	0.90±0.08	45
21	0.96±0.04	48	0.80±0.03	40
28	0.82±0.03	41	0.61±0.02	30.5
35	0.70±0.02	35	0.59±0.01	29.5
42	0.64±0.03	32	0.50±0.02	25
49	0.60±0.04	30	0.46±0.05	23
56	0.57±0.02	28.5	0.28±0.07	14
66	0.51 ±0.01	25.5	0.20±0.05	10
90	0.42±.0.04	21.5	0.14±0.02	7
DT_{50}	15.4 days		7.8days	

The lower half-life observed compared to the temperate data can be attributed to enhanced photochemical reactions near or on the sediment surface and the extent of these reactions depend on light intensity and sediment characteristics. Solar radiation intensity is relatively high throughout the year in Maseno, which makes photodegradation an important dissipation pathway for pesticides. Photosynthesizers such as organic molecules with benzoic or alkenoic bonds in humic circumstances often found in natural waters and in sediment environments can also absorb solar energy and transfer it to pesticide molecules and consequently effect reactions in compounds that would not normally undergo photochemical transformation on their own as reported by Lalah *et al*. (2001).

Volatilization mechanisms can also explain the lower half-life of the pesticides in thesediment.The loss by volatization is a function of weak van der Waals forces of attraction resulting from short dipole-dipole interactions of several kinds, mainly in non-ionic, non-polar pesticides or portions of several molecules, hydrogen bonding: a special dipole-dipole interaction in which the H-atom serves as a bridge between two electronegative atoms, one being held by covalent bond and the other by electrostatic forces, charge transfer mechanisms

which involve formation of charge transfer complexes where electrostatic attraction occurs when electrons are transferred from one electron rich donor to an electron deficient acceptor within a short distance of separation, and ion-exchange (e.g. pesticide interactions with the –COOH, -OH groups of the organic matter) and coordination bonding, where pesticide molecules cluster around metalions as ligands. The last three types of interactions are not expected to influence persistence of DDT and chlorpyrifos. This is in agreement with research done by Lalah *et al*. (2001).

Abiotic chlorpyrifos hydrolysis is influenced by the pH of the matrices, temperature and elevated concentrations of some metallic ions such as Cu^{2+}. The pH of the sediment samples used in this study was 5.7 (Table 3.2) and did not lead to significance increase in hydrolysis since the rate of alkaline hydrolysis has been reported to increase when pH exceeds 7.5 by Macalady and Wolfe (1983).

Biotic factors probably had a greater influence on degradability observed in this study because sediment had nutrient materials which enhanced microbiol activities. Microbial metabolism could have been promoted by biostimulation with microbial nutrients and microbial-mediated chlorpyrifos hydrolysis and degradation and contributed significantly to its dissipation in sediment (Berry *et al.*,1993)

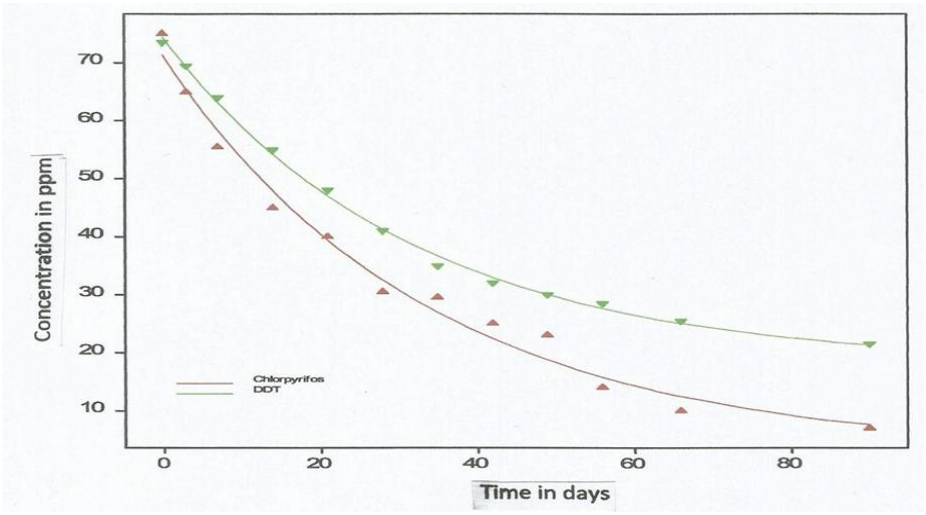

Figure 4.1: Concentration in ppm expressed as percentage recovery versus time in days when sediment was treated with chlorpyrifos and DDT

Chlorpyrifos had a shorter half-life than DDT. This implies that it is less persistent in sediment and therefore if used as a pesticide against mosquito larvae, it would have lower susceptibility to resistence by the vector. Chlorpyrifos can therefore be a suitable replacement of DDT with respect to being environmentally friendly and less susceptible to mosquito laval resistence.

4.1.2: Dissipation of DDT and chlorpyrifos from water after treatment

The physico-chemical properties of water used in the experiment are shown in the (Table 3.3). Table 4.2shows the results of dissipation of DDT and chlorpyrifos from water dosed with DDT and chlorpyrifos. The concentration for DDT and chlorpyrifos in water dropped by about 49% and 47.5% ,respectively, within the first 6 hours. After 3 days, the concentrations had dropped by about 91.5% and 89% for DDT and chlorpyrifos respectively. DDT is hydrophobic in nature, therefore it is expected that there would be no residues found in water compartment of the ecosystem. The half-lives of dissipation obtained for water were (DT_{50} = 6.12 h) for chlorpyrifos and (DT_{50}=6.3 h) for DDT, respectively.

Table 4.2: Dissipation of chlorpyrifos and DDT from water after treatment

Residues recovered (ppm), mean± S.D, hours after treatment				
Time in hours (h)	DDT in water (ppm)	Percentage of residue recovered	Chlorpyrifos in water (ppm)	Percentage of residue recovered
0	1.98±.0.01	99	1.79±0.03	89.5
2	1.40±0.02	70	1.56±0.01	78
6	1.12±0.04	51	1.05±0.04	52.5
12	0.76±0.04	38	0.70±0.01	35
18	0.39±0.04	19.5	0.33±0.03	16.5
24	0.33±0.05	16.5	0.28±0.01	14
48	0.20±0.02	10	0.26±0.02	13
72	0.17±0.03	8.5	0.22±0.01	11
168	0.140.04±	7	0.10±0.04	5
336	0.08±0.02	4	0.07±0.01	3.5
504	0.05±0.01	2	0.03±0.00	1.5
DT_{50}	6.1 h		6.3 h	

Although the solubility of DDT in pure water is lower than these concentrations, its solubility in natural water can increase because it can partition into particulate matter or dissolved organic matter in the water column.

Losses of DDT and chlorpyrifos residues in the set up in glass could be attributed to volatization, adsorption on glass walls and dissolved organic matter, and to complete degradation. In other studies DDT residues have been found to volatilize rapidly in water under tropical conditions and only little loss due to decomposition and desorption (Wandiga *et al*., 2002). They obtained half-life of 2.5 hours in sea water in tropical seawater ecosystem after 24 hours. Dissipation graph of concentration in ppm expressed as percentage recovery versus hours when water was treated with chlorpyrifos and DDT respectively are shown in Figure 4.2.

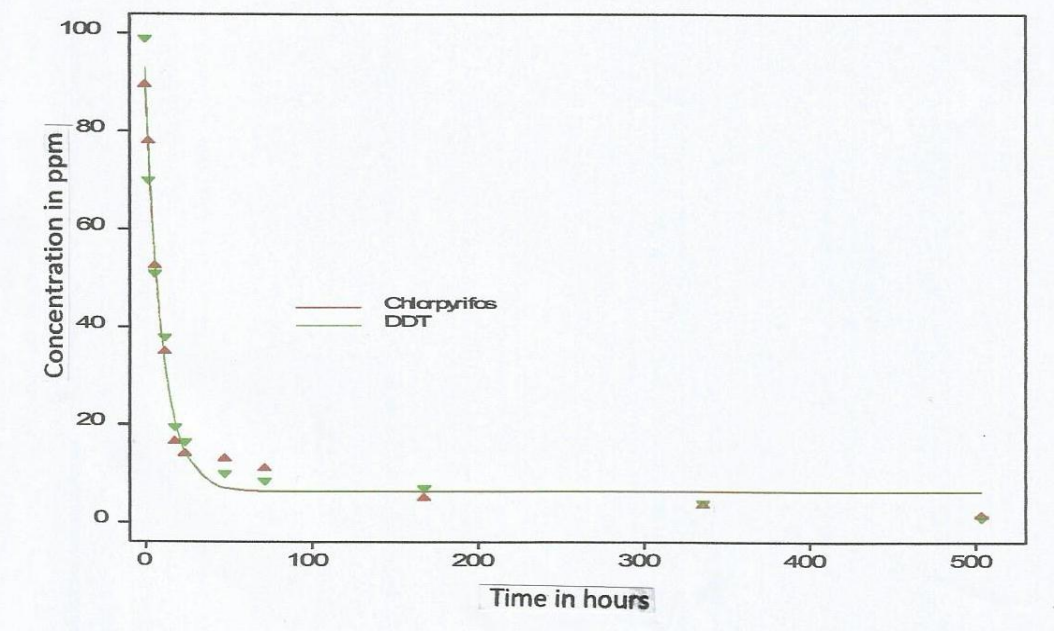

Figure 4.2: Concentration in ppm expressed as percentage recovery versus time in hours when water was treated with chlorpyrifos and DDT

USEPA (1989) reported that the half-life of DDT is 28 days in river water and 56 days in the lake water. The faster dissipation in this work could be due to higher volatization in tropical environment since the former study was conducted in temperate environment. ATSDR (1994a) also reported that the main pathways for pesticide loss in water are: adsorption to water-borne particles and sedimentation, photodegradation, volatilization and aquatic organism that absorb and store it and its metabolites. Similar pathways could have contributed to degradation and dissipation of DDT and chlorpyrifos in this work.

The observed shorter half-life of 6.12 hours of chlorpyrifos in water was attributed to the fact that chlorpyrifos has low water solubility (0.7 mg/L), moderate volatility (2×10^{-5} mm Hg at

25°C), and moderate hydrophobicity (log K_{oc} of 3.73, log K_{ow} of 4.82), and it adsorbs fairly strongly to sediments (USEPA, 2000). It, therefore, has a propensity to partition to organic matrices in aquatic systems, with little tendency to exist in dissolved form in surface waters. Abiotic and biotic degradation of chlorpyrifos in water led to formation of 3, 5, 6-trichloro-2-pyridinol (TCP), which is hydrolytically degraded in water in less than one hour. Dilling *et al*. (1984) also reported similar results. Racke (1993) reports water half-lives of ≤5 days and sediment half-lives of ≤16.3 days for chlorpyrifos. The faster dissipation in this work could be due to higher volatisation in tropical environment since the former study was conducted in a temperate environment. Giesy *et al*. (1999) reported that in water, the half-life of chlorpyrifos is affected by sediment/particle binding, biodegradation, volatilization, hydrolysis and photolysis.

The loss of chlorpyrifos from water was bi-phasic, with an initial rapid loss of chlorpyrifos over the first 24 hours, followed by a slower rate of loss over subsequent sampling days. Bi-phasic patterns of dissipation were also noted in laboratory aquaria and out door mesocosms by Mazanti *et al*. (2003) and in outdoor enclosures by Giddings *et al.* (1997). The rapid loss of chlorpyrifos in phase 1 represents loss from the system due to photolysis, hydrolysis and volatization.

Organophosphate insecticides, undergo an hydrolysis reaction in the presence of alkaline water (at pH value greater than 7), which reduces the effectiveness of the pesticide's active ingredient (Fred, 2002). Water with a lower pH also contains a higher number of suspended solids and dissolved minerals. This is because the suspended material typically has high salt concentrations. These substances also affect the performance of pesticides. The degradation and breakdown of the pesticides depend on the specific chemical properties of the pesticide, the pH of the mix water and the length of time that the pesticide is in contact with the water. Spray-mix water with a pH value between 8 and 9 can cause a rapid hydrolysis to the point that the degree of pest control is greatly diminished or lost (Fred, 2002). Thus, the result from this study shows that chlorpyrifos can be more effective when applied in water since it has a moderate pH value (Table 3.3).

Meikle *et al*. (1983) also reported that the rate of loss of chlorpyrifos due to photolysis is likely to be similar to the rate of loss of hydrolysis at circum-neutral pH and in many fate

studies, photolysis and hydrolysis are considered together. Neely *et al*. (1976) modelling suggested that loss by hydrolysis may be greater (76%) than loss by volatization (11%) however, empirical estimates from littoral enclosure studies suggest that losses due to volatization could be between 16% and 34% of the initial dose as reported by Knuth and Heinis (1992). Despite attempts to minimize volatization during application, deionised water was added to compensate for evaporation of water, volatization of chlorpyrifos may have been high, particulally given the large surface area to volume ratio of the glass jars. These loses would be higher in larval habitats outdoors where wind and other weather conditions are diverse.

4.1.3: Dissipation of DDT and chlorpyrifos from *Justicia flava* leaf

Table 4.3shows results of dissipation of DDT and chlorpyrifos from leaf. Chlorpyrifos and DDT dosed on leaf surfaces dissipated rapidly. The concentration on the leaf dropped by 49.5% and 40% for chlorpyrifos and DDT respectively, after two days and less than one tenth remained after 16 days. After 16 days, 94.5% and 91% of chlorpyrifos and DDT respectively, had dissipated. The percentages given in the Table 4.5 were calculated based on total DDT and chlorpyrifos added at the start of the experiment. The half-lives of dissipation obtained for leaves were (DT_{50}=48.48 h) for chlorpyrifos and (DT_{50}= 74.88 h) for DDT respectively.

Table 4.3: Dissipation of chlorpyrifos and DDT from *Justicia flava* leaf after treatment

Residue recovered (ppm), mean± S.D, hours after treatment				
Time (h)	Chlorpyrifos on leaf ppm	Percentages of residues recovered	DDT on leaf Ppm	Percentages of residues recovered
0	1.80±**0.03**	90	1.82±**0.02**	91
2	1.63±**0.02**	81.5	1.66±**0.03**	83
4	1.38±**0.04**	69	1.57±**0.04**	78.5
12	1.18±**0.04**	59	1.40±**0.02**	70
24	1.08±**0.02**	54	1.32±**0.03**	66
48	1.01±**0.02**	50.5	1.20±**0.01**	60
72	0.87±**0.02**	43.5	1.04±**0.02**	52
96	0.58±**0.03**	29	0.65±**0.02**	32.5
192	0.36±**0.03**	18	0.44±**0.03**	22
384	0.11±**0.01**	5.5	0.18±**0.04**	9
504	0.07±**0.01**	3.5	0.13±**0.02**	6.5
672	0.01±**0.00**	0.5	0.08±**0.02**	4
DT_{50}	**2 days**		**3.1 days**	

The amount of DDT and chlorpyrifos dicreased with time. The variation in disappearence from DDT and chlorpyrifos can be explained by strong adsorption and low water solubility of DDT as compared with chlorpyrifos. Some of the applied DDT might have been lost from the surface of the plant by penetrating into the plant and possibly metabolised but this is only a small fraction of the total loss from the surface. Thus, mainly volatisation and wind erosion accounted for the observed loss of pesticides from leaf surfaces. Bell and Failey, (1991) reported that chemical move from soils to plants by; root uptake into conduction charnels and subsequent translocation, uptake from vapor in the surrounding air and uptake by external contamination of shoots by soil and dust, followed by retention in the cuticle or penetration through it. Therefore, some DDT taken into plant persist longer than that on the surface and action as a stomach poision to sucking insects may last longer than as a contact poision (Trapp, 1993). Analysis by gas chromatography showed that atleast 90% of the applied DDT and chlorpyrifos in the current study was lost after 16 days. Thus both the two pesticides are not expected to give prolonged protection against insects, under the climatic condition in Maseno, where they evaporate rapidly. However, application of these pesticides for larval control was done while considering growth pattern of various stages of larvae.

Though some chlorpyrifos may be taken up by plants through leaf surfaces, much of the applied chlorpyrifos is usually lost through volatilization, and very little is translocated throughout the plant. Work by Kamrin (1997) also found similar results. Chlorpyrifos taken up by plant tissues was primarily metabolized to TCP, which was then stored as glycoside conjugates. Foliar applied chlorpyrifos is lost primarily by volatilization. The faster dissipation rate of chlorpyrifos (DT_{50}=48.48 h) in this work could be due to high volatization rates in tropical environments since the former study was done in temperate environment. Although most of the chlorpyrifos applied to plants is lost through volatilization or converted to TCP and sequestered, desulfuration to the chlorpyrifos oxon on plant surfaces has been reported by Roberts and Hudson, (1999). Dissipation graph of concentration in ppm expressed as percentage recovery versus time in days when *Justicia flava* was treated with chlorpyrifos and DDT respectively are shown in Figure 4.3.

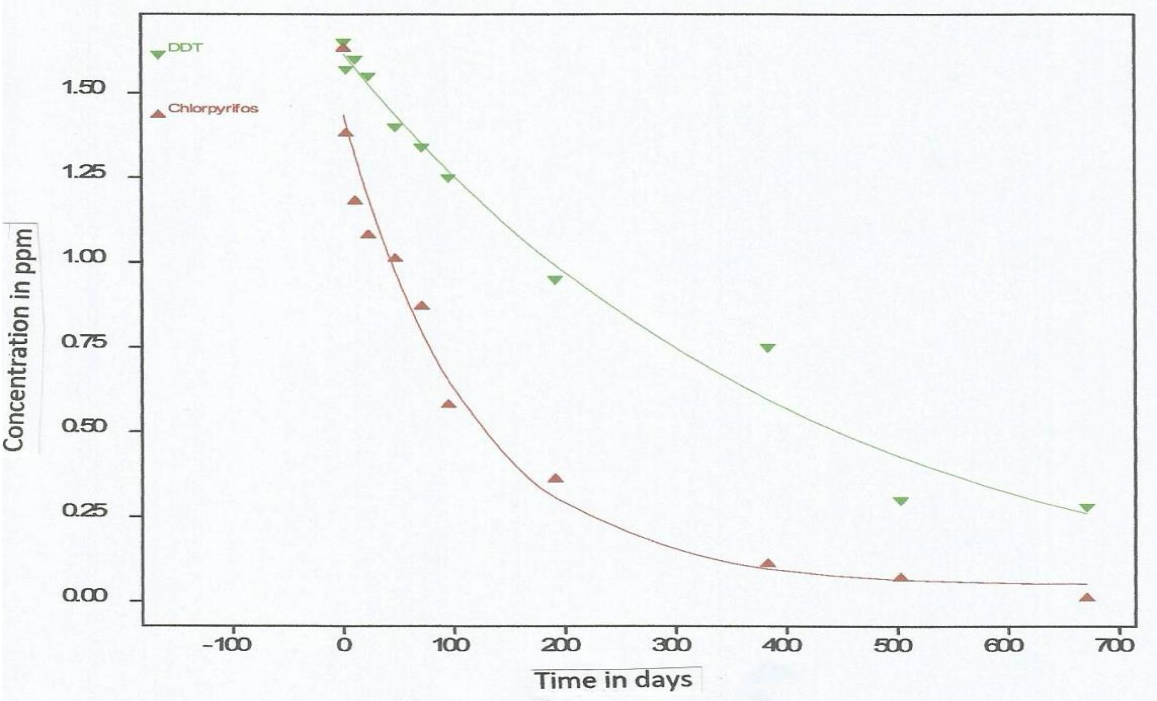

Figure 4.3: Concentration in ppm expressed as percentage recovery versus time in days when *Justicia flava* leaves were treated with chlorpyrifos and DDT

Ideally, plant pollutant concentrations should be normalized to the plant lipid concentration when directly computing different species. The behavior of plants as passive accumulators was expected. In general, lipophilic pollutants such as DDT are not translocated within plants and metabolism is not significant. Pesticides enter in plants tissue through uptake with the

help of their roots from soil or by absorption or adsorption when the foliar spray of pesticides is applied. Through these routes pesticides can accumulate in different parts of the plant tissue(Safi *et al*., 2002). Thus, it was concluded that decrease in concentration of the applied pesticides on *Justicia flava* leaf was majorly due todessipapation and not translocation.

Van den Berg *et al.* (1999) reported that pesticide volatilization from plant surfaces may occur very quickly after treatment. They recorded volatilization of more than 90% of the applied dose. Even though the rate of volatilization from plants seems to be higher than that from soil, little data is available, as pointed out by many authors as reported by Willisand McDowell(1987). Gottschild *et al.* (1995) reported data collected by Siebers in 1993 showing that volatilization of lindane within the first 24 hours after treatment is much greater from sugar beet leaves than from bare soil (89% and 13% of the application dose, respectively). Therefore, it may be concluded that most of pesticides applied on the leaves were lost through volatization in the present study.

Chlorpyrifos is not expected to be taken up by plants through its roots from soil as reported by Tomlin (2006). In his study, chlorpyrifos was applied to the leaves and fruit of orange and grapefruit trees, and residues and dissipation on the fruits were measured using gas chromatography. Chlorpyrifos residues on fruits were found to dissipate quickly, with initial mean half-lives of 2.8 days in oranges and 3.7 to 6.7 days in grapefruit, at which point residues were at or below 2 ppm. These half- lives are close to the ones calculated from this work which is 48.48 h.

Chlorpyrifos reacts with photochemically-produced hydroxyl radicals in the atmosphere and degrades to chlorpyrifos oxon. An atmospheric vapor half-life of 4.2 hours has been estimated for this reaction by HSDB (2005). In one study, researchers estimated an outdoor air residence time of 4 and 11 h for chlorpyrifos and chlorpyrifos- oxon, respectively. However, these calculations are based on approximate hydroxyl radical concentrations in a specific geographical area (Aston, and Seiber, 1997). Therefore, there is possibility of distillation of chlorpyrifos-oxon near the area where chlorpyrifos was applied initially and this may lead to increased half-life of chlorpyrifos. In a recent study, Hayward *et al*. (2010) found that the half life of chlorpyrifos is 14 h in the atmosphere indicating that it degraded more quickly in air and show much shorter atmospheric resistance time. Therefore, half-life values of chlorpyrifos are not expected to increase significantly as a result of distillation of

chlorpyrifos-oxon.Thus, generally chlorphyrifos will deissipate faster in tropical environment .

Generally, there was higher volatization from leaf surface than from sediment surface. Several possible explanations for these differences in the volatilization from sediments and crops have been proposed by Waymann and Rüdel (1995). First, turbulence above and inside the foliar coverage increases the convection exchange rate between leaves and air. Secondly, pesticide/leaf interactions may be different from pesticide/sediment interactions, with a much higher adsorption on sediment than on plants. This explanation is also suggested by Boehncke *et al*. (1990) and lastly water evaporation may also be different from leaves and sediment (due to differences in temperature and moisture levels).

4.2: Chlorpyrifos and DDT degradation rates

By taking the ln of the concentrations, the data for both pesticides were fitted to reaction orders and the relationship was found to be significant in a first order rate equation. The dissipation was assumed to be pseudo first-order kinetic for chlorpirifos when it was spiked in sediment and leaf. However, second-orderkinetics noted for water samples. Overall, dissipation was second order for DDT. This is the approach usually assumed for the interpretation of residue decline experiments (Aguilera del Real *et al.,* 2003). The kinetics of dissipation were considered to be 'pseudo' first-order since it is known that under field conditions the rate of dissipation would not be dependent on concentration interacting alone but would be dependent on concentration interacting with other factors including climatic and sediment, water and plant conditions.Dissolved organic matter could have influenced the reaction both as a reactive component of natural water and by potentially binding to chlorpyrifos inhibiting the reaction. Previous studies have determined second-order rate constants chlorpyrifos in a well-defined "clean" aqueous solutions (Wu, 2006).

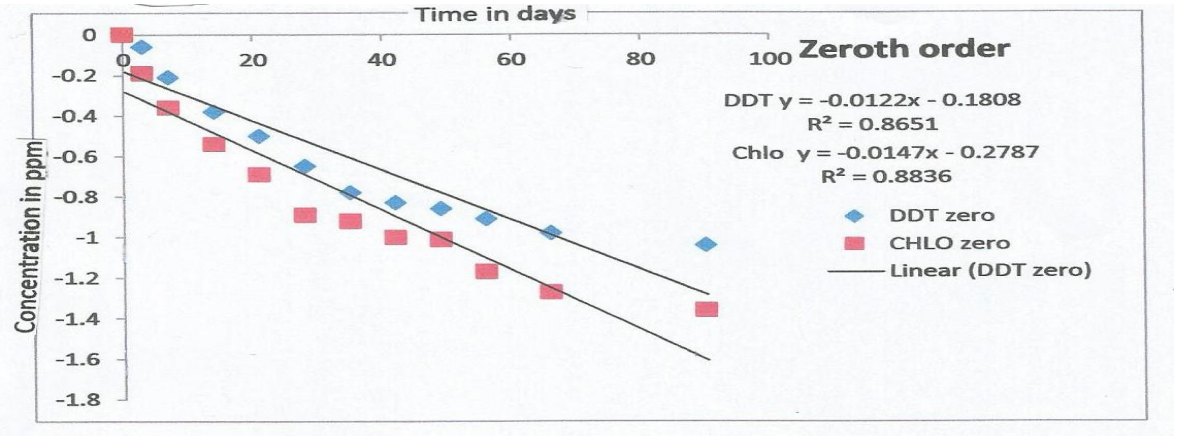

Figure 4.4.1: Zeroth–order linear regression between the concentration in ppm versus time in days when sediment was treated with DDT and chlorpyrifos

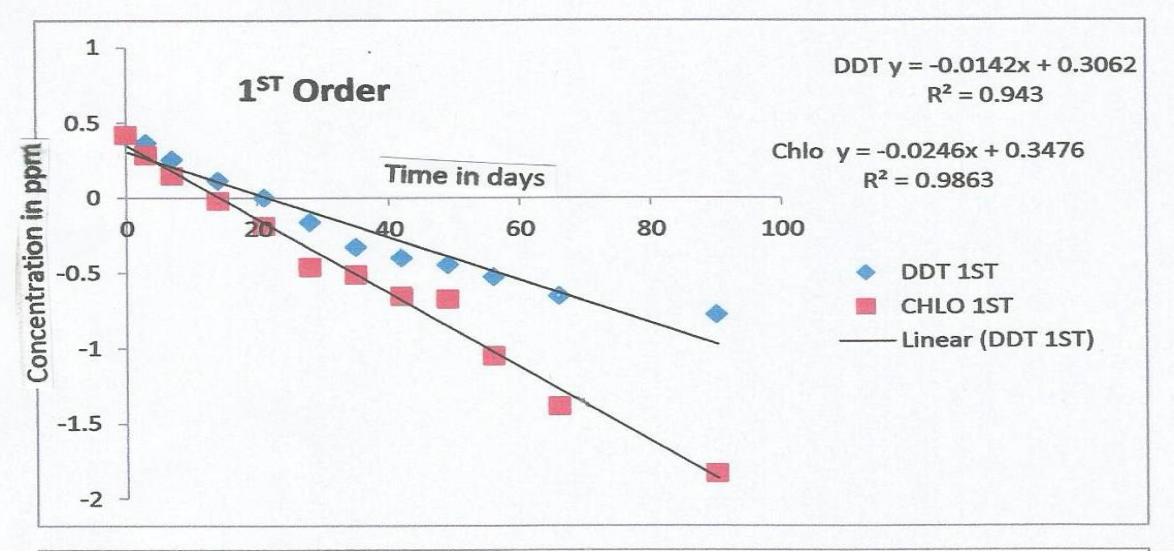

Figure 4.4.2: First-order linear regression between the concentration in ppm versus time in days when sediment was treated with DDT and chlorpyrifos

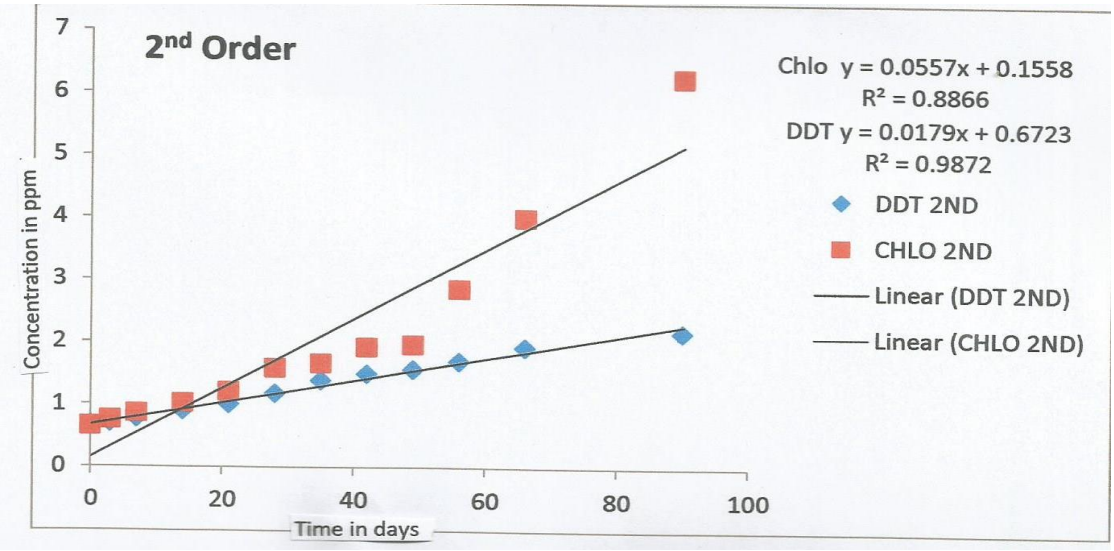

Figure 4.4.3: Second-order linear regression between the concentration in ppm versus time in days when sediment was treated with DDT and chlorpyrifos

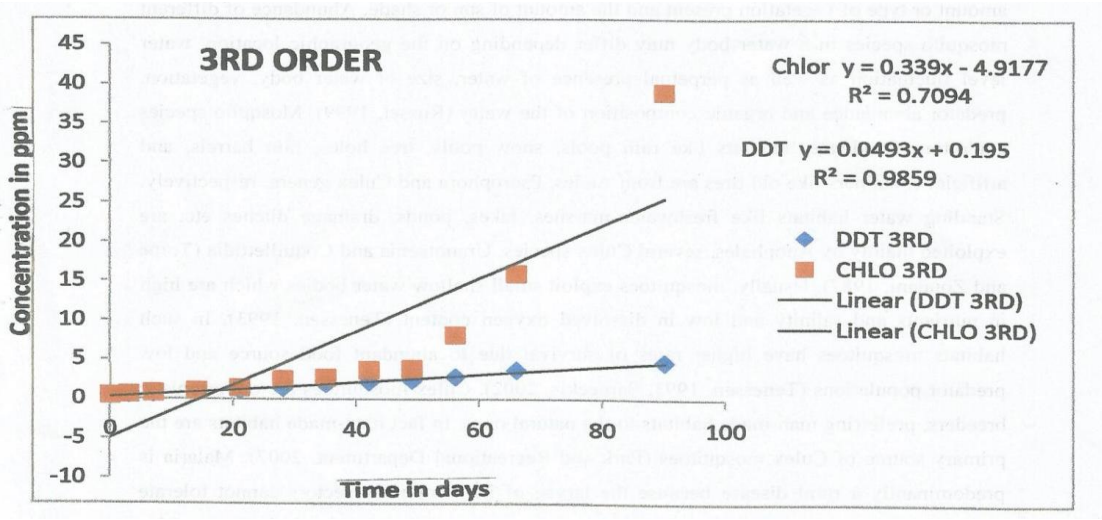

Figure 4.4.4: Third-order linear regression between the concentration in ppm versus time in days when sediment was treated with DDT and chlorpyrifos

Figures 4.4.1, 4.4.2, 4.4.3, 4.4.4,show linear regression between the ln of concentration in ppm versus days when sediment were treated with DDT and Chlorpyrifos, 4.5.1, 4.5.2, 4.5.3, 4.5.4 are linear regression between the ln of concentration in ppm versus days when sediment were treated with DDT and Chlorpyrifos, while 4.6.1, 4.6.2, 4.6.3 and 4.6.4, arelinear regression between the ln of concentration in ppm versus days when sediment were treated

with DDT and Chlorpyrifos. In addition to concentration, varying processes, including volatization and formation of bound residues as well as degradation reactions, are likely to be responsible for dissipation observed in this study. Thus, the fact that the data fits a first-order equation indicates that concentration is a major factor but it is also recognized that; other factors will obviously have been important and the dissipation itself is the result of several different processes and not just transformation reactions alone.

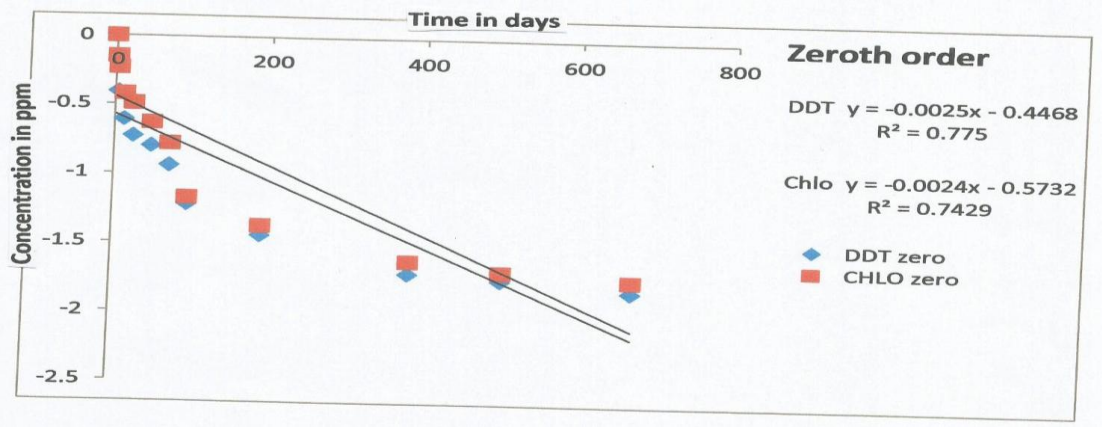

Figure 4.5.1: Zeroth-order linear regression between the concentration in ppm versus time in days when leaf was treated with DDT and chlorpyrifos

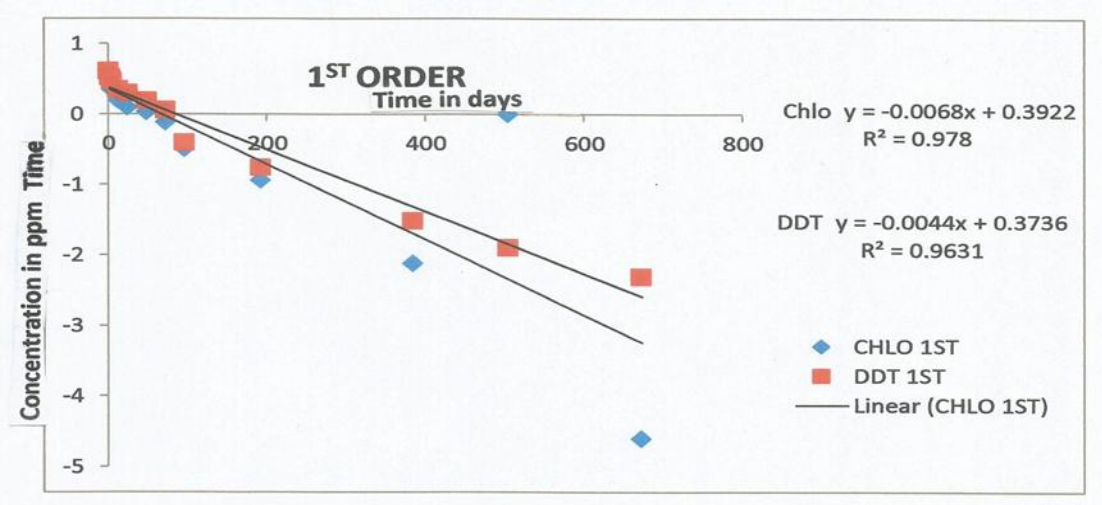

Figure 4.5.2: First-order linear regression between the ln (natural logarith) of concentration in ppm versus time in days when leaf was treated with DDT and chlorpyrifos

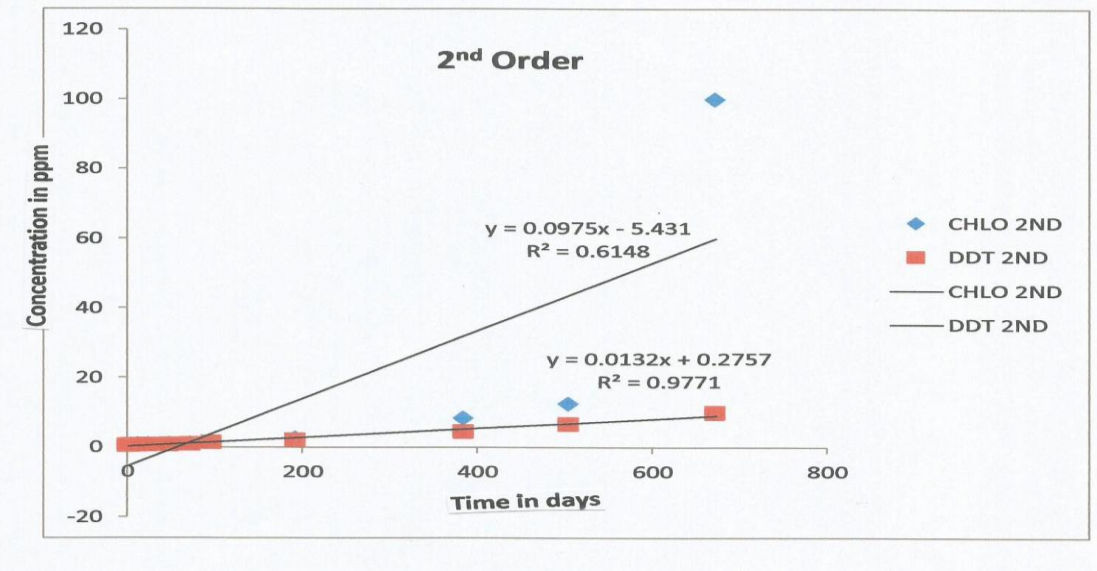

Figure 4.5.3: Second-order linear regression between the ln (natural logarith) of concentration in ppm versus time in days when leaf was treated with DDT and chlorpyrifos

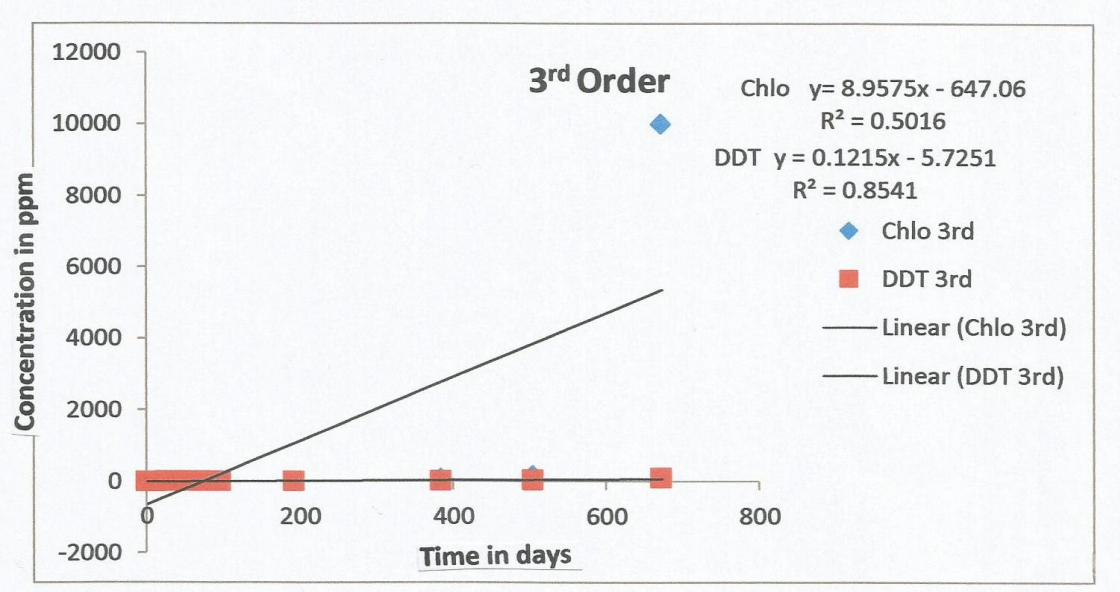

Figure 4.5.4: Third-order linear regression between the ln (natural logarith) of concentration in ppm versus time in days when leaf was treated with DDT and chlorpyrifos

The first-order half-lives and the recovery percentages from sediment, water and leaves treated with either chlorpyrifos or DDT are shown in Tables 4.1, 4.2and 4.3respectively. The rapid degradation of the pesticide in moist sediment is consistent with result from previous

investigations (Samuel *et al.*, 1988). Normally we would expect a faster decay rate of DDT and chlorpyrifos in moist sediment because microorganisms contribute to their degradation and microbial activity should be greater in sediment with higher moisture contents. This makes chlorpyrifos a suitable larvicide since it will be applied on moist environments where mosquito normally lay their eggs awaiting hatching to form larvae. It can therefore be suitable as a replacement for DDT which is known to be effective in mosquito larval control.

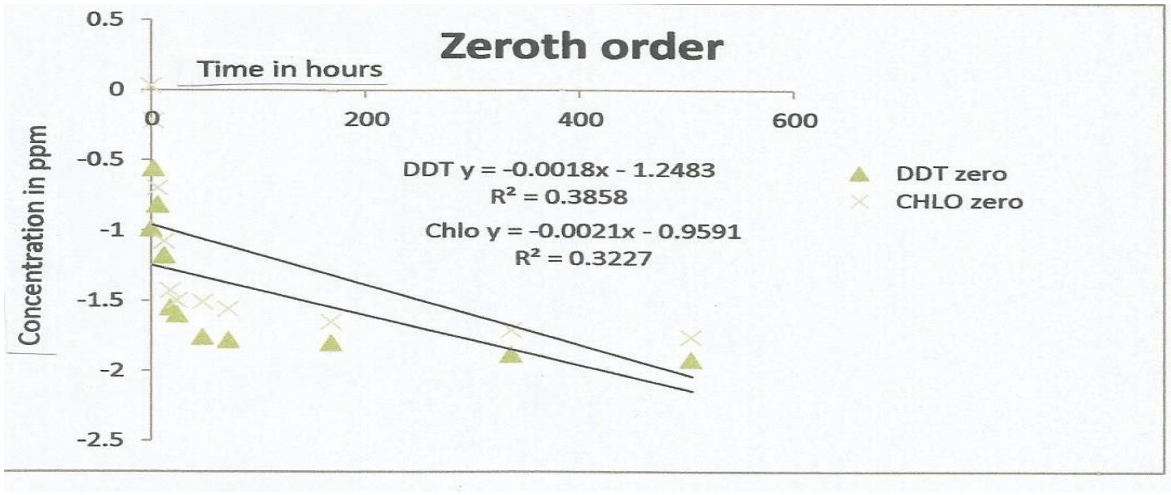

Figure 4.6.1: Zeroth-order linear regression between the concentration in ppm versus time in days when water was treated with DDT and chlorpyrifos

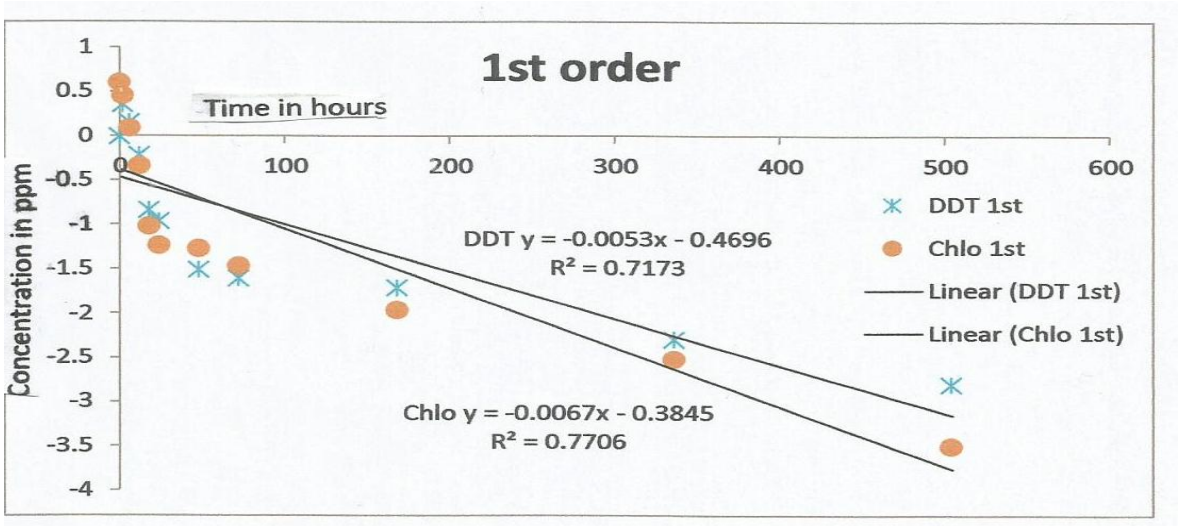

Figure 4.6.2: First-order linear regression between the concentration in ppm versus time in days when water was treated with DDT and chlorpyrifos

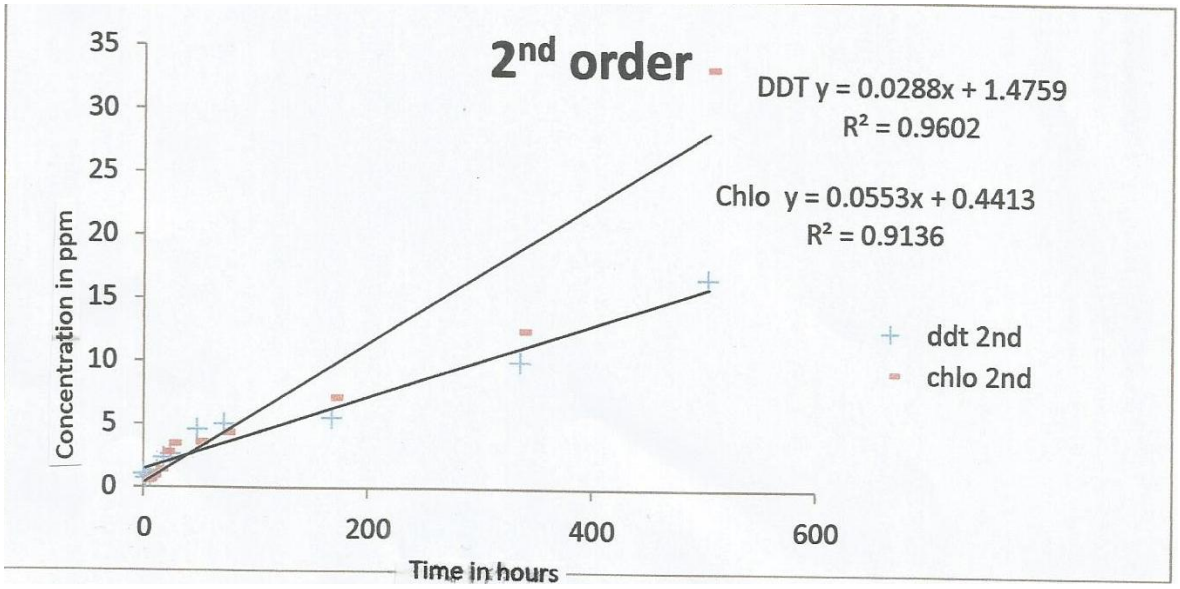

Figure 4.6.3: Second-order linear regression between the concentration in ppm versus time in days when water was treated with chlorpyrifos and DDT

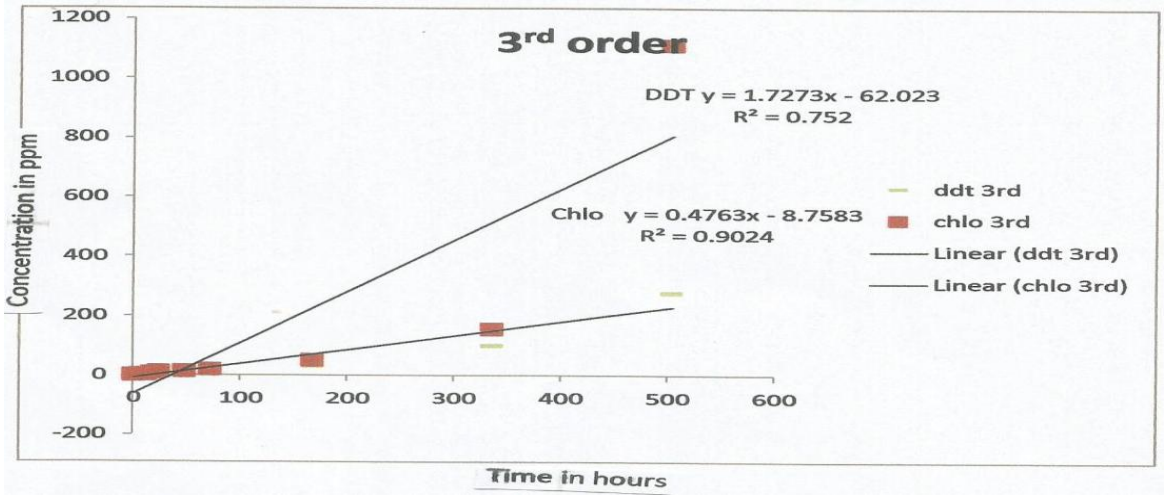

Figure 4.6.4: Third-order linear regression between the concentration in ppm versus time in days when water was treated with DDT and chlorpyrifos

4.3: Susceptibility of mosquito larvae to DDT and chlorpyrifos

The potential of chlorpyrifos and DDT to evoke toxic effects on *Anopheles gambiae* larvae in which majority of larvae showed poisoning symptoms after 24 hours of exposure was determined. Susceptibility of *Anopheles gambiae* to chlorpyrifos and DDT varied considerably (Table 3.4). The larvae displayed darting; shuddering for some hours after

which they became moribund and died. The dead or the motionless and those that were able only to make a few jerky motions of the body, without actual locomotion, were scored as dead. Between the two extremes, those that were capable of limited locomotion, but only in a very sluggish manner, were classed as moribund. Values LC_{50} after 24 hours exposure were; 0.0014 ppm, and 0.075 ppm for chlorpyrifos and DDT respectively. While LC_{90} were; 0.132 ppm and 0.21 ppm for chlorpyrifos and DDT, respectively (Table 4.4). Considering $LC_{90,}$ chlorpyrifos was about 16 times and 1.5 times more toxic than DDT.

Table 4.4: Comparative toxicity of DDTand chlorpyrifos to larvicides of laboratory rearedlate second instars and early third instars of *A. gambiae*

Larvicides	LC_{50}	95%LC	LC_{90}	95%LC
Dursban	0.0075	0.004-0.1	0.0187	0.012-0.21
Chlorpyrifos	0.0014	0.0015-0.0051	0.0132	0.0045-0.0193
DDT	0.0398	0.042-0.055	0.19	0.149-0.219

Note; LC_{50} – Lethal concentration to kill half of population concerned

LC_{90} -Lethal concentration to kill 90% of population concerned

Higher toxicity of chlorpyrifos compared to DDT is as a result of its mode of action (Simon *et al*., 1999; Glaser *et al.*, 2005). An organophosphate produces two distinct toxic effects in target organisms: direct cholinergic toxicity, with anti-AChE mechanism and neuropathic response, termed organophosphate-induced delayed neuropathy (OPIDN) (Chambers and Levi, 1992; Klaassen, 2001). If two or more insecticides are used concurrently, possible toxicological interactions between those insecticides should be considered. Insecticides of the same class may produce additive toxic effects; organophosphates, for example, reduce acetyl cholinesterase activity. Other forms of interaction include synergistic (supra-additive) and antagonistic effects, which may be caused by different classes of pesticides, for example because of metabolic interactions (N'guessan *et al*., 2007; Sharp *et al*., 2007).

DDT owes its excellent insecticidal activity particularly from its residual effect to its low vapor pressure ($1.1x10^{-7}$ at 20°C, Torr) high fat solubility (approximately 100,000 ppm), extreme low water solubility (0.025 mg/L) and stability against photooxidation. It is these very factors which cause its high environmental persistence (Metcalf, 1973). This has led to its being ubiquitously distributed in the environment on the scale that would be detected at a

latter point of time. The structural variability of chlorpyrifos is reflected in its wide range of physico-chemical properties and also in its wide considerable diversity of mechanisms through which it can be attacked by enzymes. The varying physico-chemical properties include different vapor pressure at a given temperature, different water solubilities and their structural chemical properties. These properties are almost similar to those of DDT thus suitability of chlorpyrifos as a larvicide (Table 2.1 and 2.2). The results of the toxicity study with *Anopheles gambiae* together with that of GC analysis show that the rate of dissipation would determine the extent of exposure and this would further determine the lethal and acute toxic effect of chlorpyrifos, dursban and DDT on *Anopheles gambiae* larvae. Therefore, larvicide was applied based on the knowledge of larvicide half-lives (DT_{50}) in the compartment and the various stages of growth of the larvae.

Chlorpyrifos is much more persistent at higher concentrations used for termite control, with half-lives in four sediments at a concentration of 1000 mg/kg ranging between 116 and 335 days, extending to 1575 days in the Florida sand (Racke *et al*., 1993). Therefore, suitable persistence could be increased by applying chlorpyrifos at a relatively higher concentrations given that LC_{50} and LC_{90} for malaria larvae control are very low i.e. 0.0014 ppm and 0.0132 ppm respectively (Figures 4.7). Thereafter, these concentrations would dissipate to reach the calculated LC_{50} and LC_{90} levels. Chlorpyrifos is not subject to enhanced degradation associated with a previous application history (Racke *et al*., 1990). This is a great benefit to the environment during its application in larval control since when chlorpyrifos is repeatedly applied, it will not degrade rapidly and it will still control target organisms. It is also of significance to note that the concentration of the larvicide during toxicity test falls since toxicity is done over 24 hours and concentration of the applied pesticides also decreased.

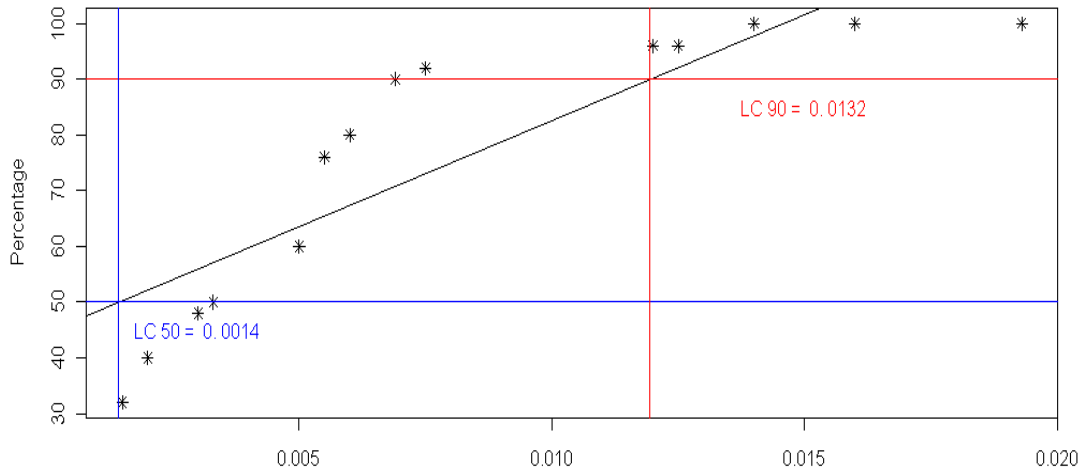

Figure 4.7: Percentage mortality of mosquito larvae versus concentration of Chlorpyrifos in ppm

When chlorpyrifos was applied, 100% mortality was recorded while for DDT, some larvae pupated and emerged to adults. This was an indication of the larval resistance to applied DDT. Earlier studies indicated thata relatively small foci of resistance has been documented in Western Kenya in an area on the shores of Lake Victoria adjacent to Uganda. Multiple resistance mechanisms have been described in the *An. gambiae s.s.* populations. (Vulule *et al.*, 1999; Strode *et al.*, 2005). These observed resistance can also be due to the fact that the larvicide concentrations has suddenly fallen before the larval death has occurred.

Frequent application of pesticides has led to the development of resistance, for instance, in *Anopheles gambiae* Giles, to DDT and fenitrothion. No such resistance was observed for pesticides not normally used for pest control, such as dieldrin and malathion (Okendi, 1988). The development of resistance to insecticides has been a contributor to the resurgence of malaria in many regions. However, since resistance is only developed when direct exposure to insecticides is applied over a period of time, replacement of a pesticide or stoppage of application reduces development of resistance (Okendi, 1988). Since chlorpyrifos has not been used widely in Kenya for mosquito larvae control, it is unlikely to face resistance when used and could also solve the problem of malaria resurgence associated with mosquito larvae resistance to DDT. The concentration of DDT to evoke 100% mortality is higher than

chlorpyrifos and dursan (Figures 4.8). This could be as a result of A Gambie resistsnce to DDT.

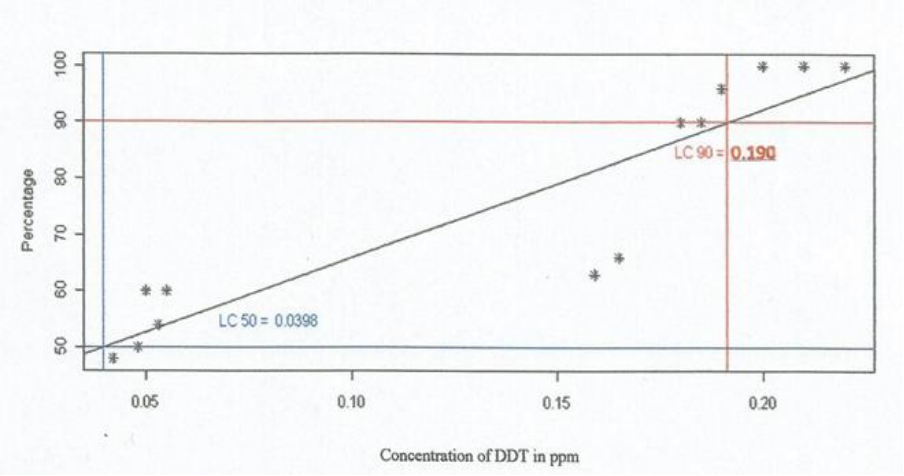

Figure 4.8: Percentage mortality of mosquito larvae versus concentration of DDT in ppm

Of the various groups of insecticide recommended by WHO for mosquito control (WHO, 2006) the most cost-effective, DDT, is compromised by its negative environmental impact, and the most widely used, the pyrethroids, will surely accelerate the selection of resistance and undermine other great tool for malaria prevention, the long-lasting pyrethroid-treated net (N'guessan *et al*., 2007; Sharp *et al*., 2007; Feachem., 2009). The ideal compound for malaria vector control would come from an entirely different class of insecticide to pyrethroids or organochlorines. The formulations of organophosphates and carbamates currently recommended for Indoor residual spraying (IRS) are relatively short-lived (WHO, 2006) and this had limited their deployment by malaria control programs. However, chlorpyrifos application can provide solution to larval control outdoors as suggested in this study.

CHAPTER FIVE

SUMMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1: Summary

The half-lives for dissipation obtained for chlorpyrifos (DT_{50}=7.77 days) and DDT (DT_{50}=15.4 days) were lower than the values reported in temperate sediments.Chlorpyrifos had a shorter half-life than DDT. This implies that it is less persistent in sediment and therefore if used as a pesticide against mosquito larvae, it would have lower susceptibility to resistence by the vector. Chlorpyrifos can therefore be a suitable replacement of DDT with respect to being environmentally friendly and less susceptible to mosquito laval resistence.

The half-lives of dissipation obtained for water were (DT_{50} = 6.12 h) for chlorpyrifos and (DT_{50}=6.3 h) for DDT, respectively. These were lower than reported in temperate regions. The faster dissipation in this work could be due to higher volatization in tropical environment since the former study was conducted in temperate environment.The loss of chlorpyrifos from water was bi-phasic, with an initial rapid loss of chlorpyrifos over the first 24 hours, followed by a slower rate of loss over subsequent sampling days

The half-lives of dissipation obtained for leaves were (DT_{50}= 48.48 h) for chlorpyrifos and (DT_{50}= 74.88 h) for DDT respectively.Atleast 90% of the applied DDT and chlorpyrifos in the current leaves was lost after 16 days.There was higher volatization from leaf surface than from sediment surface.

The degradation data for both chlorpyrifos and DDT in sediment, water and leaves fitted a first-order equation indicating that concentration is a major factor, however, it is also recognized that other factors would obviously be important and the dissipation was the result of several different processes and not just transformation reactions alone.

Considering $LC_{90,}$ chlorpyrifos was about 16 times and 1.5 times more toxic than DDT.When chlorpyrifos was applied, 100% mortality was recorded while for DDT, some larvae pupated and emerged to adults. This was an indication of the larval resistance to applied DDT

5.2: Conclusions

Chlorpyrifos dissipates faster than DDT in all the tested mosquito laval habits (sediment, water and leaves) in Maseno and therefore more environmental friendly and would be less susceptible to mosquito larval resistence.

The degradation data for both chlorpyrifos and DDT in sediment, water and leaves fitted a first-order equation indicating that concentration is a major factor in mosquito larval control

Chlorpyrifos was about 16 times and 1.5 times more toxic than DDT and therefore a more potent replacement of DDT

5.3: Recommendations

With its good safety profile, low mammalian toxicity and residual activity, chlorpyrifos meets the profile of a cost-effective replacement for DDT in mosquito larval control. The ministry of Public Health and Sanitation should adopt a larvicide strategy that incorporates chlorpyrifos inorder toreduce the selective pressure generated by DDT.

5.4: Suggestion for future research

There is need to conduct more detailed studies on microbial degradation pathways and kinetics and also to identify the type of microorganisms responsible for chlorpyrifos degradation. This is to determine whether sediment microbes capable of degrading chlorpyrifos are the same in tropical and temperate sediments. These microorganisms could then be used for remediation of sediments contaminated by pesticides.

Additional research is required to assess the role that dissolved organic matter and organo-sulfur compounds play in the transformation of chlorpyrifos in natural environment. Experiments with model compounds in well-defined aqueous solutions with varying levels of DOM could help determine at what concentration thiols enhance the reactivity of organophosphate compounds in natural mosquito lavae habitat.

Further studies are recommended to investigate bioconcentration factor for various plants to come up with suitable plant species that can be used in phytoremediation of the chlorpyrifos contaminants from the environment.

There should be continued efforts by scientists towards developing environmentally friendly and safer compounds for both agriculture and public health use. Furthermore, there is need to define cellular and molecular mechanisms of neurotoxicity and to develop mechanistically relevant biomarkers. There should also be valid measurements of exposure to organo-phosphate pesticides covering long periods to be able to make good assertions about the health and environmental effects.

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APPENDICES

Appendix I: Banned pesticides in Kenya

No.	Common name	Use	Date Banned
1.	2,4,5 Trichlorophenoxybutyric acid	Herbicide	1986
2.	Chlordane	Insecticide	1986
3.	Chlordimeform	Insecticide	1986
4.	DDT (Dichlorodiphenyl Trichloroethane)	Agriculture	1986
5.	Dibromochloropropane	Sediment Fumigant	1986
6.	Endrin	Insecticide	1986
7.	Ethylene dibromide	Sediment Fumigant	1986
8.	Heptachlor	Insecticide	1986
9.	Toxaphene (Camphechlor)	Insecticide	1986
10.	5 Isomers of Hexachlorocyclohexane (HCH)	Fungicide	1986
11.	Ethyl Parathion	Insecticide All formulations banned except for capsule suspensions	1988
12.	**C**aptafol	Fungicide	1989
13.	Methyl Parathion	Insecticide All formulations banned except for capsule suspensions	1988
14	Aldrin	insecticide	2004
15.	Benomyl, Carbofuran, Thiram combinations	Dustable powder formulations containing a combination of Benomyl above 7%, Carbofuran above 10% and Thiram above 15%	2004
16.	DNOC and its salts (such as Ammonium Salt, Potassium salt & Sodium Salt)	Insecticide, Fungicide, Herbicide	2004
17.	Binapacryl	Miticide/Fumigant	2004
18.	Chlorobenzilate	Miticide	2004
19.	Dieldrin	insecticide	2004
20.	Dinoseb and Dinoseb salts	Herbicide	2004
21.	Ethylene Dichloride	Fumigant	2004
22.	Ethylene Oxide	Fumigant	2004
23.	Fluoroacetamide	Rodenticide	2004

24.	Hexachlorobenzene (HCB)	Fungicide	2004
25.	Mercury Compounds	Fungicides, seed treatment	2004
26.	Pentachlorophenol	Herbicide	2004
27.	Phosphamidon	Insecticide, Soluble liquid formulations of the substance that exceed 1000g active ingredient/L	2004
27.	Monocrotophos	Insecticide/Acaricide	2009
28.	All Tributylin Compounds	All compounds including tributyltin oxide, tributyltin benzoate, trybutyltin fluoride, trybutyltin lineoleate, tributyltin methacrylate, tributyltin naphthenate, tributylin chloride	2009
29	Alachlor	Herbicide	2011
30.	Aldicarb	Nematicide/Insecticide/Acaricide.	2011

Appendix II: Rectricted Pesticides in Kenya (PCBP, 2008)

Common name	**Remarks**
Benomyl, Carbofuran/Thiram Combinations	Dustable powder formulations containing a combination of Benomyl below 7%, Carbofuran below 10% and Thiram below 15%.
DDT (Dichlorodiphenyl trichloroethane)	Insecticide, restricted use to Public Health only for mosquito control for indoor residual spray by Ministry of Health. Banned for agricultural use.
Ethyl Parathion	Insecticide, capsule suspension formulations allowed in 1998.
Methyl parathion	Insecticide, capsule suspension formulations allowed in 1998.
Phosphamidon	Insecticide, Soluble liquid formulations of the substance that is below1000g active ingredient/L.

AppendixIII: Causes of death in all ages in Kenya, 2002.

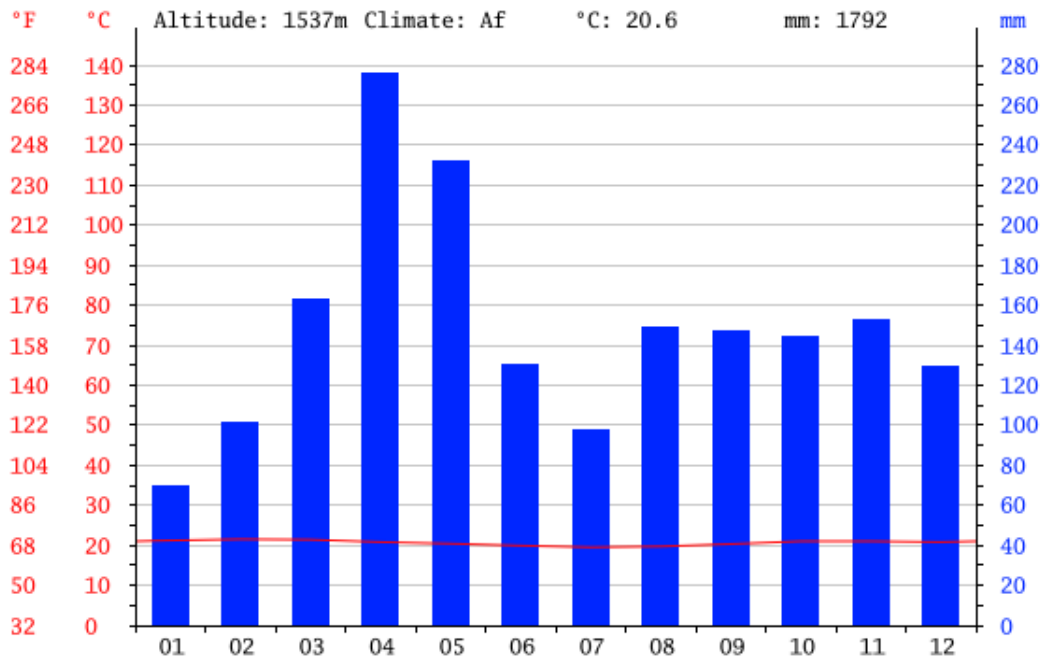
Causes	Deaths		Years of life lost (100%)
	(000)	(%)	
All causes	376	100	100
HIV/AIDS	144	38	40
Lower respiratory infection	37	10	11
Diarrhoeal diseases	24	7	8
Tuberculosis	19	5	5
Malaria	18	5	6
Cerebrovascular disease	14	4	1
Ischaemic heart disease	13	4	1
Perinatal conditions	13	4	5
Road traffic accidents	7	2	2
Chonic obstructive pulmonary disease	6	2	1

Source; Death and DAILY estimates by cause, 2002

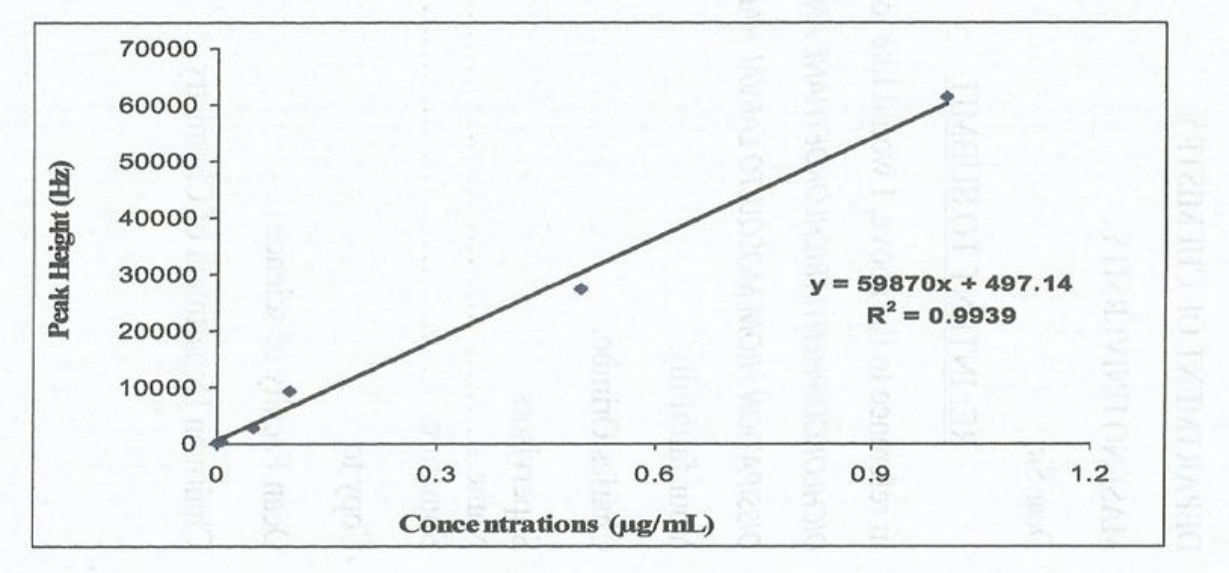
Appendix IV: Mean monthly temperture in Maseno in 2011

month	1	2	3	4	5	6	7	8	9	10	11	12
mm	70	101	163	276	232	130	98	149	147	144	153	129
°C	21.2	21.5	21.4	20.8	20.4	19.9	19.5	19.7	20.3	21.0	21.0	20.8
°C (min)	13.2	13.5	13.7	13.8	13.5	12.9	12.3	12.5	12.8	13.4	13.5	13.2
°C (max)	29.3	29.6	29.2	27.9	27.3	26.9	26.8	27.0	27.8	28.6	28.5	28.5
°F	70.2	70.7	70.5	69.4	68.7	67.8	67.1	67.5	68.5	69.8	69.8	69.4
°F (min)	55.8	56.3	56.7	56.8	56.3	55.2	54.1	54.5	55.0	56.1	56.3	55.8
°F (max)	84.7	85.3	84.6	82.2	81.1	80.4	80.2	80.6	82.0	83.5	83.3	83.3

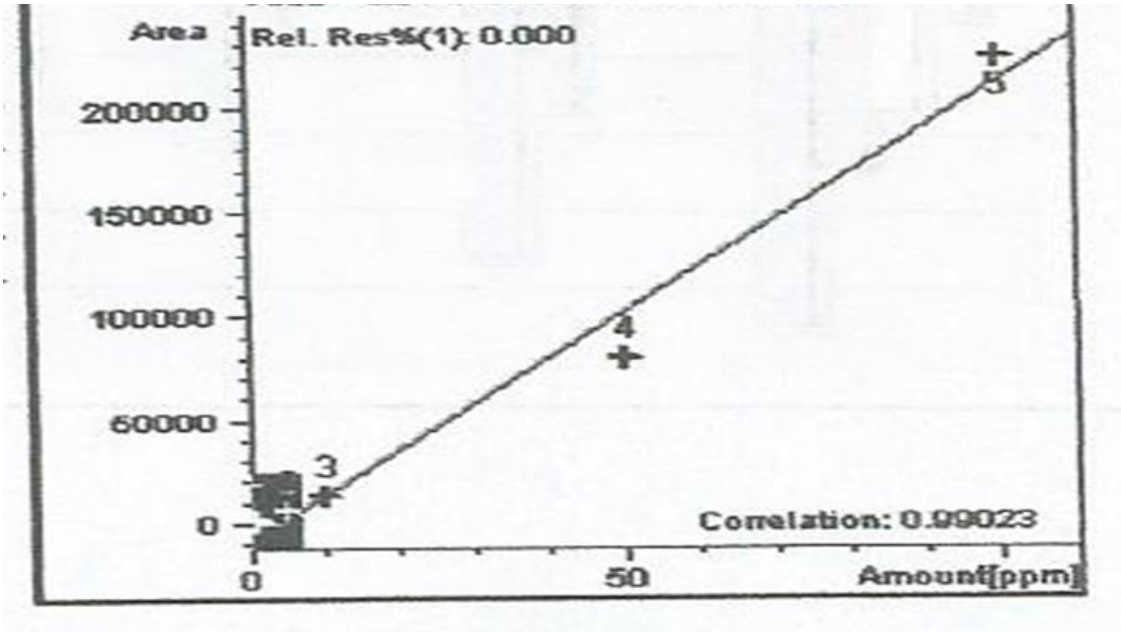
Appendix V: Monthly rainfal distribution in Maseno in the year 2011



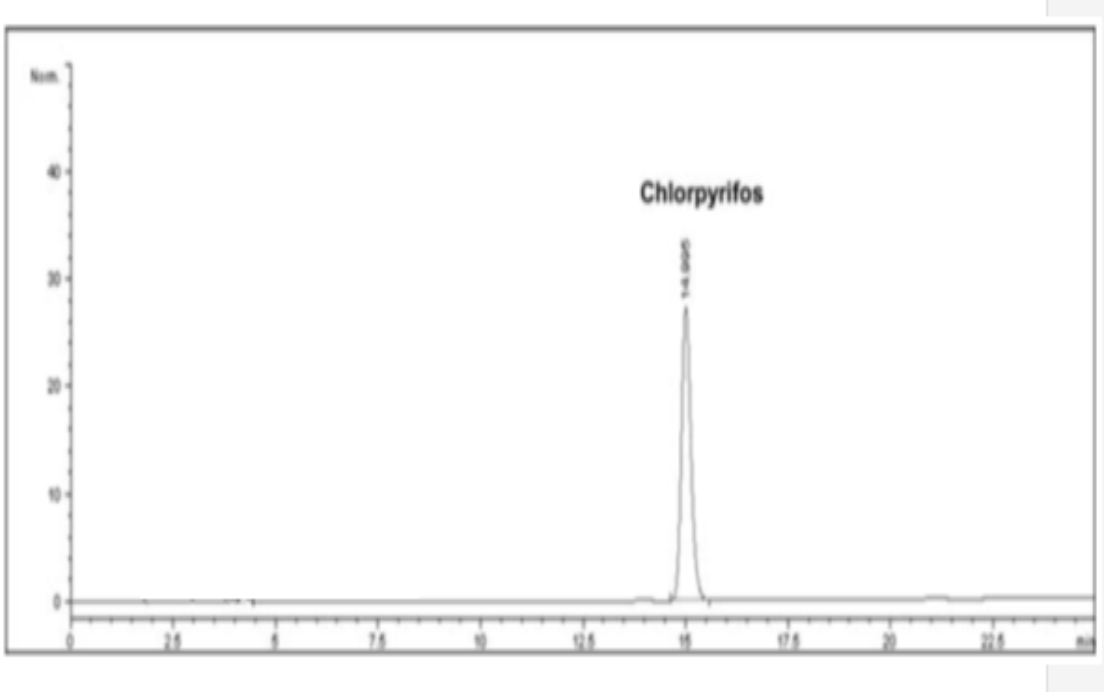
Appendix VI: Callibration curve of chlorpyrifos



Appendix VII: Callibration curve of p,p' DDT



Appendix VIII: GC-NPD Chromatogram of chlorpyrifos



Appendix IX: GC-ECD chromatogram of p,p'DDT standard