STUDIES ON DECAY PROFILES OF QUINALPHOS AND THIRAM PESTICIDES

A THESIS

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CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis entitled **STUDIES ON DECAY PROFILES OF QUINALPHOS AND THIRAM PESTICIDES** in partial fulfilment of the requirements for the award of the Degree of Doctor of Philosophy and submitted in the Department of Chemistry of the Indian Institute of Technology Roorkee, Roorkee is an authentic record of my own work carried out during a period from July 2007 to January 2012 under the supervision of Dr. Bina Gupta, Associate Professor, Department of Chemistry, Indian Institute of Technology Roorkee, Roorkee.

The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other Institute.

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This is to certify that the above statement made by the candidate is correct to the best of my knowledge.

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ABSTRACT

Pesticides are among the few toxic materials deliberately disbursed into the environment to preserve the agricultural produce. Population is growing explosively and as a consequence of this the need for more food is increasing steadily and simultaneously. The farmers are getting aware of the benefits that can be harvested by the application of agrochemicals without understanding the implications of the enhanced rate of usage of these chemicals. This intrinsically dangerous practice is being promoted in a setting without technical and human resources to control it properly. Large volumes of pesticides are used in developed countries and their use in developing countries is on a fast increase. This relentless use of pesticides results in irrevocable havoc on the health and environment of a country.

The issue of pesticides and their use is actually quite complex, involving sometimes the conflicting interests of more crop and environmental safety. The topic is important as all of us are affected in some way or the other by their presence in our environment. Genetic damage, reproductive problems and possible links to cancer are just some of the risks associated with the use of pesticides. Sometimes the metabolites are more toxic than the parent compound. Lower rate of degradation of pesticides results in bioaccumulation in organisms which causes various diseases.

Once the pesticides are released into the environment, they residually impair the quality of water, soil and air depending on their persistence. The degradation of the pesticides and the types of metabolites formed involve complex mechanism and depend upon the chemical nature of pesticide, type of medium and the environmental conditions. The medium can be air, water, soil, plant and animal. In order to keep a proper track of the cycle of these materials a careful study on their persistence in different parts of ecosystem under different environmental conditions is required.

The pesticides can be classified on the basis of target use like, fungicide, insecticide, and herbicide or their chemical composition. The most acceptable classification includes the following five major categories based on their chemical composition-

1. Organochlorine pesticides
2. Organophosphate pesticides
3. Carbamate pesticides

4. Inorganic pesticides

5. Botanical and biopesticides

Among the pesticides, organophosphates and carbamates enjoy a favoured position due to their relatively fast decomposition and low accumulation in the biological food chain. The economic considerations also dictate their use particularly, in the developing countries. Market and field survey shows that nowadays quinalphos and thiram are the most commonly used pesticides. The indiscriminate use of these pesticides in agriculture, forestry and public health leaves considerable amount of residues and their metabolites in the environment. A survey of literature reveals that scanty reports are available on the decay profiles of the above mentioned pesticides in water and soil under laboratory conditions. The effects of various parameters affecting the decay and the identification of metabolites have not been systematically investigated. However, the field data on the persistence profiles under different field conditions are scarce. Inadequate studies are documented on the degradation of these pesticides in Wistar albino rats.

In view of the above premise it was planned to investigate the decay of quinalphos and thiram under controlled laboratory and field conditions and identify the metabolites formed. It was also important to look into the effect of biological conditions on the decay pattern of quinalphos in Wistar albino rats.

For the sake of clarity and convenience in presentation the work embodied in the thesis has been divided into the following five chapters

I. General introduction

II. Experimental methodology

III. Decay profiles and metabolic pathways of quinalphos in water, soil and plants

IV. Decay profiles and metabolic pathways of thiram in water, soil and plants

V. *In vitro* and *in vivo* studies on the degradation of quinalphos in rats

Chapter I deals with the role of the pesticides and their classification. The problem of pesticides pollution is highlighted. The parameters affecting the decay of the pesticides are discussed. Finally the aims and objectives of the present study are defined. The relevant literature on different aspects has been included in the respective chapters.

Chapter II details out the optimum operating conditions developed for the analysis of quinalphos and thiram. Both have been analysed using RP- HPLC with a UV detector. This is

followed by a description on the extraction procedures adopted in the present investigations. Different procedures were explored to develop a method for each pesticide and the percentage recovery was noted. Ethyl acetate is found to be a good extractant for the recovery of quinalphos from water and soil. QuEChERS (quick, easy, cheap, effective, rugged and safe) method using acetonitrile with slight modification has been employed for the quantitative recovery of quinalphos from plants. For the best recovery of quinalphos from biological fluids, methanol is employed. The quantitative extraction of thiram from water, soil and plants is carried out using ethyl acetate, acetonitrile and dichloromethane, respectively.

For studies on Wistar albino rats the animals have been divided into different groups and the details are discussed in the present chapter. Details of digestion methods for simulated gastric and intestinal phases have also been explained in the chapter.

Chapter III describes the decay profiles and metabolic pathways of quinalphos pesticide in water and soil in laboratory conditions and in plants under field conditions. Effect of temperature, pH and organic content (humic acid) on the degradation of quinalphos in water is discussed. Results of the decay profiles of the pesticide in three different types of soil are also illustrated. The degradation was monitored for nearly four half lives. In all the cases the decay is exponential in nature. In the case of field study, pesticide solution of appropriate concentration was spotted on the different parts of radish and tomato plants, namely leaf, fruit and root and the spotted parts were harvested for the analysis at various time intervals. The pesticide from spotted part of plant was extracted and analysed using HPLC. For the identification of metabolites of quinalphos GC-MS was used. The results indicate that the different metabolites are formed by de-estrification, hydrolysis and oxidation. On the basis of metabolites identified in different matrices metabolic pathways of quinalphos have been proposed.

Chapter IV presents results of the effect of temperature, pH and organic content (humic acid) on the decay of thiram in water. The findings about the decay profiles of the pesticides in three different types of soil are also included. The samples were spiked with a known amount of the pesticide, extracted at different time intervals and analysed using HPLC. The degradation, in all the cases, was monitored for nearly four half lives and the decay follows first order kinetics. The degradation rate in water is found to increase with the increase in the temperature, pH and humic acid content. A similar effect of pH is observed in the soil. The

chapter IV also incorporates the degradation study of thiram on/in radish leaf, radish root and tomato fruit in field conditions. Pesticide solution of appropriate concentration was spotted on the desired parts of the plant which were then harvested for the analysis at various time intervals. The pesticide was extracted from the spotted part of the plant and analysed using HPLC as described in chapter II. Finally metabolic pathways of thiram in different matrices were presented by identifying the individual metabolites using LC-MS. The results indicate that the different metabolites are formed by N-dealkylation, hydrolysis, oxidation. However, different metabolites are observed with the change in the matrix or its characteristics.

Chapter V describes the *in vitro* and *in vivo* studies of quinalphos in albino rats. The degradation of quinalphos in simulated gastric and intestinal phases was studied. The metabolic intermediates of quinalphos in simulated gastric and intestinal phases as well as in blood serum and urine were identified at different time intervals after dosing the animals. All the samples were lyophilized, extracted and analysed by HPLC/GC-MS. In simulated *in vitro* studies some isomerisation derivatives have been identified which are missing in the blood and urine of the treated animals.

The thesis concludes with a brief discussion on the findings of the present investigations.

The proposed study will be helpful in assessing the safety aspects of use of these commonly used pesticides. The decay profiles give an idea as to when it is less harmful to consume the food articles sprayed with them. The systematic studies carried out on the degradation of these two pesticides would give a better insight in understanding the role of various environmental parameters in choosing this period. By identifying the different metabolites it may be possible to comment on the toxicity of the metabolites as compared to the parent pesticide. In addition to above, the studies carried out on albino rats are significantly important to indicate the behaviour of ingested pesticide in human systems. The results may be helpful to forensic scientists investigating cases of homicide and suicide.

LIST OF PAPERS
PUBLISHED/ACCEPTED/COMMUNICATED

1. Decay profile and metabolic pathways of quinalphos in water, soil and plants by Bina Gupta, **Manviri Rani**, Rahul Kumar and Prem Dureja, Chemosphere 85(10), 710–716 (2011).
2. Degradation of thiram in water, soil and plants: a study by high-performance liquid chromatography by Bina Gupta, **Manviri Rani** and Rahul Kumar, Biomedical Chromatography 26(1), 69–75 (2012).
3. Dynamics of some toxic heavy metals in different compartments of a highly urbanized closed aquatic system by Bina Gupta, Rahul Kumar, **Manviri Rani** and Tripti Agarwal, Journal of Environmental Monitoring (*in press* DOI: 10.1039/c2em10505e).
4. Identification of degradation products of thiram in water, soil and plants using LC-MS technique by Bina Gupta, **Manviri Rani,** Rahul Kumar and Prem Dureja, Journal of Environmental Science and Health Part B (*in press*).
5. *In vitro* and *in vivo* studies on degradation of quinalphos in rats by Bina Gupta, **Manviri Rani,** Rajani Salunke and Rahul Kumar, Journal of Hazardous Materials (*in press*).
6. Partitioning of some trace elements in the different segments of a highly degraded urban river stretch by Bina Gupta, Rahul Kumar and **Manviri Rani,** Environmental Monitoring and Assessment (Under revision)
7. Equilibrium studies on persistent organic pollutants in waters and sediments of Bhoj Wetland, India" Bina Gupta, Rahul Kumar and **Manviri Rani** (Under preparation)
8. Studies on polynuclear aromatic hydrocarbons and organochlorine pesticides in waters and sediments of Delhi stretch of the Yamuna by Bina Gupta, Rahul Kumar and **Manviri Rani** (Under preparation).

LIST OF PAPERS PRESENTED AT NATIONAL/ INTERNATIONAL CONFERENCES

1. Parameters Affecting the Decay of Dithiocarbamate Pesticide: A Study by HPLC by Bina Gupta and **Manviri Rani**, Proceedings of Indian Analytical Science Congress, Indian Society of Analytical Scientists, Munnar, Kerela (India), pp. 71, Nov. 21-23, 2008.

2. Parameters Affecting the Decay Profile of Thiram. A Dithiocarbamate Pesticide by Bina Gupta and **Manviri Rani**, Proceedings of 27th Annual Conference of Indian Council of Chemists, Gurukul Kangri Vishwavidyalaya Haridwar, Uttarakhand (India), pp. 220, Dec. 26-28, 2008.

3. Degradation of Thiram in Vegetables in Field Conditions by **Manviri Rani** and Bina Gupta, Proceedings of 4th Uttarakhand State Science and Technology Congress, G.B. Pant University of Agriculture & Technology, Pantnagar, Uttrakhand (India), pp. 154, Nov. 10-12, 2009.

4. Persistence Study of Quinalphos on Plants in Field Conditions by **Manviri Rani** and Bina Gupta, Proceedings of 5th Uttarakhand State Science and Technology Congress, Doon University, Dehradun, Uttrakhand (India), pp. 4, Nov. 10-12, 2010.

5. Persistence of Quinalphos in Water and Soils by Bina Gupta and **Manviri Rani**, Proceedings of International Congress on Analytical Science, Indian Society of Analytical Scientists & Cochin University of Science and Technology, Kochi, Kerela (India), pp. 49, Nov. 24-27, 2010.

6. Decay Study and Identification of Metabolites of Thiram–A Dithiocarbamate Fungicide in Water by LC-MS Technique by Bina Gupta and **Manviri Rani**, Proceedings of 4th Conference on Recent Trends in Instrumental Methods of Analysis, IITR Roorkee, Uttrakhand (India), pp. 35, Feb.18-20, 2011.

7. Degradation and Metabolic Pathways of Thiram in Water and Soils by Bina Gupta, **Manviri Rani** and Rahul Kumar, Proceedings of International Conference on Chemistry and the Environment (ICCE 2011), ETH Zurich, Switzerland, pp. 298, Sep. 11-15, 2011.

8. Dynamics of Chromium and Lead in Water, Sediment and Biota of an Urban River Bina Gupta, Rahul Kumar and **Manviri Rani**, Proceedings of International Conference on Chemistry and the Environment (ICCE 2011), ETH Zurich, Switzerland, pp. 299, Sep. 11-15, 2011.

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1. National workshop on "Techniques and Challenges for Structure Solution in Chemical Crystallography" in Department of Chemistry, IITR, Roorkee, Uttrakhand (India), Aug. 31 and Sep. 1, 2007.
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3. National workshop on "Global Climate Change and Its Impact on Water Resources" in Department of Water Resources and Development, IITR, Roorkee. Uttrakhand (India), Dec. 06, 2008.
4. National workshop on "Empowering Teachers: Effective Pedagogy and Learner Profiles" in Department of Humanities and Social Sciences, IITR, Roorkee Uttrakhand (India), Jan. 18-19, 2010.
5. International workshop on "Chemical Evolution and Origin of Life" in Department of Chemistry, IITR, Roorkee Uttrakhand (India), March 05-07, 2010.

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ACRONYMS

HCH	hexa chlorohexane
AChE	acetylcholinesterase
DTCs	dithiocarbamate
HPLC	high performance liquid chromatography
GC	gas chromatography
GPC	gel permeation chromatography
FID	flame ionization detector
ODS	octadecyl silane
LC-MS	Liquid Chromatography-Mass Spectrometry
GC-MS	Gas Chromatography- Mass Spectrometry
ESI	electrospray ionization
rpm	revolution per minute
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
b.wt.	body weight
PBS	phosphate buffered saline

CHAPTER 1

INTRODUCTION

1.1 Introduction

India is one of the largest growers of the agricultural produce and to protect them from the damage by pests, invariably, a large amount of variety of pesticides is needed. Pesticides constitute a group of chemicals deliberately disbursed into the environment to preserve the agricultural produce. The population is growing explosively and as a consequence of this the need for more food is increasing at a very fast rate. The farmers are getting aware of the benefits that can be harvested by the application of these agrochemicals but without realizing the implications of their enhanced rate of usage. Nowadays about 85% of the crops are protected by the usage of pesticides. Despite such a large consumption of pesticides, it is estimated that the crop losses vary between 10–30% due to pests alone. In monetary terms, these losses in India alone amount to Rs. 2, 90,000 million per year. This clearly exposes the inadequacy of the measures taken for the storage and protection of the crops. Giddings [1] has very rightly stated *"The dream of the man that synthetic chemicals could dismantle the universal scourge of insects lies scattered – a monumental ecological absurdity."* Large volumes of pesticides are used in developed countries and their use in developing countries is also on a fast increase. This relentless use of pesticides results in the irrevocable havoc on the health and the environment of a country.

No doubt, the use of pesticides is an important input for the safety of the agriculture produce but it leaves an everlasting problem for human safety. Once the pesticides are released into the environment they residually impair the quality of water, soil and air depending on their persistence. A problem inherent in current pesticide application technology is the drift or disposal of the pesticide away from the site of application. Only 10 to 15% of the applied pesticides actually reach the target with the remaining 85 to 90% dispersed off from the target to air, soil and water. The problem of environmental pollution, arising due to their

excessive and nonjudicious use has already started restricting the boundaries of growth of the pesticides industry. Hazards and environmental contamination through the abuse of a variety of pesticides has attracted global attention. Pesticides are inherently toxic and have to be used with caution to avoid environmental pollution and hazards to the biota. The toxic effects from the accidental use, spillage or misuse of these chemicals damage practically all the segments of the environment. Moreover; any accident in the process of manufacture and lapse in the use can cause tragedies like leakage of methyl isocynate in Bhopal (India) disaster [2] or epidemic isomalathion poisoning of malaria control workers in Pakistan [3]. The use of pesticide in agricultural products is a major source of involuntary exposure of the general public to suspected carcinogenic compounds due to contamination of the fresh and processed food by the persistent pesticide residues some of which cannot be washed off or degraded by cooking. In India, 51% of the food commodities are contaminated with pesticide residues [4]. Strangely many of the pesticides related accidents in the developing nations remain unreported. An idea about the magnitude of the pesticides pollution can be had by Table 1.1. The use of pesticides is not only slowly poisoning the population but gradually jeopardizing the future of the coming generations. The environmental scenario may be slightly better in the developed nations but not necessarily free from pesticide problems. In USA even the milk available from various sources was reported to be contaminated with pesticides [5]. Interestingly, many of the pesticides which have been banned for use in the developed countries find entry in the developing nations. But to some extent they find their way back to the country of the origin through the imported food products. This phenomenon is aptly dubbed as the circle of poison.

Table 1.1: Some important pesticide related accidents in India

Pesticide	Place	Year	Causes
Parathion	Kerala	1958	Contaminated food due to leakage
	Bombay	1962	Inhalation in manufacturing plant
HCH	Bombay	1963	Contaminated rice
Endrin	Bombay	1964	Contaminated food
DDT	Punjab	1965	Contaminated chutney
Diazinon	Pune	1968	Contaminated food
HCH	UP (Lakhimpur)	1976	Mixed with wheat
Endrin	Karnataka	1977	Contaminated crabs in rice field
Aluminum Phosphide	Rajasthan	1983	Contaminated food grain
Methyl Isocyanate	Bhopal	1984	Storage tank leakage
Cartap hydrochloride	Tamil Nadu	1988	Factory workers
Endosulfan	Kerala	1997	Contamination due to aerial spray of endosulfan
Phorate	Kerala	2001	Spray drift from banana field
Endosulfan	Jabalpur (MP)	2002	Contaminated wheat flour

The pesticides have a tendency to bioaccumulate in the living cells. Kenega [6] has emphasised that low water solubility, high fat solubility, high partitioning coefficient from water to environmental components and high stability under the various hydrolytic, light, heat and microbiological conditions are some of the properties of pesticides which lead to bioaccumulation. Bioconcentration and biotransfer potentials of various pesticides and their interaction with the various components of the environment play a very important role in deciding the fate of these pesticides. The complex interrelationship of these systems has been illustrated in Figure 1.1

The degradation of the pesticides and the types of metabolites formed involve a complex mechanism and depend upon the chemical nature of the pesticide, type of medium and the environmental and biological conditions. The medium can be air, water, soil or plant and human or animal. Even in the simplest case like that of water there are variations in different physiochemical parameters which affect the persistence of the pesticide. The climatic factors such as temperature, humidity, rainfall, wind velocity etc. also affect the decay rate. Enzymes present in plants or animals can also play an important role in deciding the fate of the pesticides. The various monitoring programmes need to fulfil the obligation to know as to where do pesticides go after they are released into the environment, what happen to them subsequently, where do they accumulate if at all, how fast they break down, what compounds are formed and which can be disregarded and what are the effects of these residues? These are some of the important aspects for which answers should be available.

In order to understand the role of the pesticides in totality there is a need for determining the pesticide residue in soil, water and plants in different conditions. It will also be interesting to see as to what happens to the pesticides when they go inside the body. The problem of

tracing the pathways of the pesticides in the different matrices is difficult as they are usually present in very small quantities say from ppm down to ppb levels.

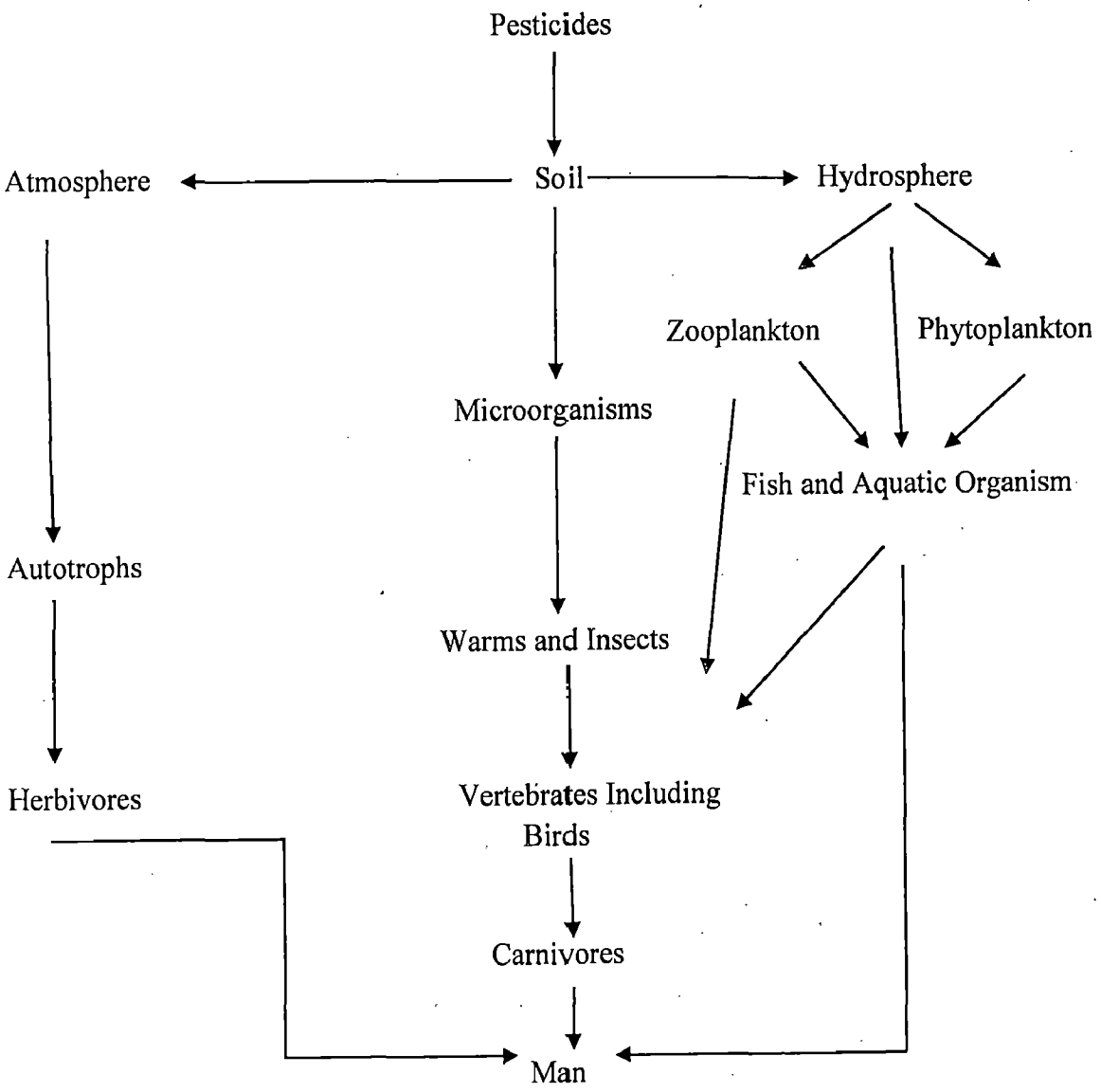


Figure 1.1: Biological transfer of pesticides to man

The situation is further complicated by the presence of an array of metabolites formed during the decay of the parent compound. A meaningful evaluation of the toxicity of the pesticides can only be made if the metabolites are identified and their toxicity evaluated. The metabolites may have different degrees of toxicity. Some of these are more toxic than their parent compounds. For example, oxons show more mammalian toxicity than their parent compounds [7]. In the cases where metabolites are more toxic it is imperative to include them for the final evaluation of the pesticide toxicity.

Reliable data on the usage of pesticides in any country are not easily available as the manufacturers are usually reluctant to disclose such information. In developing countries data are even sparser than in other parts of the world because records are often incomplete and there is a little control on the use of pesticides by the different agencies of the government. In India, the production of pesticides has increased by manifold. According to a Green Peace Report India is now producing 90,000 metric tons of pesticides. Currently, the consumption of pesticides is showing a slight declining trend, probably, due to the shift of farmers towards safer alternatives of the pest control.

The pesticides are numerous and sold under various trade names but they can be classified on the basis of target use as fungicide, insecticide, and herbicide or on their chemical composition. The most acceptable classification includes the following five categories:-

1. Organochlorine pesticides
2. Organophosphate pesticides
3. Carbamate pesticides
4. Inorganic pesticides
5. Botanical and biopesticides

The **organochlorine pesticides** include the chlorinated ethane derivatives, of which DDT and methoxychlore are the well known examples. These compounds do not breakdown into the original elements or harmless compounds. Once applied, they remain in the environment for longer duration and find their way into waterways, air, soil and finally the food chain. Most of these chemicals are banned for utilization but their illegal use continues particularly in the developing countries.

Organophosphate pesticides, derivatives of phosphoric acid, are generally more toxic to vertebrates. They inhibit acetyl cholinesterase (AChE) enzymes of the nervous system and have been responsible for a number of intentional or unintentional poisoning cases, sometimes resulting into deaths. They are absorbed by skin as well as by the respiratory and gastro-intestinal track. Most of them are easily decomposed in the environment. The commonly used pesticides in this category are malathion, methyl parathion, dimethoate and quinalphos.

Carbamates are the derivatives of carbamic acid and are used in different situations including homes, gardens and farms. Their toxicity to mammals and their persistence in the environment may vary from low to medium. They also inhibit the vital enzymes like cholinesterase. Some commonly used carbamates are carbofuran, carbaryl, thiram and mancozeb.

A large number of elements like arsenic, copper, lead and mercury are the basic constituents of several **inorganic agrochemicals**. Copper compounds such as copper sulphate and copper oxychloride are effectively used as fungicides. Sulfur dust and lime sulfur also find application as fungicides. There are several compounds of arsenic and mercury which are very poisonous. Inorganic pesticides have practically lost ground to the organic compounds due to their persistence and lethal effects.

The widespread use of the above category of chemical pesticides has resulted into several environmental problems and there is an emphasis to develop safer alternatives. A significant advancement in this direction is the development of **botanical and biopesticides.** Botanical or phytochemical pesticides are the derivatives of plants traditionally used by farmers to ward off household insects and pests. A handful of botanical insecticides are approved for use as plant protectants [8, 9] and a number of others are under development. These are mainly pyrethrum (*Chrysanthemum cineraiaefolium*), neem (*Azadirachta indica*), nicotine (*Nicotiana sp.*), rhyania (*Derris lonchocarpus*). Biopesticides formulation containing bacteria, fungi, virus, protozoa or nematodes are now becoming available in the market. Some of these are dipel (*Bacillus thuringiensis*, bacteria), bicon (*Paecillomyces lilacimis*, fungi) and spod-X (Insect virus). A large scale use of these biopesticides by a common farmer is still awaited due to the cost constraints and their availability.

After the ban on organochlorines the organophosphates are getting more popular and being used worldwide. The organophosphate pesticides, no doubt, are effective but they cannot be rated as safe or less toxic. These compounds inhibit the enzyme AChE resulting into the accumulation of acetylcholine in nerve tissue. The nerve starts firing in an uncontrolled manner and as a result organism (pest/insect) is quickly killed [10-12]. Several studies have been carried out by earlier workers for evaluating the toxicity of organophosphates and their metabolites. Matsumura [13] has suggested that the metabolic products of organophosphorus pesticides are more toxic to plants and animals than the parent compound. However, Vasilic et al. [14] observed that the presence of their diester metabolites in the human serum and urine is an indicator of the exposure rather than of the toxic effect. Studies on the suicidal/homicidal effects of the organophosphate pesticides poisoning are of immense importance in the field of

toxicology [15-17].The organophosphorus poisoning is always indicated by a significant depression of the serum and or red blood cell cholinesterase activities.

A literature and field survey shows that among the organophosphate pesticides malathion, methyl parathion, dimethoate and quinalphos are extensively used in India due to their relatively fast decomposition and low accumulation in the biological food chain. However, they are reported to be sufficiently toxic to mammals. Several attempts have been made to assess the toxicology of these pesticides. Workers who use malathion have higher levels of chromosomal damage than unexposed individuals [18]. Snamchaiskul [19] studied the systematological toxicity and the residual effect of methyl parathion and concluded that it caused longer convulsive effects. Investigations have shown that the repeated exposure of rats to dimethoate altered the functioning of the central nervous system [20]. *In vitro* and *in vivo* studies on malathion and its metabolite malaoxon in mammals showed a pattern of induction of chromosomal damage [18]. The oxons are highly toxic compounds which account for the profound cytotoxic effects of organophosphorus pesticides [21]. For example, paraoxon and malaoxon are more toxic than parathion and malathion, respectively [22].

Quinalphos when administered to pregnant rats produced enzymatic changes in liver and brain along with a significant inhibition of AChE activity in fetal brain and placenta indicating the possible transfer of the pesticide from dams to foetuses [23]. In studies on humans poisoned by quinalphos, the cholinesterase activity was affected not only by the amount of pesticide absorbed but also by its retention in the body and excretion of metabolites [14]. It was observed that 2-hydroxy quinoxaline, a metabolite of quinalphos, photocatalytically destroys the antioxidant vitamins and biogenic amines [24]. A more elaborate information on the toxicology of quinalphos and its metabolites is presented in chapter III and V.

The economic conditions prevailing in developing countries dictate the use of cheaper broad spectrum carbamate pesticides. In India they as a group are most widely used. Amongst these carbaryl, carbofuran, thiram and mancozeb are more popular due to their easier availability and faster decomposition. The indiscriminate use of these pesticides in agriculture, forestry and public health leaves considerable amount of residues and their metabolites in the environment.

A number of reports are available on the toxicology of these pesticides. Carbaryl is an AChE inhibitor which acts by carbamylating the esteratic active site of AChE [25-30]. Lin et al. [31] studied the toxicity and cardiac effects of carbaryl in the early developing zebrafish (*Danio rerio*) embryos. The reproductive toxicity of carbofuran to the female mice showed the specific action of high toxicity as well as hormonal imbalance [32]. The carbamate pesticide mancozeb has been reported for the induction of gonadal toxicity to female rats after a chronic exposure [33]. Along with these cyto- and reproductive toxicities, microbial toxicity is also common with carbamates. The metabolites of carbamates are also reported to have a toxicological concern [34].

Thiram is a member of dithiocarbamates (DTCs) a subgroup of carbamates. It is an important organosulfur compound which acts as an inhibitor of metal dependant and sulphydryl enzyme. It has a serious consequence on the biological systems [35]. Cereser et al. [36] reported the cytotoxic effects of thiram on cultured human skin fibroblasts. A number of *in vitro* short term assays including the ames test and assays on mammalian cell systems have shown the genotoxic activity of thiram [37-39]. It is metabolized in the body to toxic metabolites, dimethyldithiocarbamate and carbon disulfide, responsible for the observed hepatotoxicity.

The above reports act as a pointer to project the toxicity of organophosphates and carbamates. The toxic effects with some reservation can be extrapolated to different terrestrial and aquatic forms. In order to assess the effects of these pesticides quantitatively the decay profiles and the metabolites formed should be precisely known. It is ultimately important to know that with the time how much of the pesticide remains in the body and what are the metabolites formed.

As indicated earlier the decay of pesticides follows a very complex mechanism and depends on a number of parameters. The simplest approach to the problem should be to follow the decay under controlled laboratory conditions and identify the metabolites at various stages of decay. Once the effects of different variables on the decay and metabolites formation are known the study may be extended to the field and finally on animals. A reasonable amount of literature is available on the residue analysis of organophosphate and carbamate pesticides in different components of the ecosystem. Freed and coworkers [40] evaluated the degradation of some organophosphate pesticides in water and soil and indicated that half life is pH and temperature dependent. Sardar and kole [41] investigated the persistence and metabolism of chlorpyriphos in gangetic alluvial soil to evaluate their effect on the availability of the major plant nutrients N, P and K. They reported significant decrease in the available N and P content in soil treated with chlorpyriphos. Wolfe and his group [42] found that the degradation of malathion in laboratory water is strongly influenced by the temperature and pH and the decay rate is slower in water than in soil. Holm et al. [43] evaluated the laboratory ecosystem for assessing the chemical fate of methylparathion. The contribution of Kaur et al. [44] on the degradation of malathion and methyl parathion is very significant and noteworthy for the proposed studies. They observed that the decay of organophosphorus pesticides in water, soil

and plant follows a different pattern. Moreover, the decay is dependent on temperature, pH and organic content. A strange profile of decay is observed in the case of field conditions.

Degradation kinetics of dithiocarbamate in aqueous media has been investigated and it was observed that the dithiocarbamates are degraded by simple air exposition. It was reported that carbaryl degrades in a short time in water, soil and on various plants [45] and the decay kinetics depend on various factors.

A survey of literature reveals that a number of studies have been conducted on the toxicology of organophosphorus and carbamate pesticides. Reports are also available on the degradation of some of the compounds of the above two classes of pesticides. However, a comprehensive degradation study on the two popular pesticides, namely, quinalphos and thiram remains unavailable. Both are themselves highly toxic and to add to it is the toxicity of their metabolites.

Scattered references are documented on the degradation of these pesticides under laboratory conditions. Most of the work reported in the literature is on the photochemical and microbial degradation of these compounds. However, the field data on the persistence profiles under different field conditions are scarce. There has been a growing concern about the nature and toxicity of the breakdown products. The effects of various parameters on the formation of different metabolites at different stages of the decay have not been systematically investigated. However, some isolated studies are available on the decomposition and metabolic pathways of these pesticides which merely act as a pointer.

The most important aspect of the persistence study of a toxin is its behaviour in a mammalian system. The pesticide is no exception to it. Generally not much attention is paid to this particular aspect of the investigation. A careful pharmacokinetic study in a mammalian system should be carried out to assess its decay and transformation to different metabolites.

Generally, these studies are carried out by choosing albino rats as the subject for exposure. As a matter of scientific ritual the *in vivo* studies are generally supported by *in vitro* investigations. The literature on the pharmacokinetic studies on pesticides, in general, is scarce. Some biomonitoring studies have been carried out on a few organophosphates and carbamates other than the two compounds under investigations [46-51]

The detailed literature on all the above aspects is reviewed in different chapters.

In the light of above discussion it was planned to carry out investigations on the decay profiles and identification of the metabolites of quinalphos and thiram. It is interesting that out of the chosen pesticides one is representative of the typical toxic organophosphates and other one belongs to the series of hazardous carbamates. In the first phase, studies were undertaken on the degradation of quinalphos and thiram under controlled laboratory conditions. Subsequently investigations on the persistence of both of these pesticides were extended to plants in field conditions. Finally the metabolites of the two were identified at the various stages of decay in water, soil and plants. The plants chosen for the study were radish (*Raphanus sativus*) and tomato (*Lycopersicon esculentum*). The choice for these two plants is particularly based on the fact that some part of the plant is frequently consumed uncooked and the sprayed pesticide may be ingested directly and may pose threat to life. To make the entire study as a meaningful packet it was planned to carry out in *vitro* and *in vivo* pharmacokinetic studies on the degradation of both the pesticides in albino Wistar rats. But during the analysis of thiram in rats there were serious interferences due to matrix constituents and the proposed investigations on this pesticide could not be pursued. Moreover, it was metabolized in the body to volatile products which were not easy to trap. In view of these facts, the author had to confine the investigation on quinalphos. The degradation and identification of metabolites in biological fluids of albino Wistar rats have been studied.

For the measurement of the concentration of the pesticide various analytical technique mainly chromatography in its different forms and spectrophotometry are in vogue. Amongst chromatographic techniques high performance liquid chromatography (HPLC) and gas chromatography (GC) are more popular and whatever improvements are taking place in the pesticide analysis are mainly directed towards these techniques [52-55]. Superiority of these techniques in the identification of byproducts of pesticides is established after hyphenating them with mass spectrometer. The pertinent information on the utility of these techniques and the methods employed for the extraction has been carefully surveyed and is presented in the appropriate chapter.

For the sake of clarity and convenience in presentation the work embodied in the thesis has been divided into the following five chapters

I. General introduction

II. Experimental methodology

III. Decay profiles and metabolic pathways of quinalphos in water, soil and plants

IV. Decay profiles and metabolic pathways of thiram in water, soil and plants

V. *In vitro* and *in vivo* studies on the degradation of quinalphos in rats

This division has been restorted to make the studies more lucid and comprehensive. Finally the thesis ends with a brief discussion on the findings of the present investigations in the form of conclusions.

At the end the author would like to mention that though utmost care has been taken to cite the relevant literature, however, if there are any omissions they are inadvertent. Furthermore, in presenting the work, care has been taken to minimize repetition. In order to maintain the continuity and clarity in the text, at times, it has not been possible to do so.

1.2 References

[1]. Giddings, J. C. and Monroe, M. B., "Our chemical environment", Canfield Press, San Francisco, p.2 (1972).

[2]. Broughton, E., "The Bhopal disaster and its aftermath: a review", Environ. Health **4**, 6 (2005).

[3]. Baker, E. L., Warren, Mc. W., Zack, M., Bobbin R. D., Miles, J. W. and Miller, S., "Epidemic malathion poisoning in Pakistan malaria workers", Lancet **311**, 31 (1978).

[4]. Gupta, P. K., "Pesticide exposure – Indian scene", Toxicology **198**, 83 (2004).

[5]. Calabrese, E. J., "Human breast milk contamination in the United States and Canada by chlorinated hydrocarbon insecticides and industrial pollutants: current status", Int. J. Toxicol. **1**, 91 (1982).

[6]. Kenega, E. E., "Factors related to bioconcentration of pesticides", In: Environmental Toxicology of Pesticides, Matsumura, F., Bousch G. M. and Misato, T. (Eds.), Academic Press, New York, p.193 (1972).

[7]. Salazar-Arredondo, E., Solis-Herediaa, M. J., Rojas-Garcia, E., Hernandez-Ochoaa, I. and Quintanilla-Vega, B., "Sperm chromatin alteration and DNA damage by methyl-parathion, chlorpyrifos and diazinon and their oxon metabolites in human spermatozoa" Reprod. Toxicol. **25**, 455 (2008).

[8]. Isman, M. B., "Botanical insecticides", Pestic. Outlook **5**, 26 (1994).

[9]. Isman, M. B., "Neem and other botanical insecticides: barriers to commercialization", Phytoparasitica **25**, 339 (1997).

[10]. De, A. K., Environmental chemistry, Willey Eastern Ltd., New Delhi, p.76 (1987).

[11]. Ecobichon, D. J., "Pesticides", In: Casarett and Doull's Toxicology: The Basic Science of Poisons (4th edn. ed.), Amdur, M. O., Doull, J. and Klaassen, C. D. (Eds.), Pergamon Press, New York, p. 580 (1991).

[12]. Padilla, S., Wilson, V. Z. and Bushnell, P. J., "Studies on the correlation between blood cholinesterase inhibition and 'target tissue' inhibition in pesticide-treated rats", Toxicology **92**, 11 (1994).

[13]. Matsumura, F., "Toxicology of insecticides", 2nd ed. Plenum Press, New York, USA, (1985).

[14]. Vasilic, Z., Drevenkar, V., Rumenjak, V., Stengl, B. and Frobe, Z., "Urinary excretion of diethylphosphorus metabolites in persons poisoned by quinalphos or chlorpyrifos", Arch. Environ. Contam. Toxicol. **22**, 351 (1992).

[15]. Laham, S., Long, G. W. and Broxup, B. R., "Subchronic oral toxicity of tributoxyethyl phosphate in the Sprague–Dawley rat", Arch. Environ. Health **40**, 12 (1986).

[16]. Fautz, R. and Miltenburger, H. G., "Influence of organophosphorus compounds on different cellular immune functions *in vitro*", Toxicol. In Vitro **8**, 1027 (1994).

[17]. Teixeira, H., Proença, P., Alvarenga, M., Oliveira, M., Marques, S. P. and Vieira, D. N., "Pesticide intoxications in the centre of portugal: three years analysis", Forensic Sci. Int. **143**, 199 (2004).

[18]. Flessel, P., Quintana, P. J. and Hooper, K., "Genetic toxicity of malathion: a review", Environ. Mol. Mutagen. **22**, 7 (1993).

[19]. Snamchaiskul, C., "Studies on the systematological toxicity and the residual effect of pesticide on malberry leaves to the silkworm (*bombyx moril*)", Leaves, 61, Bangkok, Thailand, (1986).

[20]. Nagymgtenyi, L., Schulz, H., Papp, A. and Desi, I., "Developmental neurotoxicological effects of lead and dimethoate in animal experiments", Neurotoxicology **19**, 617 (1998).

[21]. Vijayaraghavan, M. and Nagarajan, B., "Mutagenic potential of acute exposure to organophosphorus and organochlorine compounds", Mutat. Res. **321**, 103 (1994).

[22]. Perry, A. S., Yamamoto, I., Ishaaya, I. and Perry, R. Y., "Insecticides in agriculture and environment: retrospects and prospects", Springer, Berlin, Heiddelberg, p. 261 (1998).

[23]. Srivastava, M. K. and Raizada R. B., "Assessment of the no-observed-effect level (NOEL) of quinalphos in pregnant rats", Food Chem. Toxicol. **37**, 649 (1999).

[24]. Riediger, S., Behrends, A., Croll, B., Vega-Naredo, I., Hänig, N., Poeggeler, B., Böker, J., Grube, S., Gipp, J., Coto-Montes, A., Haldar, C. and Hardeland, R., "Toxicity of the quinalphos metabolite 2-hydroxyquinoxaline: growth inhibition, induction of oxidative stress, and genotoxicity in test organisms", Environ Toxicol. **22**, 33 (2007).

[25]. Watts, P. and Wilkinson, G. R., "The interaction of carbamates with acetylcholinesterase", Biochem. Pharmacol. **26**, 757 (1977).

[26]. Scaps, P., Demuynck, S., Descamps, M. and Dhainaut, A., "Effects of organophosphate and carbamate pesticides on acetylcholinesterase and choline acetyltransferase of the polychaete *Nereis diversicolor*", Arch. Environ. Contam. Toxicol. **33**, 208 (1997).

[27]. Gruber, S. J. and Munn, M. D., "Organophosphate and carbamate insecticides in agricultural waters and cholinesterase (ChE) inhibition in common carp (*Cyprinus carpio*)", Arch. Environ. Contam. Toxicol. **35**, 391 (1998).

[28]. Lazartigues, E., Freslon, J. L., Tellioglu, T., Brefel-Courbon, C., Pelat, M., Tran, M. A., Montastruc, J. L. and Rascol, O., "Pressor and bradycardic effects of tacrine and other acetylcholinesterase inhibitors in the rat", Eur. J. Pharmacol. **361**, 61 (1998).

[29]. Mora, B. R., Martinez-Tabche, L., Sánchez-Hidalgo, E., Hernández, G. C., Ruiz, M. C. G. and Murrieta, F. F., "Relationship between toxicokinetics of carbaryl and effect on acetylcholinesterase activity in Pomacea patula snail", Ecotoxicol. Environ. Saf. **46**, 234 (2000).

[30]. Masuda, Y. and Kawamura, A., "Acetylcholinesterase inhibitor (donepezil hydrochloride) reduces heart rate variability", J. Cardiovasc. Pharmacol. **41**, 67 (2003).

[31]. Lin, J-. H., Kao, W-. C, Tsai, K-. P. and Chen, C-. Y., "A novel algal toxicity testing technique for assessing the toxicity of both metallic and organic toxicants", Water Res. **39**, 1869 (2005).

[32]. Baligar, P. N. and Kaliwal, B. B., "Reproductive toxicity of carbofuran to the female mice: effects on estrous cycle and follicles", Ind. Health **40**, 345 (2002).

[33]. Baligar, P. N. and Kaliwal, B. B., "Induction of gonadal toxicity to female rats after chronic exposure to mancozeb" Ind. Health **39**, 235 (2001).

[34]. Chapalamadugu, S. and Chaudhry, G. R., "Microbiological and biotechnological aspects of metabolism of carbamates and organophosphates", Crit. Rev. Biotechnol. **12**, 357 (1992).

[35]. Vettorazzi, G., Almeida, W. F., Burin, G. J., Jaeger, R. B., Puga, F. R., Rahde, A. F., Reyes, F. G. and Chvartsman, S., "International safety assessment of pesticides: dithiocarbamate pesticides, ETU, and PTU--a review and update", Teratog. Carcinog. Mutagen. **15**, 313 (1995).

[36]. Cereser, C., Boget, S., Parvaz, P. and Revol, A., "Thiram-induced cytotoxicity is accompanied by a rapid and drastic oxidation of reduced glutathione with consecutive lipid peroxidation and cell death", Toxicology **163**, 153 (2001).

[37]. Franekic, J., Bratulic, N., Pavlica, M. and Papes, D., "Genotoxicity of dithiocarbamates and their metabolites", Mutat. Res. **325**, 65 (1994).

[38]. Agrawal, R. C., Shukla, Y. and Mehrotra, N. K., "Assessment of mutagenic potential of thiram", Food Chem. Toxicol. **35**, 523 (1997).

[39]. Ardito, G., Bigatti, P. and Lamberti, L., "Increased frequencies of sister chromatid exchanges and micronuclei in '*in vitro*' lymphocyte cultures treated with the fungicides thiram and ziram", Boll. Soc. Ital. Biol. Sper. **73**, 1 (1997).

[40]. Freed, V. H., Chiou, C. T. and Schmedding, D. W., "Degradation of selected organophosphorus pesticides in water and soil", J. Agric. Food Chem. **27**, 706 (1979).

[41]. Sardar, S. and Kole R. K., "Metabolism of chlorpyrifos in relation to its effect on the availability of some plant nutrients in soil", Chemosphere **61,** 1273 (2005).

[42]. Wolfe, N. L., Zepp, R. G., Gorden, J. A., Baughman, G. L., and Cline, D. M., "Kinetics of chemical degradation of malathion in water", Environ. Sci. Technol. **11**, 88 (1977).

[43]. Holm, H. W., Kollig, H. P., Proctor, L. M., and Payne, J. W. R., "Laboratory ecosystem for studying chemical fate-an evaluation using methyl parathion", Report EPA-600/ 3-82-020 (1979).

[44]. Kaur, I., "PhD dissertation", University of Roorkee, Roorkee, India, (1997).

[45]. Uyanik, A. and Özdemir, M., "Effect of the environmental temperature on the degradation period of carbaryl", Turk. J. Agr. Forest. **23**, 579 (1999).

[46]. Gearhart, J. M., Jepson, G. W., Clewell, H. J., Andersen, M. E. and Conolly, R. B., "Physiologically based pharmacokinetic model for the inhibition of acetylcholinesterase by organophosphate esters", Environ. Health Perspect. **102,** 51 (1994).

[47]. Liu, K. H., Sung, H. J., Lee, H. K., Song, B. H., Ihm, Y. B., Kim, K., Lee, H. S. and Kim, J. H., "Dermal pharmacokinetics of the insecticide furathiocarb in rats", Pest Manag. Sci. **58**, 57 (2002).

[48]. Timchalk, C., Nolan, R. J., Mendrala, A. L., Dittenber, D. A., Brazak, K. A. and Mattsson, J. L., "A physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for the organophosphate insecticide chlorpyriphos in rats and humans", Toxicol. Sci. **66**, 34 (2002).

[49]. Mamidi, R. N., Mannens, G., Annaert, P., Hendrickx, J., Goris, I., Bockx, M., Janssen, C. G., Kao, M., Kelley, M. F. and Meuldermans, W., "Metabolism and excretion of RWJ-333369 [1,2-ethanediol, 1-(2-chlorophenyl)-, 2-carbamate, (S)-] in mice, rats, rabbits, and dogs", Drug Metab. Dispos. **35**, 566 (2007).

[50]. Szolar, O. H. J., "Environmental and pharmaceutical analysis of dithiocarbamates", Anal. Chim. Acta **582**, 191 (2007).

[51]. Forsberg, N. D., Rodriguez-Proteau, R., Ma, L., Morré, J., Christensen, J. M. ,Maier, C. S., Jenkins, J. J. and Anderson, K. A., "Organophosphorus pesticide degradation product *in vitro* metabolic stability and time-course uptake and elimination in rats following oral and intravenous dosing", Xenobiotica. **41,** 422 (2011).

[52]. Sukul, P. and Spiteller, M., "Influence of biotic and abiotic factors on dissipating metalaxyl in soils" Chemosphere **45**, 941 (2001).

[53]. Raikwar, M. K. and Nag, S. K., "Phototransformation of alphacypermethrin as thin film on glass and soil surface", J. Environ. Sci. Health B **41**, 973 (2006).

[54]. Mahalakshmi, M., Arabindoo, B., Palanichamy, M. and Murugesan, V. "Photocatalytic degradation of carbofuran using semiconductor oxides", J. Hazard. Mater. 143, 240 (2007).

[55]. Goswami, S., Vig, K. and Singh, D. K., "Biodegradation of α and β endosulfan by Aspergillus sydoni", Chemosphere **75**, 883 (2009).

CHAPTER 2

EXPERIMENTAL METHODOLOGY

2.1 Introduction

Pesticide residue analysis is generally a time consuming and complicated exercise. The major steps in the analysis are:-

1. Collection and preservation of the sample
2. Extraction of the pesticide
3. Clean up of the undesirable constituents
4. Determination of the pesticide

Sampling is the most vital step because the collected sample must represent the exact scenario of the environment from which it is withdrawn. It is very important that the sample intended for the residue analysis does not undergo any degradation or chemical change during the collection and storage. Sample preparation, sample processing and sub-sampling to obtain analytical portions should take place before any visible deterioration occurs. Also it should not be contaminated with the impurities which may affect the results of the analysis. Invariably the environmental and biological samples cannot be subjected to analysis directly as the concentration of the pesticide encountered is very low and the levels of the interfering constituents may be too high. It is, therefore, necessary to extract the desired compound from the sample ensuring that the extraction is quantitative and there are no changes in the composition. Besides, the components of the matrix should not be coextracted with the pesticide to the extent possible. Subsequently, the sample is to be cleaned from the remaining interfering impurities before subjecting it to assay. Adsorption column chromatography finds an extensive use for the clean up of the sample and nowadays standard cartridges are available for the said purpose. For the measurement of the pesticide concentration the various analytical techniques mainly chromatography in its different forms and spectrophotometry are in vogue. Amongst chromatographic techniques HPLC and GC are more popular and whatever improvements are taking place in the pesticide analysis are mainly directed towards these techniques.

In spite of a vast literature on the extraction procedures for the pesticide residue analysis none of the methods can be rated as the standard or universal. The method will depend upon the nature of the pesticide and the medium from which it is to be extracted. On the other hand it is true that the existing information on the extraction and clean up can provide a handy guide for developing the quantization procedure. Over a period of time several changes have taken place in the steps involved in the pesticide residue analysis. It may be difficult to present the entire available information on the said aspect. However, an idea about the development can be had from a brief review of trends in the analysis of pesticides. This proved to be useful to the author in selecting the methods for the analysis of pesticides involved in the present project.

The biennial reviews by Joseph Sherma which appear in the journal "Analytical Chemistry" provide an exhaustive and upto date information on the status of the pesticide analysis. Several extraction procedures ranging from the simple to soxhlet extraction have been used to extract pesticides from water, soil and plant for the analysis. The basis for the selection of an optimal extractant for the extraction has not been very clearly defined in the literature. Dao et al. [1] presented a rationale on the solvent selection used for the extraction of residues and concluded that it is not possible to predict the suitability of a solvent as an extracting medium simply on the basis of solubility.

Prior to the residue determination, the extraction and purification of sample is required. In this respect, some of the commonly employed methods like liquid liquid extraction [2], solid phase extraction [3], solid phase microextraction [4], matrix solid-phase dispersion [5], microwave assisted extraction [6-8] and ultrasonic or sonication assisted extraction [9, 10]. For aqueous samples liquid liquid partition with non polar solvents like hexane, chloroform, ethyl acetate, dichloromethane etc. is preferred for the pesticide residue analysis. Liquid solid extraction of pesticides from soil is generally

carried out by organic solvents. The two techniques which have been widely used are the shaking and filter and the soxhlet extraction method.

The earliest analytical methods used for the pesticide analysis in vegetables were developed in the 1960s. They employed basically an initial extraction with acetone, followed by a partitioning step with the addition of a non-polar solvent and a salt. The development of technology and robotics in the 1990s allied to the aim to reduce manual interference and to allow sample preparation during non-working time has boosted the development of the automated sample preparation techniques such as supercritical fluid extraction and pressure liquid extraction. Though initially very promising, these techniques have not succeeded in the field of pesticide analysis for various reasons, namely high price and low reliability of the instruments and inability to extract different classes of pesticide in foods with the same efficiency and often requiring separate optimization for different analytes. Later a successful simplification of "traditional" solvent sample preparation, QuEChERS (quick, easy, cheap, effective, rugged and safe) was presented by Anastassiades and collaborators [11]. This procedure involving a simple extraction/partition using acetonitrile and salts followed by a simple dispersive clean up, has been adopted for the analysis of many pesticide residues in food [12-17].

A comparison of the commonly used clean up methods based on adsorption column chromatography has been presented by Tekel and Hatrik [18]. Sep-pak cartridges, gel permeation chromatography (GPC), alumina, florisil and mixed adsorbant column containing sodium sulphate, florisil, celite and charcoal (10:10:8:1) were suitable for the samples with high chlorophyll content and for purifying lipid contents of the extract. A review on official multiresidue methods of pesticide analysis in vegetables, fruits and soil was presented by Matohashi and coworkers [19]. They used florisil and GPC for the primary clean up procedures.

Analytical methods are required for the routine monitoring of the pesticides in crop matrices and the other environmental compartments. For the measurement of the pesticide concentration a number of techniques namely spectrophotometry, voltammetry, polarography, capillary electrophoresis and chromatography in its different forms (column, thin layer, gas and high performance liquid chromatography) have been employed. Amongst these, chromatographic techniques are preferred for the qualitative and quantitative evaluation of the pesticides. GC and HPLC are more popular chromatographic technique for the said purpose. Since the early 1970s most of the pesticide residue determinations in different matrices have been conducted by GC. Although it has been on the scene for more than 40 years, it is still generally considered a relatively novel technique and is yet far from being fully established. The most commonly used detection system has been the flame ionization detector (FID) followed by the mass spectrometer. Detailed descriptions of the detectors used in comprehensive GC experiments have been reported in the literature [20, 21]. With regards to the GC column, glass column was used initially, but fused silica capillary columns with different stationary phases are now in vogue. Although GC is senior and more sensitive than HPLC but for the pesticide analysis it has been partly overtaken by HPLC.

The earliest documentation on the use of HPLC for pesticide residue analysis appeared in the year 1971 for the determination of pesticide in pond water [22]. Ravelo-Perez et al. [23] presented an in depth review on the use of HPLC and capillary electrophoresis in the environmental pesticide analysis. A real breakthrough in the technique came with the introduction of versatile octadecyl silane (ODS) column for HPLC [24]. Accordingly, two third of all the desired separations in the pesticide analysis can be achieved by reversed phase (RP)-HPLC. HPLC is quite advantageous as it easily provides the option of collecting the fraction for subsequent analysis. Furthermore, HPLC quickly and accurately determines the pesticides which are not easily amenable to analysis

by GC at room temperature. Several types of detectors namely, ultra violet (UV), diode array, fluorescence, refractive index and amperometric are generally equipped with HPLC. For residue analysis, however, only the UV detector has sufficient sensitivity and some degree of specificity. The introduction of the easily operated liquid chromatograph-mass spectrometer (LC-MS) instrument provides a new way of analyzing the pesticides with better sensitivity and efficiency and greater scope [25-28].

Both GC and HPLC are very effective as far as the separation is concerned but they are not equally good for the identification or confirmation. Therefore, the need of hyphenating these techniques with mass spectrometry arose. This requirement is particularly important when the metabolites are also to be identified along with the parent compound.

Reported literature reveals that generally reversed phase columns have been used in pesticide analysis but normal phase columns have also been employed by some workers [29-32]. Reversed phase columns with the stationary phase C-8 or C-18 and packing monomeric or polymeric have been widely used. With regards to mobile phases, methanol, acetonitrile or their mixture with water/buffers are mostly used in isocratic or gradient mode. It has been reported that RP-HPLC using an octadecyl (C-18) bonded silica column with endcapping and acetonitrile-water mixture as the mobile phase gives the most efficient separation of the pesticides [33].

Both GC and HPLC are used effectively for the estimation of organophosphate residues. During the course of present study with quinalphos it was observed that GC with FID detector did not give reproducible results for its quantification. Therefore, HPLC has been preferred for its residue determination. However, for the identification of its metabolites; GC-MS has been used. It is well known that LC is preferred for the nonvolatile carbamate pesticides. Therefore, HPLC was an automatic choice to follow the decay of thiram and LC-MS has been used for the characterization of its metabolites.

Different mobile phases were tried and the optimum mobile phase composition for quinalphos and thiram is methanol and acetonitrile-water, respectively in isocratic mode. The commonly used organic solvents for the extraction of the pesticides are ethyl acetate, dichloromethane, acetonitrile, chloroform and methanol. A trial of all these solvents showed that ethyl acetate is best suited for the extraction of quinalphos from water and soil [34]. QuEChERS method using acetonitrile with slight modification has been preferred for the quantitative recovery of quinalphos from plants. Ethyl acetate [35, 36], acetonitrile [37] and dichloromethane give the desired results for the extraction of thiram from water, soil and plants, respectively. Florisil column is found to be most effective for the clean up of the samples.

The present chapter incorporates the details of the required materials, extraction methods, clean up and quantification of quinalphos and thiram from water, soil, plants and biological samples.

2.2 Experimental

2.2.1 Reagents

Quinalphos and thiram standards (99.0% purity) were purchased from Merck (Dr. Ehrenstorfer GmbH Augsburg, Germany) and their purity was checked by HPLC. The acetonitrile, methanol, dichloromethane, acetone, chloroform and ethyl acetate used were of chromatographic grade (Rankem, India). Deionised water was used throughout the studies. Anhydrous sodium sulphate (E. Merck, India) and florisil (Himedia) (mesh size 60-100) used for column chromatography were of desired analytical purity. Potassium dihydrogen phosphate and di- sodium hydrogen phosphate-2- hydrate of analytical grade (E. Merck, India) were used for preparing buffer solutions. Sodium salt of humic acid (Aldrich, USA) was used as a representative of organic matter. Phosphate buffers were used to study the effect of pH in water and it was ascertained that the buffer constituents do not affect the decay. Soil samples collected from three different places, Uttarkashi and

Roorkee (Uttarakhand) and Sriganganagar (Rajasthan), India were sieved to less than 75 mm and conditioned in an incubator at 20 ^{0}C. Clay, sand, silt and organic carbon contents of different soils were determined by the standard methods [38]. Table 2.1 shows the characteristics of the soils.

Pepsin (1130 U/mg protein), pancreatin (4 X USP activity) and bile extract were procured from Sigma Aldrich (St. Louis, MO, USA). Sodium bicarbonate and hydrochloric acid, used to maintain the pH of the deionised water, were of analytical grade from SRL (India). All the chemicals were used without further purification. Potasium chloride and sodium chloride (E. Merck, India) were used for preparing buffer solutions of pH 2.0 and pH 7.0.

A stock solution of quinalphos and thiram with a concentration of 1000 μg mL^{-1} was made in methanol for water, soil and plant studies. For pharmacokinetic studies a concentrated stock solution (10,000 μg mL^{-1} quinalphos) prepared in dimethyl sulphoxide (DMSO) was used to minimize the volume of dimethyl sulphoxide per dose given to rat. All the stock solutions were stored in dark under refrigeration. It was checked that the stock solution when stored at -4 ^{0}C is stable for four months.

2.2.2 High performance liquid chromatography (HPLC)

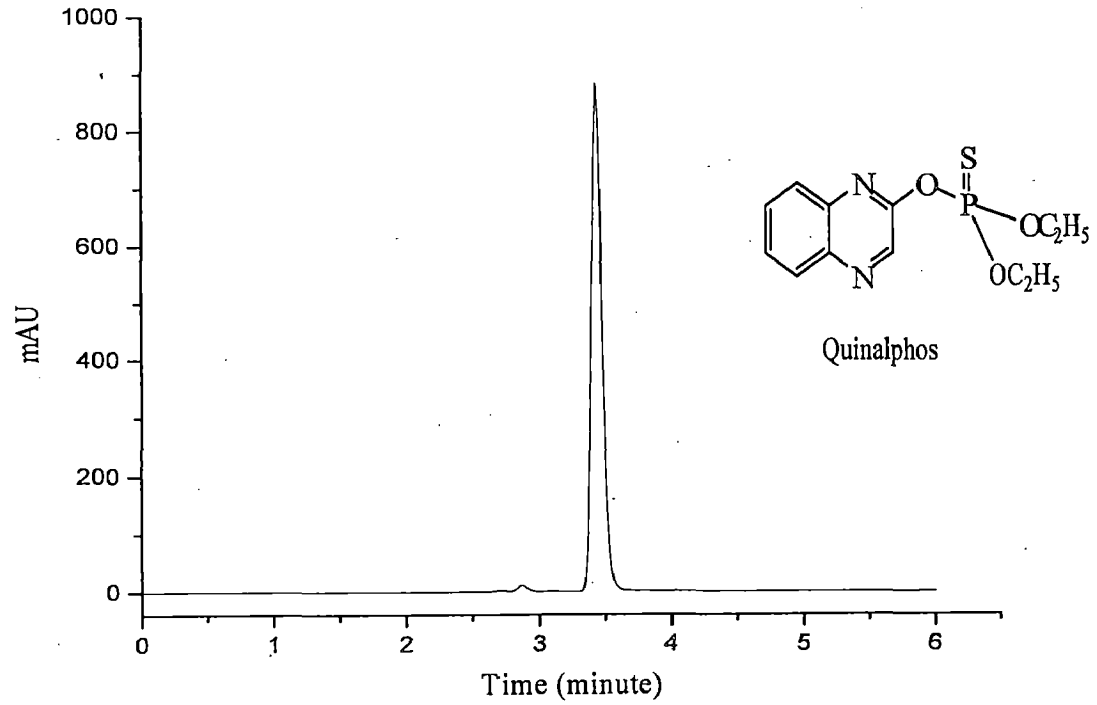
For the decay studies in water, soil and plants Knauer HPLC system (Model No D14163, Advanced Scientific Instruments Ltd. Berlin, Germany), equipped with a UV detector was employed. HPLC system consisting of two high pressure pumps with a flow capability of 9.9 mL min^{-1} and an automated gradient controller was used to programme the elution system. An octadecyl endcapped RP-C_{18} column (4.6 mm i.d × 25 cm, 5μm) and Knauer universal injector of 100 μL capacity were used. 20 μL sample was injected and a flow rate of 1 mL min^{-1} was maintained. The system was operated at the ambient temperature.

Table 2.1: Physio-chemical characteristics of the soil used for the study

Characteristic component	Uttarkashi	Roorkee	Sriganganagar
pH	5.1	6.8	8.1
Clay (%)	0.30	0.50	0.40
Sand (%)	90.0	74.5	78.3
Silt (%)	9.70	25.5	21.3
Organic carbon (%)	0.44	0.37	0.32
Moisture content (%)	13.0	16.5	12.5

2.2.3 Optimization of parameters for the analysis by HPLC

The conditions were optimized by varying different parameters mainly mobile phase composition, UV detector wavelength, flow rate of the mobile phase and injection volume. With a view to get the best recovery and resolution methanol and acetonitrile as such and in mixture with water in different ratios were tried as mobile phase. The optimum mobile phase composition for quinalphos is methanol in isocratic mode and for thiram it is a mixture of acetonitrile and water (70:30) in isocratic mode. In order to get the desired response of the UV detector wave length range from 210- 254 nm was scanned. The flow rate was varied from 0.5 to 1 mL min^{-1} for speedy elution of the peak of interest without any compromise in the resolution. The UV detector was set at 236 nm for quinalphos and 223 nm for thiram. Under these conditions the injection volume was varied from 1μL to 20 μL. The system was operated at the ambient temperature. Under the specified conditions an aliquot of 20 μL sample was injected at a flow rate of 1mL min^{-1}. A retention time of 3.6 min and 4.5 min was observed for quinalphos and thiram, respectively (Fig. 2.1). Calibration curve was constructed by plotting standard peak area versus concentration. A linear plot (r^2=0.99) was obtained in the concentration range 1- 100 μg mL^{-1} for quinalphos and 1- 200 μg mL^{-1} for thiram. Recoveries were calculated as the ratio of the peak area of the analyte from the fortified samples to the corresponding peak area of standard solutions.



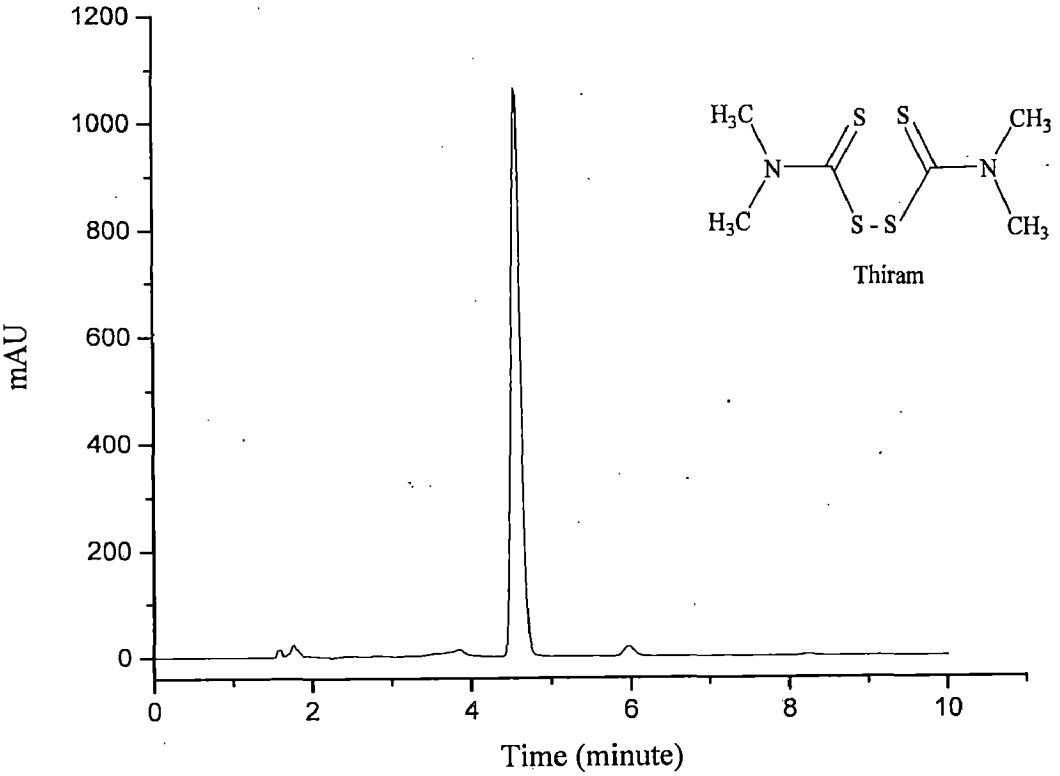


Figure 2.1: HPLC Chromatograms of quinalphos and thiram

2.2.4 Liquid chromatography with mass spectrometry (LC-MS)

LC-MS was used for the characterization of metabolites of thiram. An agilent LC-MS -1100 Series instrument (Palo Alto, CA, USA) with the Hystar software version consisting of a DE 11404298 manual injection with a 20 μL loop, a quaternary pump, and a VWD UV-VIS detector coupled in series with a mass selective detector (Bruker Esquire 4000, Bruker Daltonic, Bremen, Germany) interfaced to an elctrospray ionization (ESI) source was used for mass analysis and detection. The LC separation was achieved on an octadecyl endcapped RP-C_{18} chromatographic column (4.6 mm i.d × 25 cm, 5μm) from Merck. The optimal chromatographic and mass spectrometric conditions are listed in Table 2.2.

2.2.5 Gas chromatography with mass spectrometery (GC-MS)

Degradation products of quinalphos in different samples were identified with a gas liquid chromatograph interfaced with a mass selective detector (Perkin Elmer-Clarus 500). GC was fitted with an Elite-5 capillary column (Crossbond 5% diphenyl and 95% dimethyl polysiloxane; 30 m × 0.32 mm i.d. × 0.25 μm film thickness, Perkin Elmer ^{TM} instrument, Shelton, CT, USA). Helium was used both as the carrier (1 mL min^{-1}) and the make-up gas (40 mL min^{-1}). A split/splitless injector in the splitless mode at 250 ^{0}C was used. The transfer line temperature was set at 280 °C and oven temperature was programmed from 60 ^{0}C (2 min hold) to 300 ^{0}C at the rate of 10 ^{0}C min^{-1} (5 min hold). The injected volume and scan time were 2 μL and 0.2 s, respectively. Chromatographic data were acquired by recording the full scan mass spectra in the range m/z 50-500. The chromatographic data acquisition and processing were carried out with a Turbo mass software. All GC-MS chromatograms were extensively searched for the possible byproducts arising from quinalphos degradation, using a set of indicative fragment ions related to the parent molecule. The corresponding mass spectra are given in respective chapters.

Table 2.2: LC–MS conditions

LC separation	
Solvent A	Acetonitrile
Solvent B	Water
Flow rate (mL min^{-1})	1
Time (min)	30
MS detection	ESI
Drying gas flow (L min^{-1})	5.0
Drying gas temperture (^{0}C)	300
Nebulizer pressure (psi)	10
Capillary voltage (V)	4000
m/z range	50–400

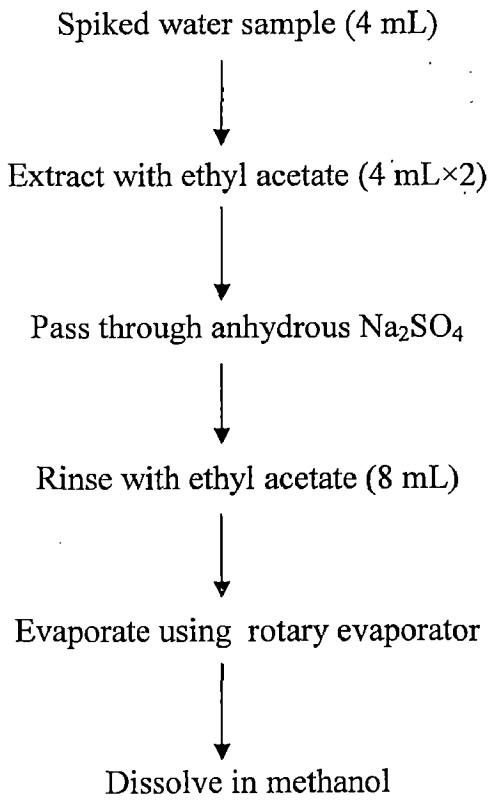
2.2.6 Extraction and clean up

Several procedures for the extraction of the pesticides from different matrices namely water, soil and plants (radish and tomato) were tried and judged on the basis of recovery and intereference encountered. Since the purpose of the determination was eventually to measure trace amount of the pesticide in the field samples it was logical to use a procedure which may give maximum recovery of the pesticide and require a minimum clean up. Extraction procedures involving the use of ethyl acetate, acetone, acetonitrile, dichloromethane and chloroform were tried for the recovery of the pesticide from water, soil and plant samples. Ethyl acetate is found to be a good extractant for the recovery of quinalphos from water and soil. For the extraction of quinalphos from plants acetonitrile followed by partitioning in the presence of salt (QuEChERS method with slight modification) [13] was used. For the best recovery of quinalphos from biological fluids methanol was employed with a recovery of 95 ± 5%. The quantitative extraction of thiram from water, soil and plants was carried out using ethyl acetate, acetonitrile and dichloromethane, respectively. The recoveries are 95 ± 5%.

Florisil column was used for the clean up of the samples. The most efficient way to recover the pesticide from water samples was found to be direct partitioning with ethyl acetate. The methods adopted for the extraction and clean up of quinalphos and thiram from water, soil and plant (radish and tomato) are summarized below:-

2.2.6.1 Water

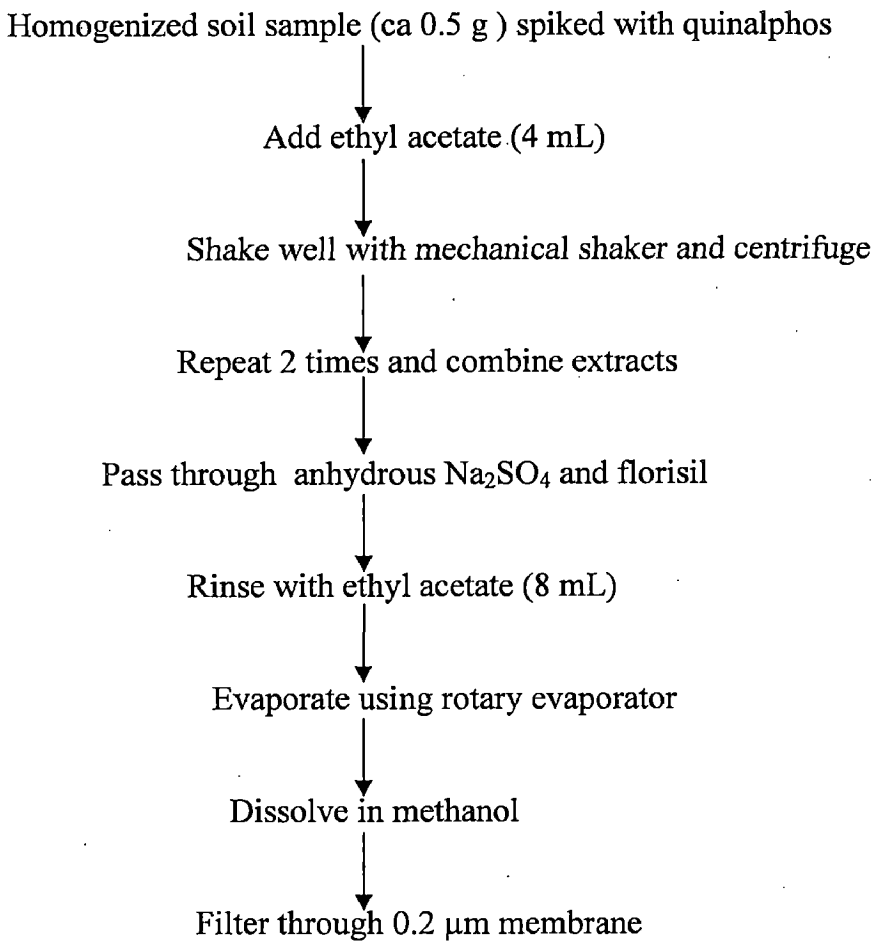
Water sample containing the quinalphos/thiram was extracted with ethyl acetate. The organic layer was passed through an anhydrous sodium sulphate column and rinsed with ethyl acetate which was evaporated at 37 ^{0}C using a rotary evaporator and the residue was dissolved in an appropriate solvent. The scheme applied for the extraction of quinalphos from water is given in Flowsheet 2.1.



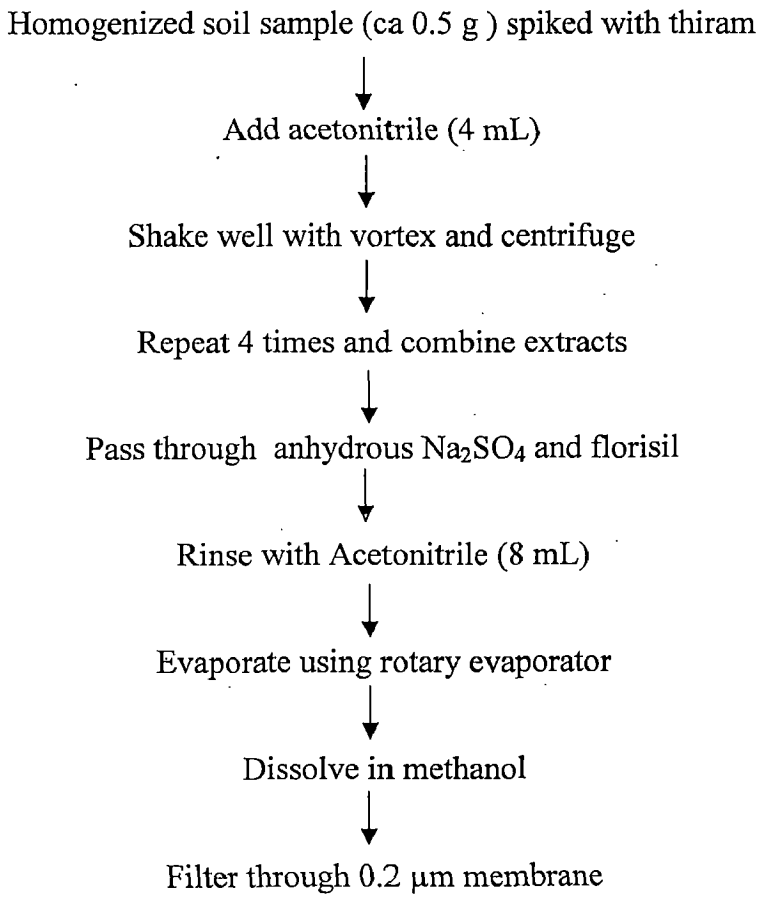
Flowsheet 2.1: Extraction procedure for quinalphos and thiram from water sample

2.2.6.2 Soil

A known amount of soil sample spiked with quinalphos/thiram was shaken with ethyl acetate (acetonitrile in the case of thiram) and centrifuged. The supernatant was taken and the extract was passed through a column containing anhydrous sodium sulphate with florisil. The eluting solvent was evaporated at 37 ^{0}C using a rotary evaporator and the residue was dissolved in an appropriate solvent and filtered through a 0.2 μm membrane. Flowsheets 2.2 and 2.3 show the details of the extraction procedure for quinalphos and thiram, respectively.



Flowsheet 2.2: Extraction procedure for quinalphos from soil sample



Flowsheet 2.3: Extraction procedure for thiram from soil sample

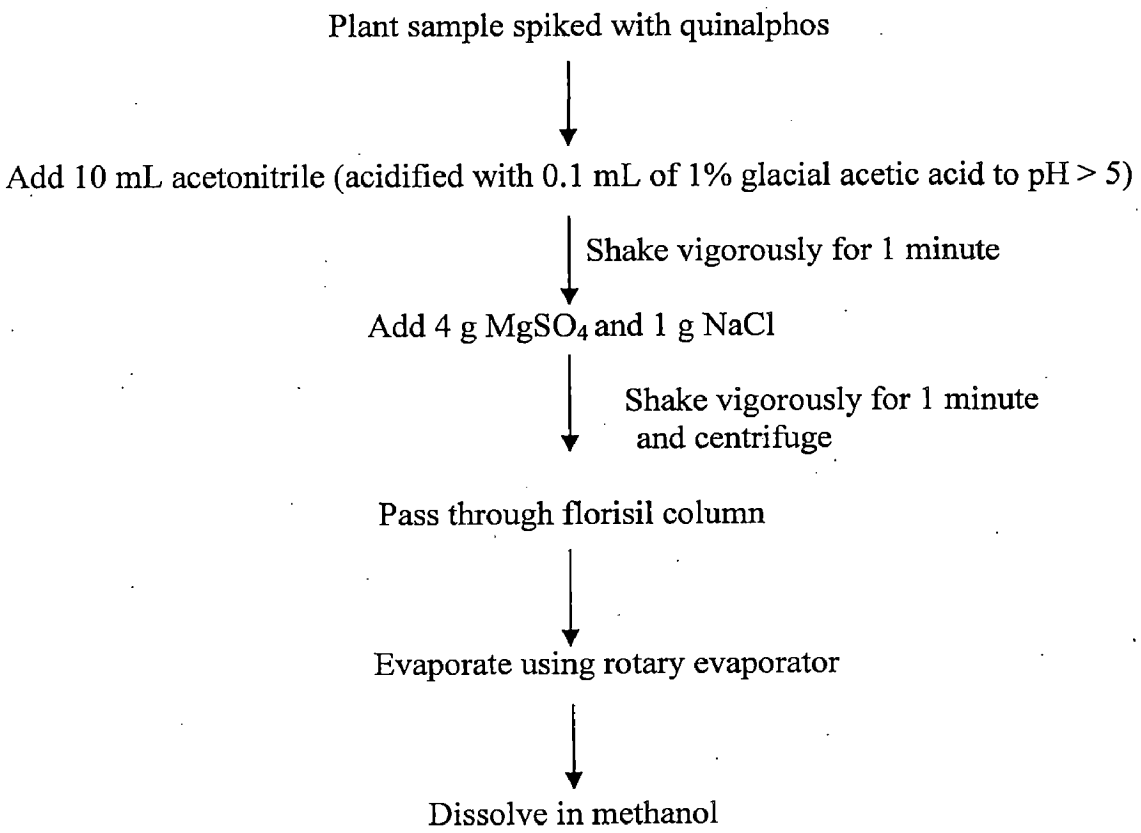
2.2.6.3 Plant (Quinalphos)

A known amount of plant sample (radish/tomato) was crushed and homogenised followed by extraction with ethyl acetate. The extract was passed through an anhydrous sodium sulphate column. Due to high pigmentation the extract was cleaned on a column containing florisil. The details of the above procedure are schematically shown in Flowsheet 2.4.

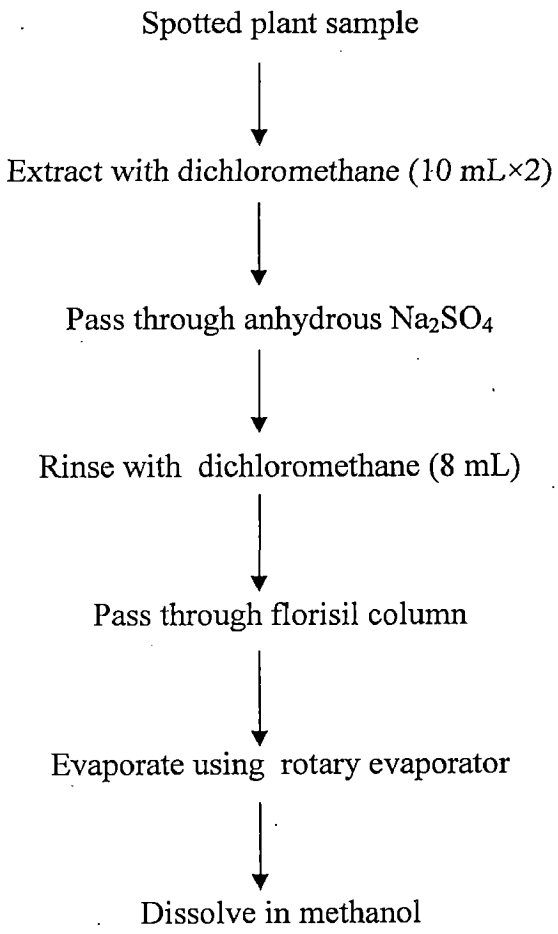
2.2.6.4 Plant (Thiram)

While performing the experiment to follow the decay of thiram on plant it was observed that when thiram is triturated with plant material to recover the pesticide it decays almost instantaneously. In order to avoid such a situation and to achieve results which may be close to the true picture it was planned to spot the parts of the plants with a known

amount of the pesticide on the surface and subsequently remove it by washing thoroughly with the solvent. The spotted plant sample (radish and tomato) was thoroughly washed with dichloromethane and shaken on a mechanical shaker for the recovery of the pesticide. The extract was passed through a column containing anhydrous sodium sulphate and florisil. The details for the extraction of thitam are given in Flowsheet 2.5.



Flowsheet 2.4: Extraction procedure for quinalphos from plant sample



Flowsheet 2.5: Extraction procedure for thiram from plant sample

The above procedures were developed by spiking water, soil and plant samples with 100 μg mL^{-1} of quinalphos and 20 μg mL^{-1} (water)/100 μg mL^{-1} (soil and plants) of thiram, respectively. Blank determinations were run but the contribution was found to be negligible.

2.2.7 Studies on quinalphos in rats

2.2.7.1 Simulated digestion

For simulation studies, method described by Bergqvis et al. [39] was used with a slight modification. An aliquot of 25 μL of quinalphos solution (10,000 μg mL^{-1}) was taken in a number of teflon tubes each for gastric and intestinal simulations along with blanks in deionised water. Half of quinalphos samples were used for gastric simulation studies and the remaining half for intestinal simulation studies. For gastric simulation

investigations the pH of each solution was adjusted to 2.0 with 5 mol L^{-1} HCl followed by the addition of 0.3 mL of freshly prepared pepsin solution (0.16 g pepsin per mL of 0.1 mol L^{-1} HCl). The volume was adjusted to 10 mL with buffer solution of pH 2.0. The mixture was incubated on an incubator shaker at 120 rpm at 37±0.5 ^{0}C. Triplicate samples for time points of 0, 0.5, 1 and 2 hours were analysed for gastric digestion. The pH of the remaining gastric digests was brought to 7.0 by dropwise addition of 1 mol L^{-1} $NaHCO_3$ followed by the addition of 1.7 mL of pancreatin–bile mixture (0.12 g bile extract and 0.02 g Pancreatin in 5 mL of 0.1 mol L^{-1} $NaHCO_3$). The resulting mixture was finally made upto 15 mL with buffer of pH 7.0 and incubated on an incubator shaker at 120 rpm at 37±0.5 ^{0}C. Triplicate samples at 0, 0.5, 1 and 2 hours intervals were analysed for intestinal digestion. The protocol of incubation times was in accordance to that of Miller et al. [40]. In both the cases the digests were placed on ice to stop the enzymatic activity. All the samples were lyophilized followed by extraction with methanol. The details of simulated digestion along with extraction are depicted in Flowsheet 2.6.

2.2.7.2 Animal groups and treatment

All the experiments were performed as per the guidelines of the Institutional Animal Ethics Committee. The national laws according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) were applicable. The project had prior approval from the same committee (registration number: 563/02/a/CPCSEA). Further, "principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed. Experiments were carried out on pathogen-free albino Wistar rats, *Rattus norvegicus*, of age group 6-8 weeks, purchased from the animal house facility of National Institute of Pharmaceutical Education and Research (Chandigarh, India). They were housed in a well-ventilated animal house at Department of Biotechnology, IIT Roorkee in polypropylene cages bedded with sterilized rice husks under a 12 hours light/dark cycle. The animals were fed *ad libitum* with a balanced animal

feed (Ashirwad Animal Feed Industries, Punjab, India) and had an access to normal drinking water at all the times. The animals were allowed to acclimatize to the laboratory environment for one week and then randomly divided into groups (n = 3) as given below:

Group I - Animals treated with the vehicle (Sham).

Group II – Animals treated with 5 mg kg^{-1} body weight (b.wt.) quinalphos and sacrificed after 0.5 hour

Group III – Animals treated with 5 mg kg^{-1} b.wt. quinalphos and sacrificed after 1 hour

Group IV – Animals treated with 5 mg kg^{-1} b.wt. quinalphos and sacrificed after 2 hours

Group V – Animals treated with 5 mg kg^{-1} b.wt. quinalphos and sacrificed after 3 hours

Group VI – Animals treated with 5 mg kg^{-1} b.wt. quinalphos and sacrificed after 6 hours

Group VII – Animals treated with 5 mg kg^{-1} bwt quinalphos and sacrificed after 9 hours

Group VIII – Animals treated with 5 mg kg^{-1} b.wt. quinalphos and sacrificed after 12 hours

Group IX- Animals treated with 5 mg kg^{-1} b.wt. quinalphos and sacrificed after 18 hours

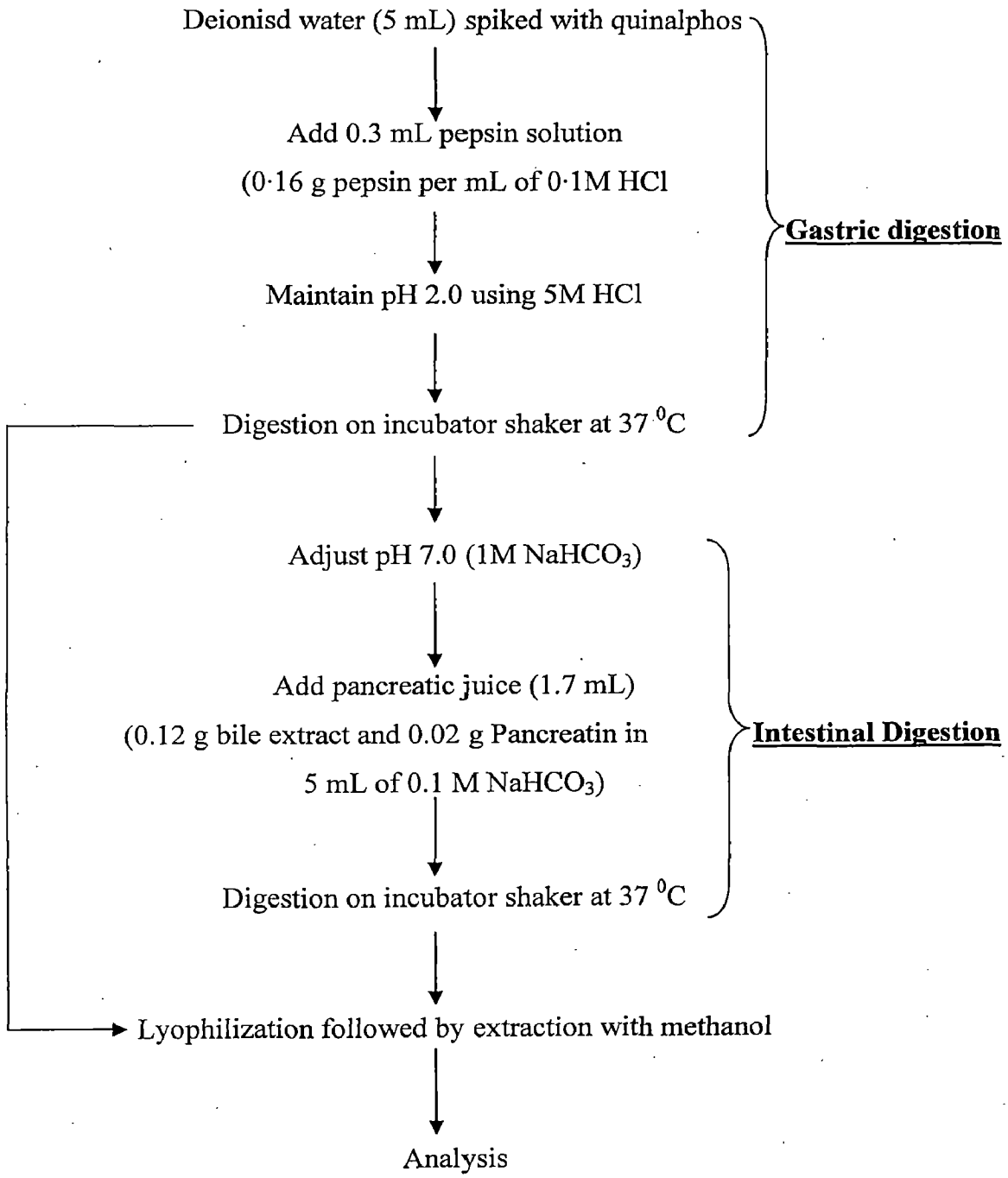
Group X- Animals treated with 5 mg kg^{-1} b.wt. quinalphos and sacrificed after 24 hours

Quinalphos in dimethyl sulphoxide suspended in a phosphate buffered saline (PBS) solution was used for dosing the animal by oral gavaging. The rats were starved for 12 hours before dosing with quinalphos.

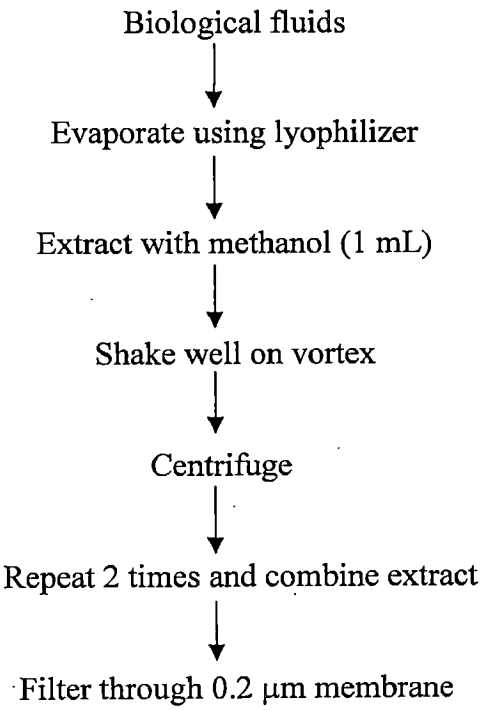
Animals were sacrificed under a pentobarbital anesthesia at various time points after urine collection following the single animal method of collection of mouse urine using a clear plastic wrap [41] with a mild intervention as described by Watts [42]. Blood was collected from the left ventricle. The urine and the serum samples were stored immediately at –40 ^{0}C till the sample processing. In order to get a good recovery all the samples from the same group were pooled before analysis. The different steps involved in the extraction procedure of *in vivo* samples are given in the Flowsheet 2.7.

The decay was measured for two hours each for simulated gastric and intestinal phases and twenty four hours in rats. Standard deviations for the concentration based on three observations at 200 $\mu g\ mL^{-1}$ in simulated and biological samples were 1.01 and 1.85, respectively.

In all the cases triplicate samples were analysed at periodic time intervals by HPLC for the determination of pesticide and GC-MS/LC-MS for the identification of metabolites. The limit of detection for HPLC was 1 $\mu g\ mL^{-1}$. Control and blank were run wherever necessary.



Flowsheet 2.6: Schematic representation of the gastric and intestinal digestion and extraction procedure for *in vitro* studies



Flowsheet 2.7: Extraction procedure for *in vivo* studies

2.3 References

[1]. Dao, T. H., Lavy, T. L. and Dradun, J., "Rationale of the solevent extraction for soil extraction of pesticide residues", In: Residue Reviews, Springer-Verlag, New York, **87**, p. 91 (1983).

[2]. Jansson, C., Pihlstrom, T., Osterdahl, B. G. and Markides, K. E., "A new multi-residue method for analysis of pesticide residues in fruit and vegetables using liquid chromatography with tandem mass spectrometric detection", J. Chromatogr. A **1023**, 93 (2004).

[3]. Stajnbaher, D. and Zupancic-Kralj, L., "Multiresidue method for determination of 90 pesticides in fresh fruits and vegetables using solid-phase extraction and gas chromatography–mass spectrometry", J. Chromatogr. A **1015**, 185 (2003).

[4]. Zambonin, C. G., Quinto, M., De Vietro, N. and Palmisano, F., "Solid-phase microextraction-gas chromatography mass spectrometry: a fast and simple screening method for the assessment of organophosphorus pesticides residues in wine and fruit juices", Food Chem. **86**, 269 (2004).

[5]. Perret, D., Gentili, A., Marchese, S., Sergi, M. and D'Ascenzo, G., "Validation of a method for the determination of multiclass pesticide residues in fruit juices by liquid chromatography/tandem mass spectrometry after extraction by matrix solid-phase dispersion", J. AOAC Int. **85**, 724 (2002).

[6]. Pylypiw, H. M., Arsenault, T. L., Thetford, C. M and Matina, M. J. I., "Suitability of microwave-assisted extraction for multiresidue pesticide analysis of produce", J. Agric. Food Chem. **46**, 5302 (1998).

[7]. Mutavdzic, D., Horvat, A. J. M., Babić, S., Kaštelan-Macan, M., "SPE–Microwave-assisted extraction coupled system for the extraction of pesticides from water samples", J. Sep. Sci. **28**, 1485 (2005).

[8]. Ho, W. -H and Hsieh, S. -J., "Solid phase microextraction associated with microwave assisted extraction of organochlorine pesticides in medicinal plants", Anal. Chim. Acta **428**, 111 (2001).

[9]. Rezic, I., Horvat, A. J. M. Babic´, S. and Kasˇtelan-Macan, M., "Determination of pesticides in honey by ultrasonic solvent extraction and thin-layer chromatography", Ultrason. Sonochem. **12**, 477 (2005).

[10]. Pan, J., Xia, X. -X. and Liang, J., "Analysis of pesticide multi-residues in leafy vegetables by ultrasonic solvent extraction and liquid chromatography-tandem mass spectrometry", Ultrason. Sonochem. **15**, 25 (2008).

[11]. Anastassiades, M., Lehotay, S. J., Stajnbaher, D. and Schenck, F. J., "Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce", J. AOAC Int. **86**, 412 (2003).

[12]. Díez, C., Traag, W. A., Zommer, P., Marinero, P. and Atienza, J., "Comparison of an acetonitrile extraction/partitioning and "dispersive solid-phase extraction" method with classical multi-residue methods for the extraction of herbicide residues in barley samples", J. Chromatogr. A **1131**, 11 (2006).

[13]. Cajka, T., Hajslova, J., Lacina, O., Mastovska, K. and Lehotay, S. J., "Rapid analysis of multiple pesticide residues in fruit-based baby food using programmed temperaturevaporiser injection-low-pressure gas chromatography-high-resolution time-of-flight mass spectrometry", J. Chromatogr. A, **1186**, 281 (2008).

[14]. Lee, Y. S., Kim, J., Shin, S. -C., Lee, S. -G. and Park, K., "Antifungal activity of Myrtaceae essential oils and their components against three phytopathogenic fungi", Flavour Frag. J. **23**, 23 (2008).

[15]. Lesueur, C., Knittl, P., Gartner, M., Mentler, A. and Fuerhacker, M., "Analysis of 140 pesticides from conventional farming foodstuff samples after extraction with the modified QuEChERS method", Food Control **19**, 906 (2008).

[16]. Walorczyk, S., "Development of a multi-residue method for the determination of pesticides in cereals and dry animal feed using gas chromatography–tandem quadrupole mass spectrometry II. Improvement and extension analytes", J. Chromatogr. A **1208**, 202 (2008).

[17]. Cunha, S. C., Lehotay, S. J., Mastovska, K., Fernandes, J. O. and Oliveira, M. B. P. P., "Sample preparation approaches for the analysis of pesticide residues in olives and olive oils", In: Olives and Olive Oil in Health and Disease Prevention, Preedy V. R. and Watson R. R. (Eds.), Oxford: Academic Press, chap. 70, p. 653 (2010).

[18]. Tekel, J. and Hatrik, S., "Pesticide residue analyses in plant material by chromatographic methods: clean-up procedures and selective detectors", J. Chromatogr. A, **754**, 397 (1996).

[19]. Matohashi, N., Nagashim, H., Parkanyl, C., Subrah, B. and Zhang, G. -W., "Official multiresidue methods of pesticide analysis in vegetables, fruits and soil", J. Chromatogr. A **754**, 333 (1996).

[20]. Adahchour, M., Beens, J., Vreuls, R. J. J. and Brinkman, U. A. Th., "Recent developments incomprehensive two-dimensional gas chromatography (GC x GC) III. Applications for petrochemicals and organohalogens", Trac-Trend Anal. Chem. **25**, 726 (2006).

[21]. Von Muhlen, C., Zini, C. A., Caramao, E. B. and Marriott, P. J., "Applications of comprehensive two-dimensional gaschromatography to the characterization of petrochemical and related samples", J. Chromatogr. A **1105**, 39 (2006).

[22]. Henry, R. A., Scmit, J. A., Dieckman, J. F., and Murphey, F. J., "Combined high speed liquid chromatography and bioassay for the evaluation and analysis of an organophosphorus larvicide", Anal. Chem. **43**, 1053(1971).

[23]. Ravelo-Perez, L. M., Hernández-Borges, J. and Rodríguez-Delgado, M. Á., "Pesticides analysis by liquid chromatography and capillary electrophoresis", J. Sep. Sci. **29**, 2557 (2006).

[24]. Kirkland, J. J., and De Stefanos, R., "High speed liquid chromatography", J. Chromatogr. Sci. 8, 309 (1970).

[25]. Picó, Y., Font, G., Moltó, J. C. and Mañes, J., "Pesticide residue determination in fruit and vegetables by liquid chromatography–mass spectrometry", J. Chromatogr. A **882**, 153 (2000).

[26]. Careri, M. Bianchi, F. and Corradini, C., "Recent advances in the application of mass spectrometry in food-related analysis", J. Chromatogr. A **970**, 3 (2002).

[27]. Picó, Y., Blasco, C. and Font, G., "Environmental and food applications of LC–tandem mass spectrometry in pesticide-residue analysis: an overview", Mass Spectrom. Rev. **23**, 45 (2004).

[28]. Andreu, V. and Picó, Y., "Liquid chromatography-ion trap-mass spectrometry and its application to determine organic contaminants in the environment and food", Curr. Anal. Chem. **1**, 241 (2005).

[29]. Hernandez, F., Serrano, R., Pitarch, E. and Lopez, F. J., "Automated sample clean-up procedure for organophosphorus pesticides in several aquatic organisms using normal phase liquid chromatography", Anal. Chim. Acta **374**, 215 (1998).

[30]. Tuzimski, T. and Soczewiński, E., "Use of database of plots of pesticide retention (RF) against mobile-phase composition. Part I. Correlation of pesticide retention data in normal- and reversed-phase systems and their use to separate a mixture of ten pesticides by 2D-TLC", Chromatographia, **56**, 219(2002d)..

[31]. Tuzimski, T. and Bartosiewicz, A., "Correlation of retention parameters of pesticides in normal and RP systems and their utilization for the separation of a mixture of ten urea herbicides and fungicides by two-dimensional TLC on cyanopropyl-bonded polar stationary phase and two-adsorbent-layer multi-K plate", Chromatographia, **58**, 781(2003).

[32]. Vodeb, L. and Petanovska-Ilievska, B., "HPLC–DAD with different types of column for determination of β-cyfluthrin in pesticide formulations", Acta Chromatographica **17**, 188 (2006)

[33]. Trajkovska, V. and Petrovska-Jovanovic, S., "Optimization of HPLC conditions for simultaneous determinationof captan, terbumeton and deltamethrin", Bulletin of the Chemists and Technologists of Macedonia **21**, 193 (2002).

[34]. Uygun, Ü., "Determination of organophosphorus pesticide residues in carrot using gel permeation chromatography", J. Liq. Chromatogr. R. T. **20**, 771 (1997).

[35]. Susane, B. E., Birgit, O. and Bengt-Goran, O., "Rapid and simple method for determination of thiram in fruits and vegetables with high performance liquid chromatography with ultraviolet detection", J. Agric. Food Chem. **46**, 5302 (1998).

[36]. Hernandez-olmos, M. A., Agiu, L., Yanaz-Sedeno, P. and Pingarron, J. M., "Analytical voltammetry in low-permitivity organic solvents using disk and cylindrical microelectrodes. Determination of thiram in ethyl acetate", Electrochim. Acta **46,** 289 (2000).

[37]. Garcia, A. L., Gonzalez, E. B. and Medel, A. S., "Determination of tetramethylthiuram disulfide (thiram) in river water by high-performance liquid chromatography: micellar versus conventional reversed phase chromatography", Chromatographia **43**, 607 (1996).

[38]. ISI Bulletin 2720, Method of Tests for Soils, India (1976).

[39]. Bergqvis, S. W., Andlid, T. and Sandberg, A., "Lactic acid fermentation stimulated iron absorption by Caco-2 cells is associated with increased soluble iron content in carrot juice", Br. J. Nutr. **96**, 705 (2006).

[40]. Miller, D. D., Schricker, B. R., Rasmussen, R. R. and Campen D. V., "An *in vitro* method for estimation of iron availability from meals", Am. J. Clin. Nutr. **34**, 2248 (1981).

[41]. Kurien, B. T. and Scofield, R. H., "Mouse urine collection using clear plastic wrap", Lab. Anim. **33**, 83 (1999).

[42]. Watts, R. H., "A simple capillary tube method for the determination of the specific gravity of 25 and 50 micro l quantities of urine", J. Clin. Pathol. **24**, 667 (1971).

CHAPTER 3

DECAY PROFILES AND METABOLIC PATHWAYS OF QUINALPHOS IN WATER, SOIL AND PLANTS

3.1 Introduction

Pesticides enter in soil or water bodies accidently or through their intended use for the control of pests. Invariably after their application a large amount of the pesticides persist in the agricultural and forest soils. They reach the soil mainly as fallout during the aerial spraying. The remains of the plants and animals which get buried into the soil also contribute towards their entry into the open environment. Waste water from the pesticide manufacturing industries further adds to the load onto the environment. The presence and the persistence of the pesticides in the soil and water affect the innocent biota and cause the unprecedented damage to the ecology of the area. The deleterious effects to a large extent will depend upon the decay rate and the toxicity of the pesticide and its metabolites. The persistence, no doubt, is primarily determined by the chemical nature but is significantly affected by the nature of the medium and the environmental factors like temperature, humidity and the pH. The interaction of the pesticide with a medium like soil is more complicated than with the water. The persistence of the pesticide in the soil may vary from a few hours to many years depending on its type. The fate or the behaviour of the pesticides in soil depends upon many factors like chemical and photochemical degradation, uptake by the soil microorganisms or plants, adsorption on the clay and organic matter, leaching with the downwards percolation of water, movements with the runoff water and volatilization. Kookana and associates [1] have presented an exhaustive review on the fate and behaviour of pesticides in Australian soil and emphasized the need to understand the behaviour of pesticide in the soil environment to minimise any adverse impact on our environment.

In a particular study, it may not be feasible to consider all the above factors resulting into the change in the concentration of the pesticide. Generally, the emphasis is laid on the chemical interaction. The degradation has been mainly reported as a first order or pseudo

first order reaction. In exceptional cases where the microbial activity in the matrix is significant a second order rate kinetics has been observed [2]. The persistence of a pesticide is expressed in terms of half life or the rate constant of the reaction.

When a pesticide is applied on the plants, it partitions itself into the adsorbed and absorbed fractions. The adsorbed fraction of the pesticide decays primarily due to the physical loss, microbial activity and photochemical degradation. The loss due to the vaporization from the surface reaches the atmosphere resulting into its deposition in the rain and fog. Organophosphates and their metabolites have been reported in the fog in the states of California and Maryland (USA) [3-6]. The absorbed fraction may be affected by the presence of enzyme present in the plant content. From the environmental point of view, the decay in all the segments of the biosphere is of great concern but the decay in the plant and its components is of primary importance. It is the plant which is invariably directly consumed by the humans. Pesticide residues are transported to various parts of the plant like root, stem, foliage and fruit in varying forms. It is ultimately important to know as to how much pesticide remains in the plant with the passage of the time.

It has already been mentioned in chapter I that following the ban on organochlorine pesticide organophosphates started getting popular. Literature and field survey shows that organophosphate pesticides are being extensively used due to their relatively fast decomposition and low accumulation in the biological food chain. However, their toxicity to the human and other biotic environment cannot be ignored [7]. In order to keep a proper track of the environmental impact of the use of these materials, a comprehensive study on their decay profiles in the various segments of ecosystem under the varying environmental conditions is needed. The study should include both the dissipation rate and metabolic pathways. Dissipation rate of pesticide may be dependent in aquatic environment on a number of natural water conditions like pH, salinity, dissolved organic matter (DOM) and metals [8]. In soil, many environmental factors such as soil type, moisture, organic matter,

microbial activity, sunlight intensity, amenability to aeration as well as weather conditions may affect the dissipation rate [9-11] (Fig. 3.1).

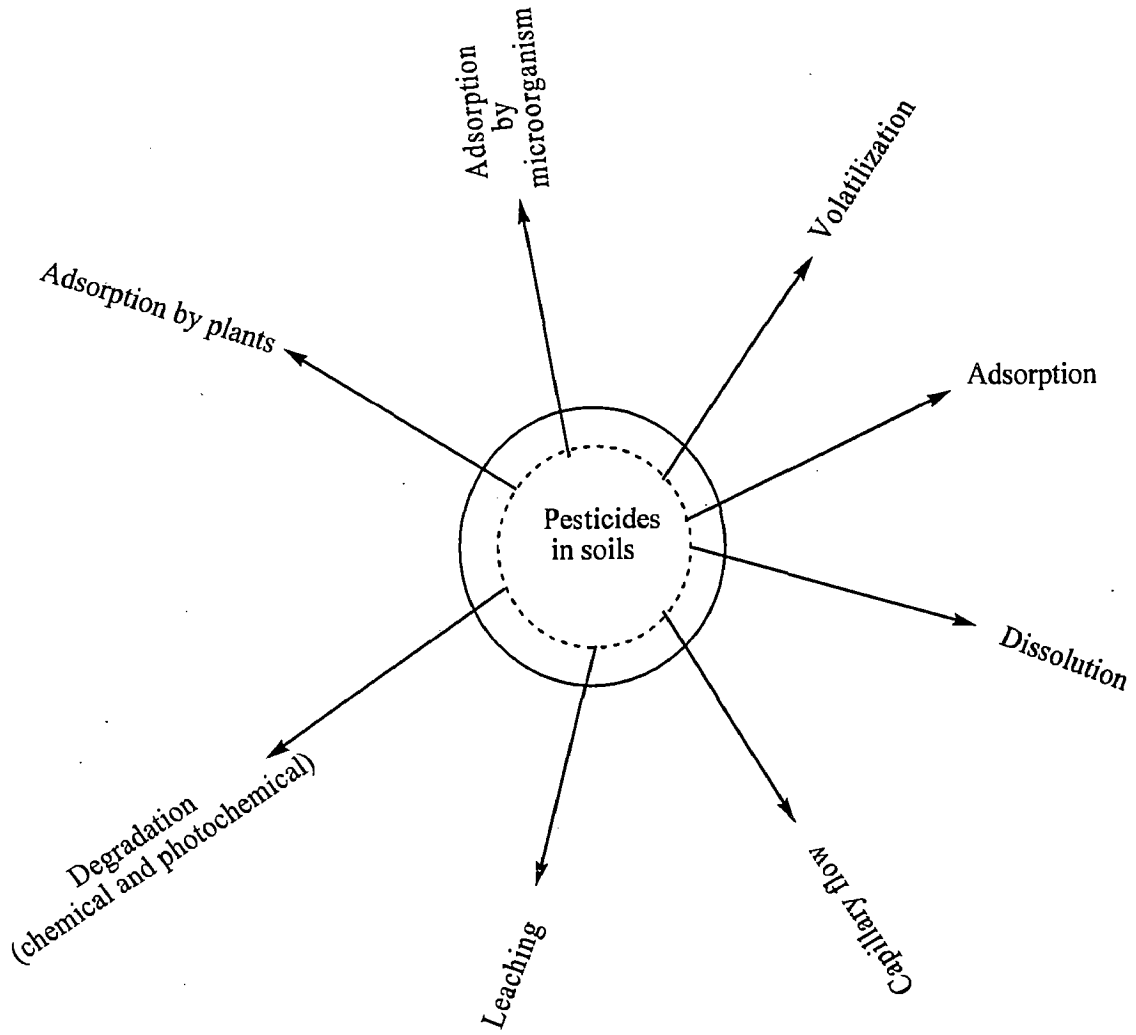


Figure 3.1: Fate of pesticide in soil environment

A survey of a certain region of India indicated that amongst the important organophosphate pesticides, quinalphos (O, O-diethyl O-quinoxalin-2-yl phosphorothioate) is quite a popular insecticide. Compared to other organophosphates, a comprehensive degradation study on quinalphos has not been done. Thus it seems to be of considerable scientific interest to investigate the degradation profile of quinalphos and comment on its persistence and decay pattern.

Quinalphos is widely used for the control of pest over certain crops such as cotton, groundnut and rice [12-14]. Owing to its good penetrating power into the plant body it can be efficiently utilized in controlling *Pegomya hyosoyami* and *Scirpophaga insertulu* [15]. The build-up of tolerance to quinalphos by the insects was attributed to the extensive usage of the insecticide [14]. In spite of quinalphos utility as an effective pesticide, its toxicological effects cannot be ignored. It is an inhibitor of AChE [16]. Tests conducted to evaluate the ovicidal activities showed that it is highly toxic to the eggs of arthropods [17]. Satpute et al. [18] observed that its toxicity increases with the temperature. Some of the degradation products of quinalphos are stronger inhibitor of AChE than the parent compound [19]. Quinalphos is a known pesticide and its toxicity is documented to some extent.

Some studies on the degradation of quinalphos under various conditions are also reported. A major portion of these investigations focuses on the photodegradation of the pesticide. Some useful information is also available on the microbial degradation and decay in other matrices. Goncalves et al. [20] investigated the effect of operational parameters on the photocatalytic degradation of quinalphos in aqueous solution in the presence of TiO_2 under UV light. Photodegradation follows a first order kinetics with a half life of 11.6 hours. The photolysis half life of quinalphos is shorter in sandy soil showing strong dependence on the composition of the irradiated mediaum. The byproducts are formed through photohydrolysis and photoisomerization processes.

Megharaj et al. [21] investigated the metabolism of quinalphos by algae isolated from soil and concluded that both blue and green algae are capable for the detoxification. Results also showed that two filamentous cyanobacteria, *Phormidium tenue and Nostoc linckia* significantly degraded quinalphos within five days. Rangaswamy and Venkateswarlu [22] conducted a study on the degradation of quinalphos insecticide by bacteria isolated from soil. The results of the investigation clearly indicate that quinalphos is more susceptible to the microbial metabolism. Both *Azospirillum lipoferum* and Bacillus species, degraded quinalphos rapidly even by the end of 7 days of incubation.aThe degradative action of two strains of Saccharomyces cerevisiae on quinalphos was studied by Cabras et al. [23]. It was found that the thiophosphates are more prone to the degradation.

Most of the available information on the metabolites of quinalphos is on its photochemical decay under different laboratory conditions. Only a few studies are available on the formation of metabolites in the biological fluids which are discussed in chapter V. Pusino et al. [24] observed that the decomposition of quinalphos on homoionic clays depends upon the nature of the exchangeable cation. The pesticide hydrolyses to 2-hydroxyquinoxaline on the Cu-, Fe- and Al-clay surfaces, whereas O-ethyl O-quinoxalin-2-yl thiophosphoric acid is the main product of hydrolysis on the Na-, K- and Ca-clay surfaces. They [25] also observed that the photolysis of quinalphos in the ethanolic solution gives O,O- diethyl- (3-ethoxy-quinoxalin-2-yl) phosphorothioate and O,O-diethyl-O-3(1-hydroxy ethyl)-quinoxalin-2-yl phosphorothioate. Banerjee and Dureja [26] characterized the photochemical decomposition products of quinalphos on clay surfaces under sunlight and UV light and identified quinalphos oxon as the main product formed by the oxidation of P=S bond. Oxon analogues are, in general, more toxic than the thioanalogues of organophosphorus pesticides. It was also observed that the irradiation of quinalphos on clay surfaces for 15 days resulted in a complex mixture of photoproducts.

Chukwudebe et al. [27] studied the toxicity of several trialkylphosphorothioates which resulted from the phototransformation of quinalphos.

It may be important to point out here that studies have also been conducted on the persistence of quinalphos in different matrices, namely water, soil and plants. A number of impurities were identified by Sanyal and Dureja [28] in a sample of technical quinalphos on storage. They concluded that technical quinalphos when stored at an ambient temperature for more than 6 months undergoes degradation and loses its bioefficacy. Prasad et al. [29] estimated quinalphos in wastewaters of organophosphate pesticide manufacturing industry and found that it persists for a long time in water. Mansour et al. [30] during the determination of agrochemicals in water and soil observed that the humic substances lead to a fast degradation of the pesticide. An increase in decay rate with increasing temperature was observed by Gopal et al. [31]. Zhang and coworkers [32] carried out dynamic degradation studies on quinalphos in wheat and soil for two years and found that the pesticide residue falls below maximum residue limit (MRL) in 21 days. In a study carried out by Schmidt [15], it was concluded that quinalphos is susceptible to hydrolysis and in soil it degrades to quinoxalin-2-ol with DT50 of about three weeks. Babu et al. [33] concluded that quinalphos persisted in soils with a half life of 2 weeks and hydrolyses to 2-hydroxyquinoxaline. Further investigations revealed that its degradation is influenced by the type of the soil. Menon and Gopal [34] investigated the degradation of quinalphos in three types of soil and 2-hydroxy quinoxaline found to be the principal metabolite but after seed treatment a thiol metabolite of quinalphos was also detected. The complete dissipation of quinalphos for all the three soil samples was observed by the end of 16^{th} day. Dureja et al. [35] reported that quinalphos is unstable under both UV and sun light. The degradation of quinalphos formulation on the tomato fruit was observed by Gajbhiye and coworkers [36]. They reported the formation of two major metabolites, oxon and 2-hydroxy quinoxalin and the residue persisted up to 14 days. Persistence studies on

quinalphos on rice plant showed that the half-life values range between 1.87 and 2.43 days [37]. In another study [38] the persistence of quinalphos residue on bitter gourd fruits at the fruiting stage was evaluated. Quinalphos persisted for 1-2 weeks above the maximum permissible limit. Nazer [39] analysed quinalphos residues on and in lemon fruits and detected the metabolite 2-hydroxy quinoxalin in the rind only and no quinalphos oxygen analogue was detected in the rind or pulp.

It is apparent from the above discussion that the decay of quinalphos is dependent on a number of parameters. In this context the studies conducted in the laboratory do not necessarily reflect on the decay profile of the pesticide in field conditions as the various environmental conditions such as temperature, humidity, wind velocity etc. affect the decay rate. In such a situation more conclusive results may emerge by carrying out decay experiments both in the laboratory and field conditions.

In the light of above a systematic study was planned on the decay of quinalphos and identification of its metabolites in water, soil and plants. In the case of water the effect of temperature, pH and organic content (humic acid) was investigated. For soil it was only possible to observe the effect of pH. The plants chosen for the study were radish and tomato which are frequently used as uncooked. Studies on the plants were carried out in conditions which were near to field conditions. However, some extra care was taken to avoid the loss of pesticide from extraneous reasons. The plants were exposed to sun light in the day time and at the sunset they were placed under a tin shade. In all the above matrices the degradation products were identified at logically pre-decided time intervals.

3.2 Experimental

The details and the operating conditions of HPLC and GC-MS used for the study are cited in chapter II.

3.2.1 Persistence studies

Laboratory experiments were conducted using deionised water and soil. Known volumes of deionised water as such, buffered to the desired pH and or containing different amounts of humic acid were placed in glass stoppered conical flasks and 100 μg mL^{-1} solution of quinalphos in methanol was added separately to each of the conical flasks. The effect of humic acid concentration on the decay profile of quinalphos in water was studied in deionised water buffered to pH 8.0. The humic acid solutions had to be buffered because its addition to deionised water shifts the pH to an alkaline range. Known amounts of soil of different pH collected from three different regions of India were placed in glass stoppered conical flasks and spiked with 100 μg mL^{-1} quinalphos solution. The water and soil samples were stored at 28±2 ^{0}C unless mentioned otherwise. The decay profile of the pesticide was followed under different conditions by determining the residual pesticide in different samples after a definite time period. The decay was measured for two to three months in water and soil.

The plants were grown in Roorkee soil contained in earthen pots. No additional manuring was done during the period of study and watering of the plants was done carefully. The plants were kept under a tin shade at the top to avoid any loss of the pesticide due to dew, rain or cyclonic activity. The plants were so covered that it allowed the passage of air and sun light. To investigate the decay profile of quinalphos on different parts of the plants, the leaves and roots of radish and tomato fruits of four weeks old plants were spotted with a known amount (100 μg mL^{-1}) of the pesticide and subsequently at certain interval of time the particular part was thoroughly crushed, homogenized and extracted to recover the remaining pesticide. The decay was followed by determining the residual pesticide in the particular part namely, leaf, root and fruit separately. The decay was measured for fifteen days in the month of October as there was a sufficient

degradation in this duration. The minimum and maximum temperatures, average humidity and wind velocity were 15±5 - 31±3 ^{0}C, 72.1±9% and 1.1±0.5 km/h, respectively.

Extraction and determination of the pesticide from water, soil and plants was done as per the procedures listed in chapter II. In the case of all the three matrices the limit of detection was 1 μg mL^{-1}. Controls and blanks were run wherever necessary. The values reported are the average of minimum of triplicate runs. Standard deviations for the concentration based on three observations at 100 μg mL^{-1} in water, soil and plant were 0.73, 1.01 and 4.85. The schemes employed for the extraction of quinalphos from water, soil and plants are given as Flowsheets 2.1, 2.2 and 2.4 in chapter II.

For the identification of metabolites the water (pH 6.0 and 8.0), soil (pH 5.1 and 8.1) and different parts of plants were spiked with 1000 μg mL^{-1} of quinalphos. At periodic intervals duplicate samples were removed for the extraction and analysed by the GC-MS.

3.3 Results and Discussion

3.3.1 Effect of temperature

To investigate the effect of temperature on the degradation of quinalphos studies were carried out at three different temperatures (10, 20 and 30 ^{0}C) in deionised water (pH 6.3). The results suggest that there is an exponential decrease (Fig.3.2) in the concentration of the quinalphos pesticide. The rate of decay increases with the increase in temperature and as a consequence of that rate constant increases (Table 3.1). This is in agreement with the observations made on the effect of temperature on the decay of malathion and parathion in water [40] and dimethoate in different soils [41]. The data plotted on the natural log scale by least square method gives a straight line indicating that the process of disappearance of the pesticide obeys the first order rate equation.

Table 3.1: Half lives of quinalphos pesticide under different conditions

Matrices	Conditions	$t_{1/2}$ (d)	k (d^{-1})
Water	**Temperature** (pH: 6.3)		
	10 ^{0}C	74.1	0.009
	20 ^{0}C	47.0	0.015
	30 ^{0}C	26.0	0.027
	pH (Temperature: 28±2 ^{0}C)		
	6.0	40.8	0.017
	7.0	38.3	0.018
	8.0	26.7	0.026
	Organic content		
	Pesticide: Humic acid		
	(pH: 8.0, Temperature: 28±2 ^{0}C)		
	1:0.1	27.5	0.025
	1:1	17.8	0.039
	1:100	15.1	0.046
Soil	Uttarkashi (pH: 5.1)	52.9	0.013
	Roorkee (pH: 6.8)	6.3	0.110
	Sriganganagar (pH: 8.1)	9.0	0.077
Plants	Tomato fruit	3.3	0.210
	Radish leaf	3.9	0.178
	Radish root	2.9	0.239

3.3.2 Effect of pH

Investigations were conducted at different pH both in water (Fig. 3.3) and soil (Fig. 3.4) at 28±2 ^{0}C. The amount of quinalphos recovered at different time intervals from water and soil samples was plotted on a log scale against time of incubation. The dissipation pattern of the quinalphos follows first order kinetics as the plots yield straight lines based on equation $C = C_0e^{-kt}$, where C is the concentration of pesticide remaining after time t, C_0 is the initial concentration and k is the first order kinetic constant. The half lives and rate

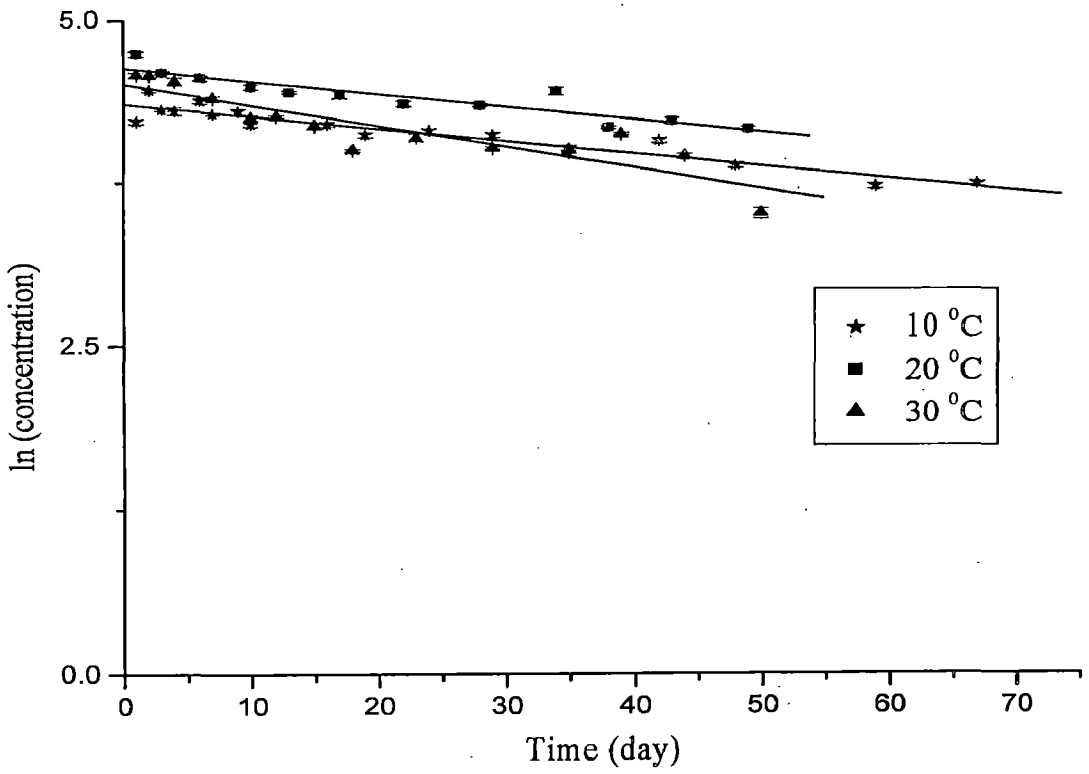


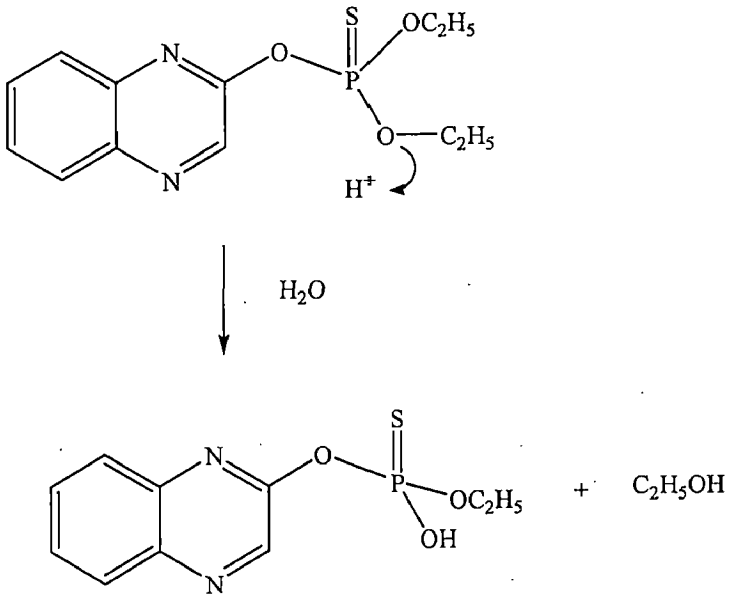
Figure 3.2: Decay of quinalphos in water at different temperatures (pH: 6.3)

constants calculated from these plots are given in Table 3.1. It is apparent from the results that both in water and soil the decay increases with the increasing pH except in soil of neutral pH. A faster decay rate in soil of neutral pH in comparison to soil of alkaline pH has also been reported by Babu et al. [33].

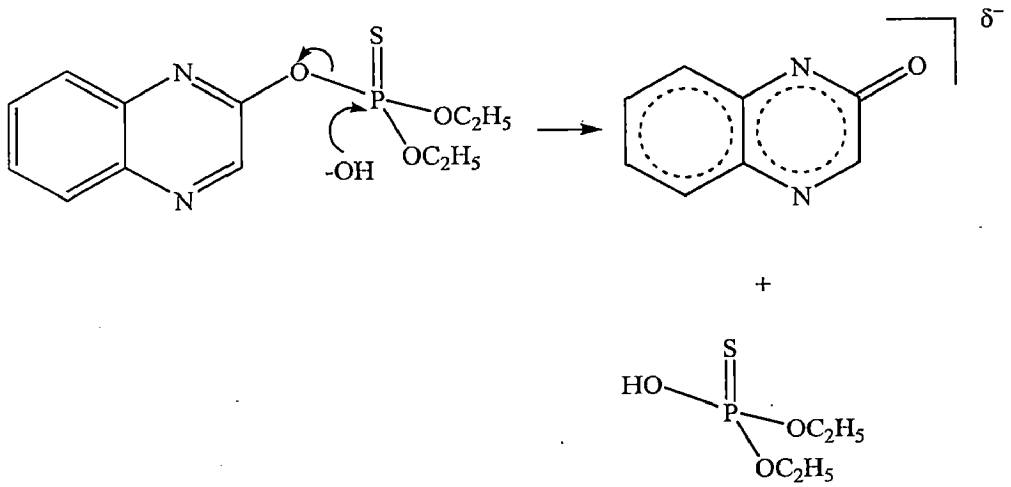
The faster decay kinetics in alkaline medium indicates that the reaction is more effectively catalyzed by the hydroxide ion than the hydronium ion. With the increase in OH^- ion concentration, the probability of rupture of P-O bond in the moiety of quinalphos increases and the resulting compound is stabilized by resonance. On the other hand in the acidic medium the hydrogen ion attack results in cleavage of O-C bond. Some earlier reports [42, 40, 43] also pointed out that the hydrolysis of organophosphate under alkaline conditions proceeds at higher rates than in acidic medium. Abnormal behavior observed in soil of neutral pH may be attributed to the fact that decay of pesticide in soil is very

complex and may be governed by different factors namely moisture, organic content and microbial activity other than the H^+ ion concentration. In this context it may be important to mention that the composition of the matrices has a definite role to play. This is aptly reflected by the fact that in acidic medium the decay in soil is slower than in water but in alkaline medium the reverse is true.

The probable mechanisms are depicted below:-



In acidic medium



In alkaline medium

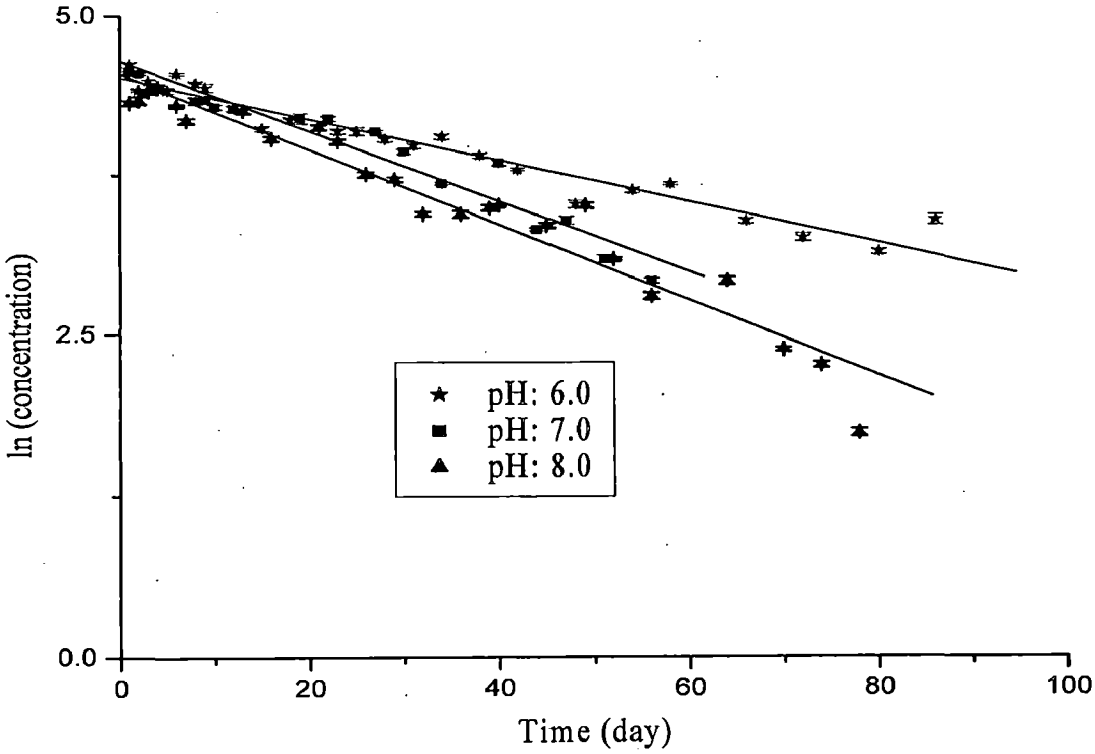


Figure 3.3: Decay of quinalphos in water at different pH
(Temperature: 28±2 ^{0}C)

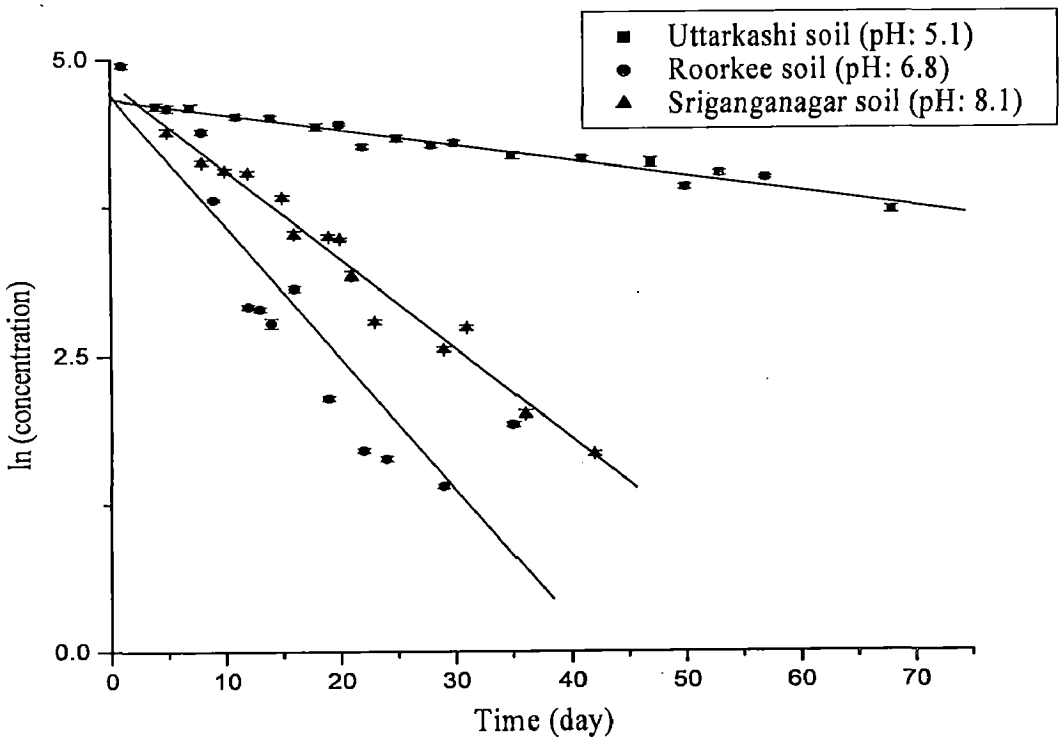


Figure 3.4: Decay of quinalphos in soils at different pH
(Temperature: 28±2 ^{0}C)

3.3.3 Effect of humic acid

Decay studies were carried out with varying ratio of humic acid to pesticide. The results (Table 3.1) indicate that the presence of humic acid increases the rate of decay as it acts as a reducing agent. The results further reveal that higher the organic content the lower is the persistence of quinalphos (Fig. 3.5).

3.3.4 Effect of environmental conditions

The decay behaviour of quinalphos on tomato fruit and different parts of radish plant has been investigated(Fig. 3.6). The results (Table 3.1) indicate a high decay rate in plants as compared to water and soil. Moreover, the degradation is faster in radish root as compared to radish leaf and tomato fruit. The faster kinetics in root can be attributed to moisture content and microbial activity of the soil with which it is in contact. Similar observation has been made by Menzie [44] for carbofuran.

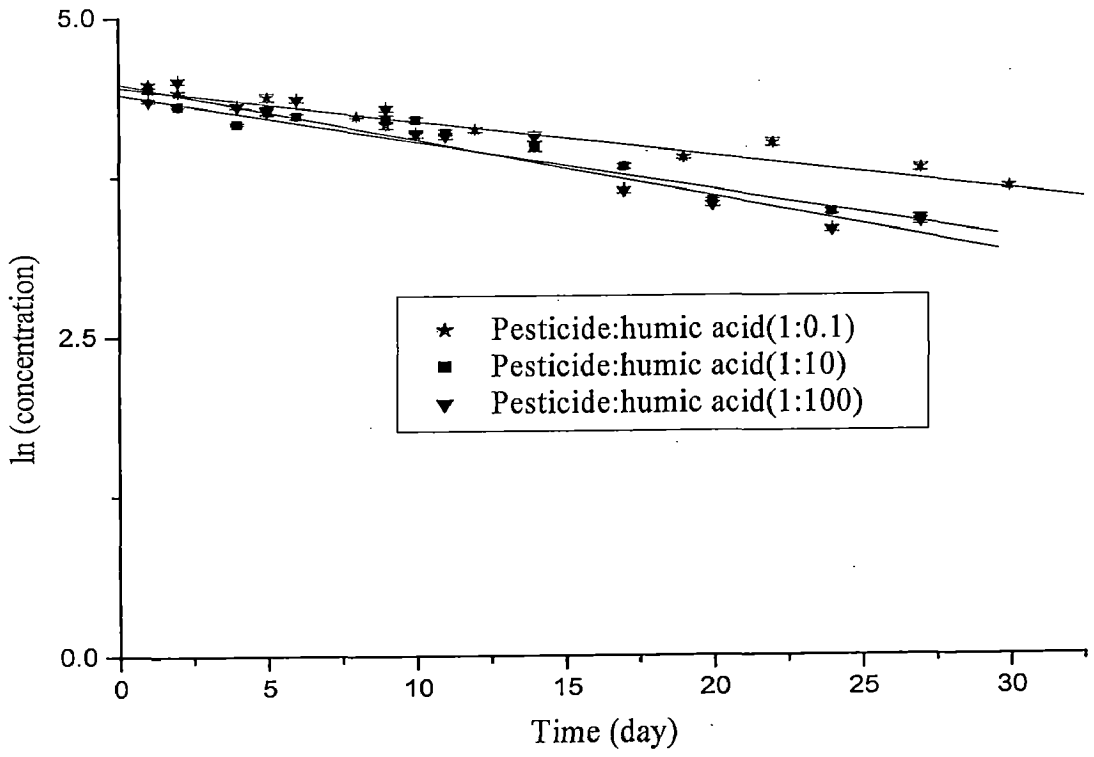
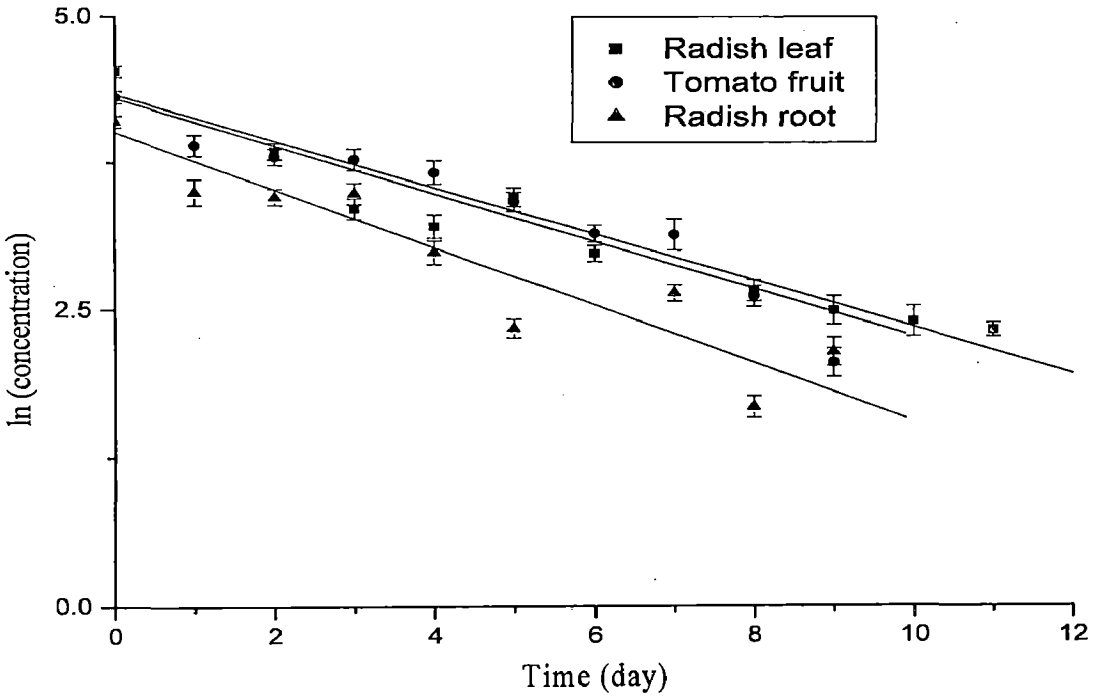


Figure 3.5: Decay of quinalphos in water at different humic acid concentrations (pH: 8.0, Temperature: 28±2 ^{0}C)



Figutre 3.6: Decay of quinalphos in plants in field conditions

3.3.5 Identification of metabolites of quinalphos

Degradation of quinalphos was studied in water (pH 8.0 and 5.5), soil (pH 8.1 and 5.1) and in plants. Water, soil and plant samples spiked with quinalphos were incubated under ambient conditions and withdrawn at different time intervals and analysed by GC-MS for identification of degradation products. The criterion for selecting the days for withdrawing the samples was based on the persistence/half life of the pesticide in different matrices observed during decay studies. The proposed metabolic pathways of quinalphos are depicted in Figure 3.7. This is based on the identification of different metabolites as discussed in the following paragraphs. Fragmentation pathways of various metabolites proposed in the following text are based on mass spectrometric fragmentations peaks.

3.3.5.1 Degradation products of quinalphos in water at pH 8.0

The samples were withdrawn after 26th, 40th, 53rd and 78th days. The GC-MS of the 26th day water sample shows the formation of four products. The mass spectrum of first

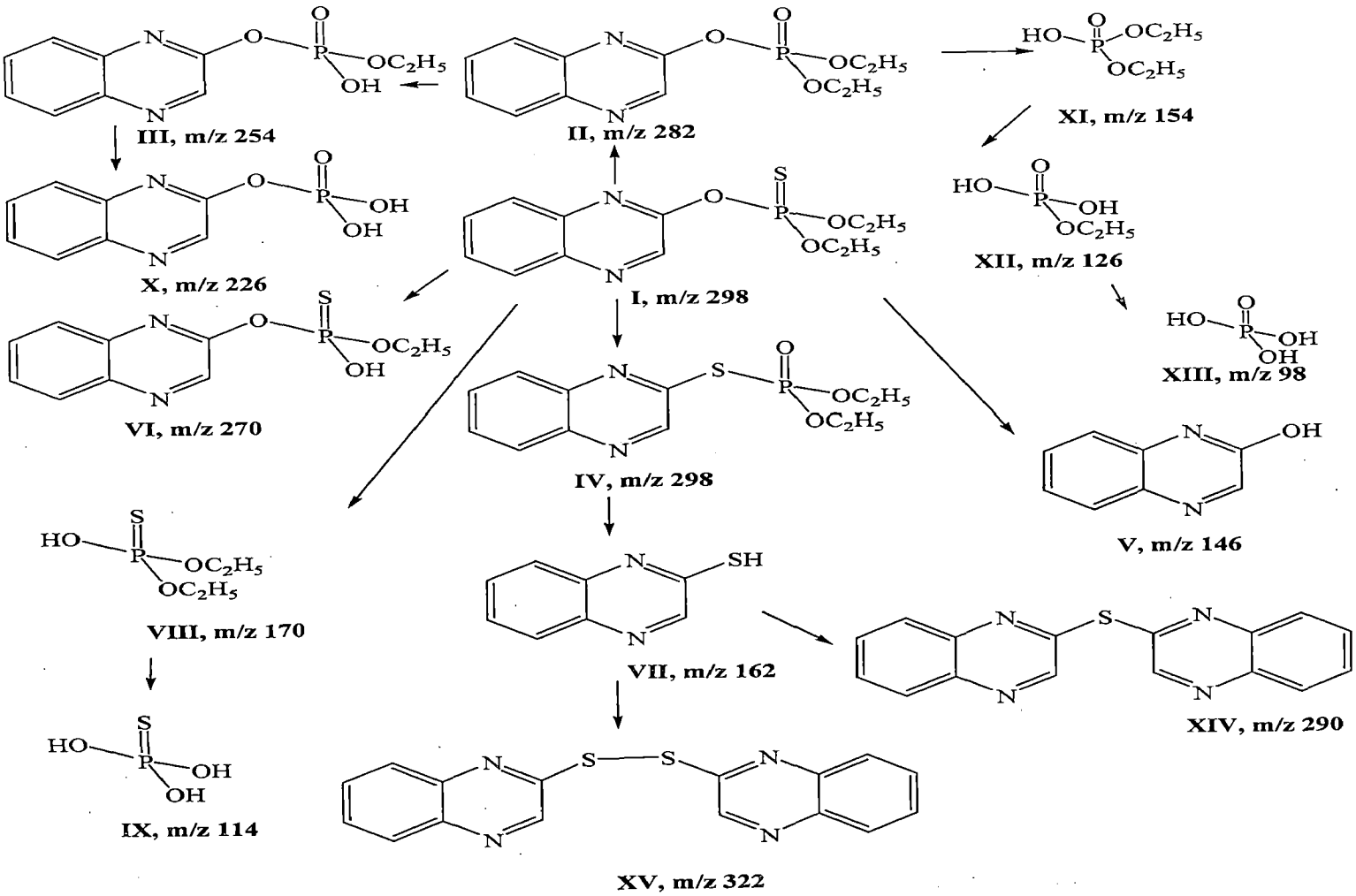
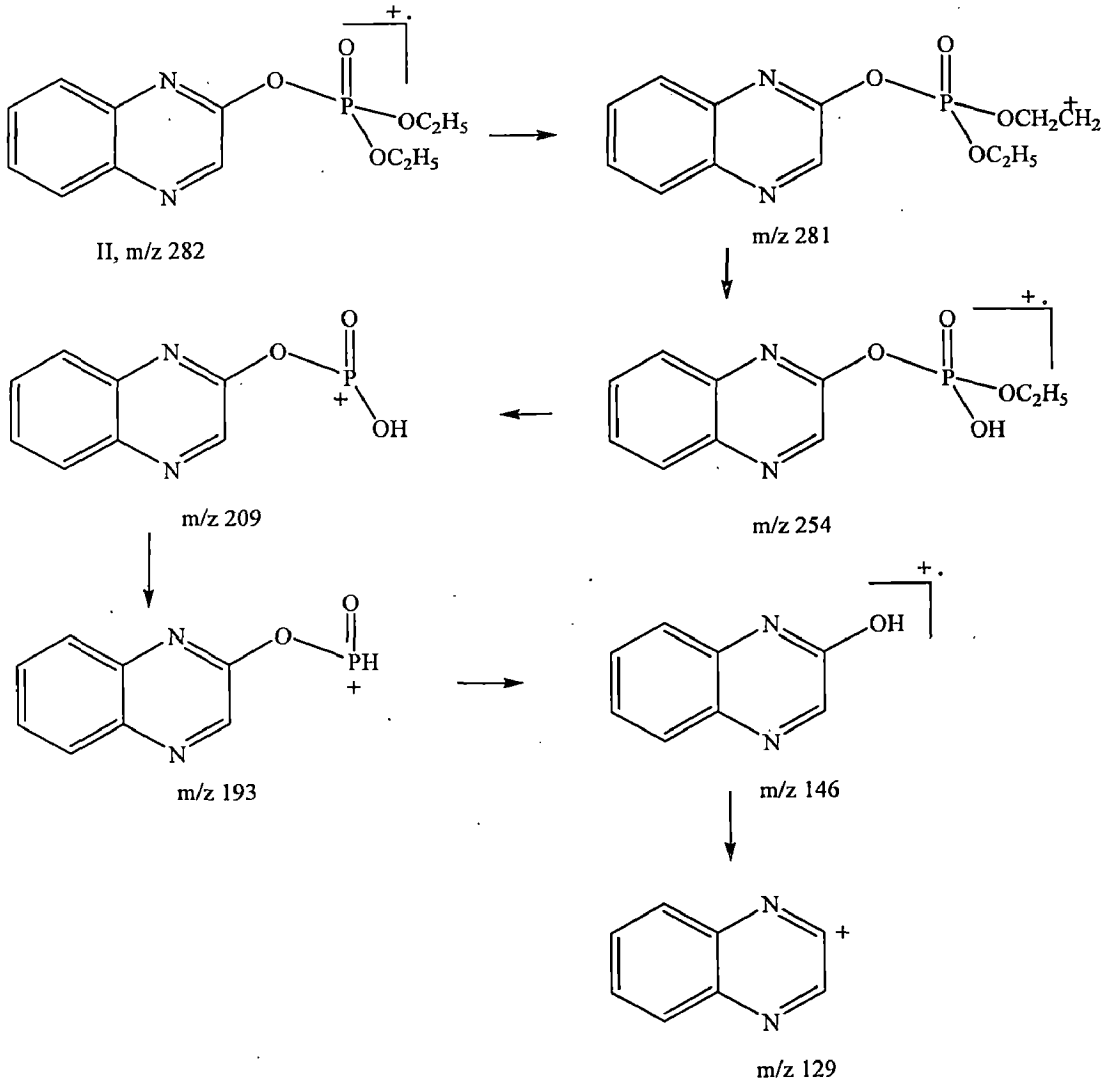
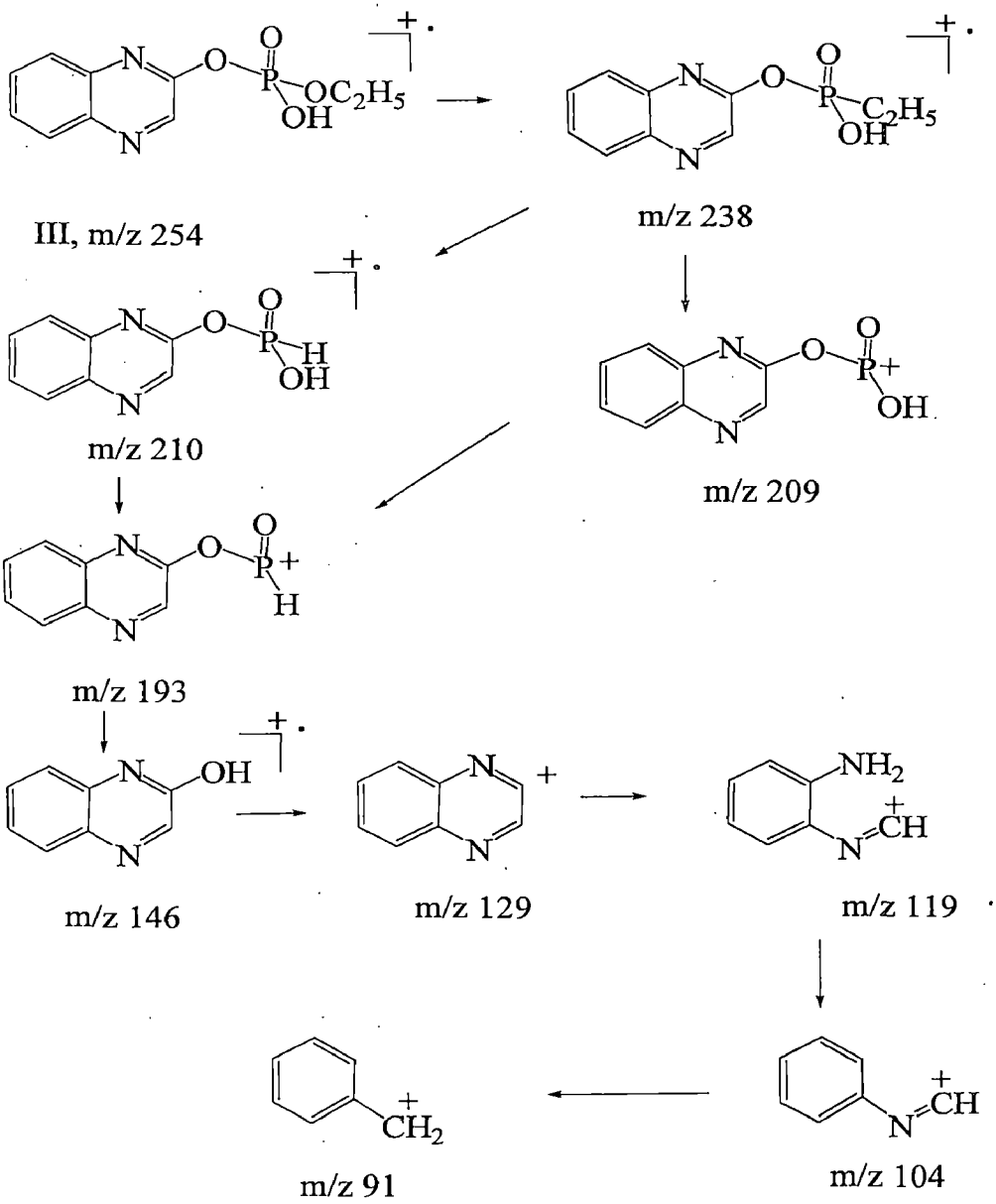


Figure 3.7: Degradation products of quinalphos identified in water, soil and plants by GC-MS

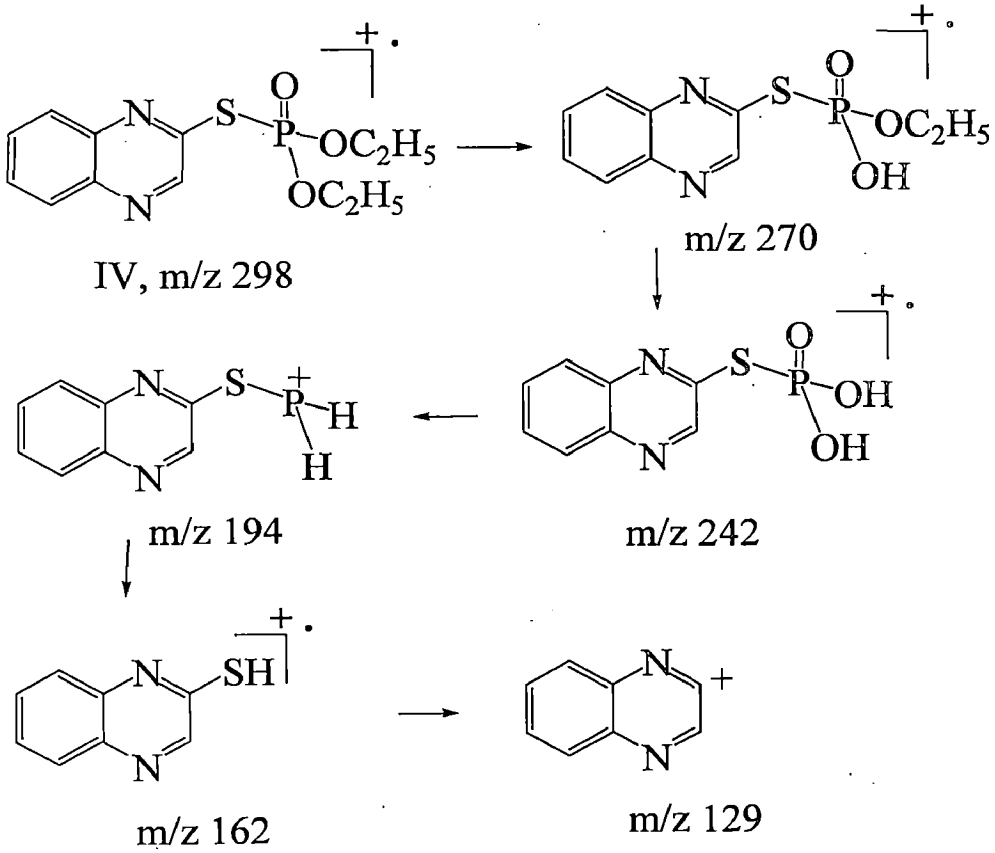
product has a molecular ion peak at m/z 282 along with fragment ion peaks at m/z 281, 209, 193, 146 and 129 (Fig. 3.8). It has been tentatively identified as quinalphos oxon (II) formed by the oxidation of P=S to P=O.



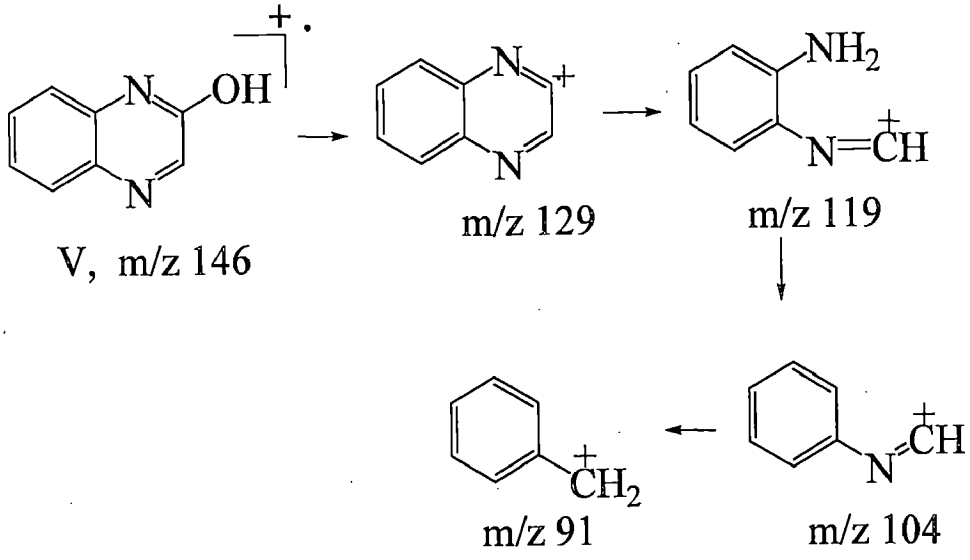
The second product has a molecular ion peak at m/z 254 with fragment ion peaks at m/z 238, 210, 209, 193, 146, 129, 119, 104 and 91(Fig. 3.9). Based on the mass spectrum, the product is identified as O-ethyl-O-quinoxalin-2-yl phosphoric acid (III), formed by dealkylation of quinalphos oxon (II).



The mass spectrum of third product has a molecular ion peak at m/z 298 with fragment ion peaks at m/z 270, 242, 194, 162 and 129 (Fig. 3.10). The mass of this peak corresponds to that of quinalphos but with different fragmentation pattern. It is identified as isoquinalphos (IV) formed by the thiono-thiolo rearrangement.

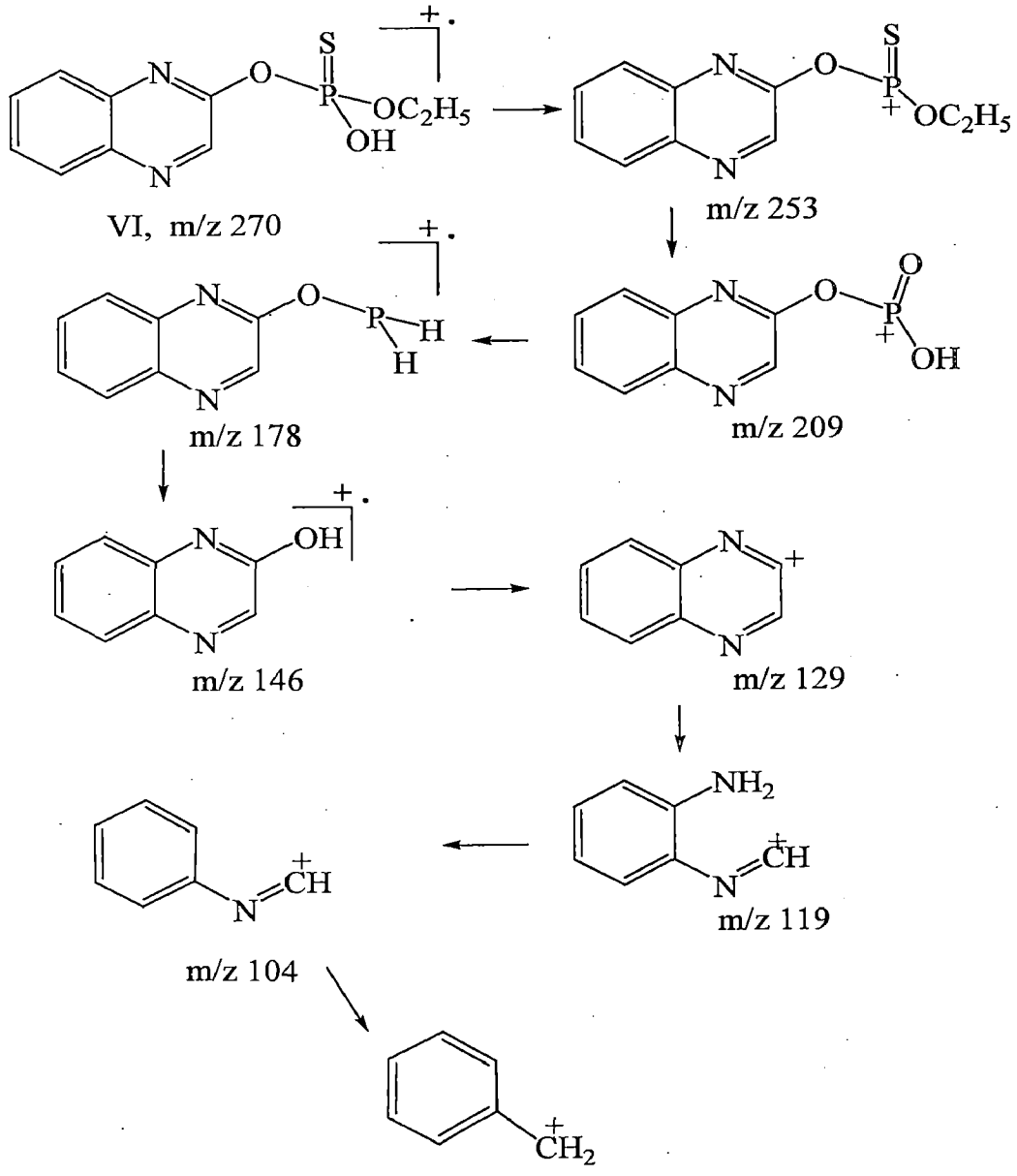


The fourth product shows molecular ion peak at m/z 146 and fragment ion peaks at m/z 129, 119, 104 and 91 (Fig. 3.11). This has been identified as 2-hydroxy quinoxaline (V) formed by the nucleophilic substitution reaction at the phosphorus via SN_2.

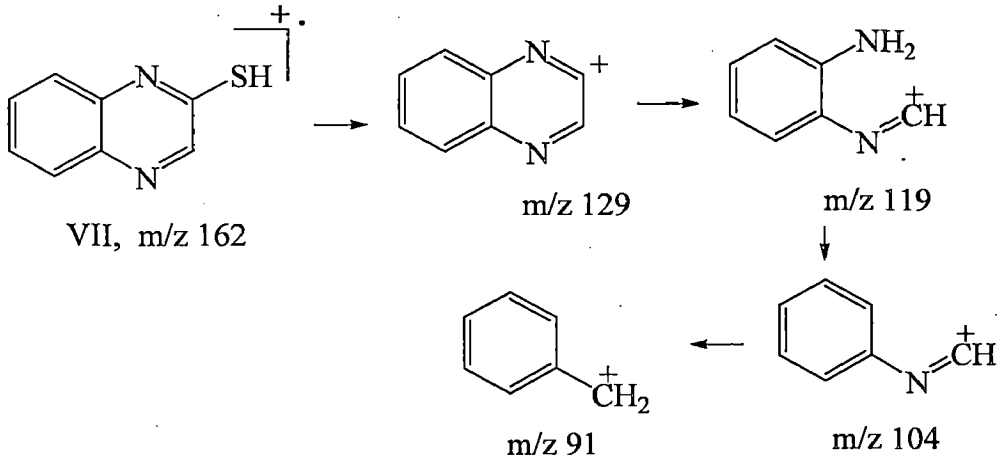


The GC-MS of 40th day sample shows besides products II, III, IV, V, a new product with a molecular ion peak at m/z 270 (Fig. 3.12) along with fragment ion peaks at m/z

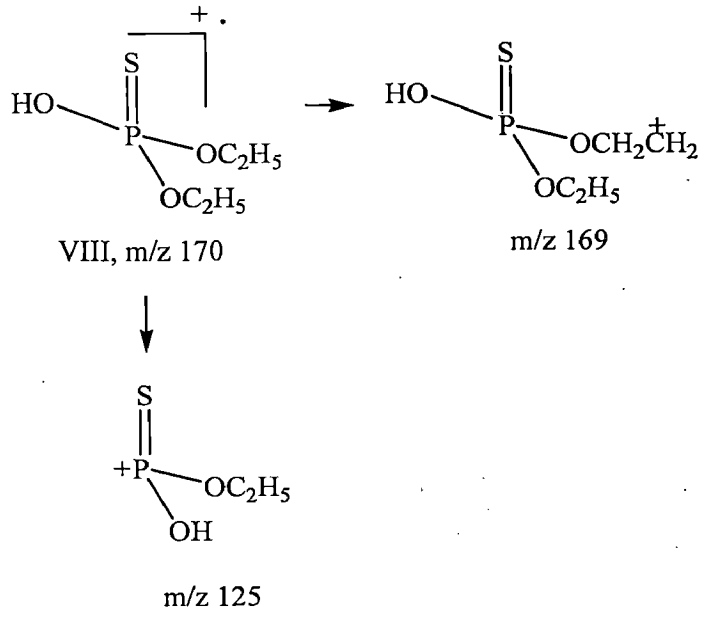
253, 209, 178, 146, 129, 119, 104 and 91. It is tentatively identified as desethyl quinalphos (VI).



The sample collected on 53rd day shows the formation of III, V and VI along with two more products. One of the products has a molecular ion peak at m/z 162 with fragment ion peaks at m/z 129, 119, 104 and 91. It is tentatively identified as quinoxaline-2- thiol (VII) (Fig. 3.13).



The mass spectrum of the second product shows a molecular ion peak at m/z 170 and fragment ion peaks at m/z 169 and 125 (Fig. 3.14). It has been identified as diethyl thiophosphoric acid (VIII). This product is formed by hydrolysis of quinalphos.

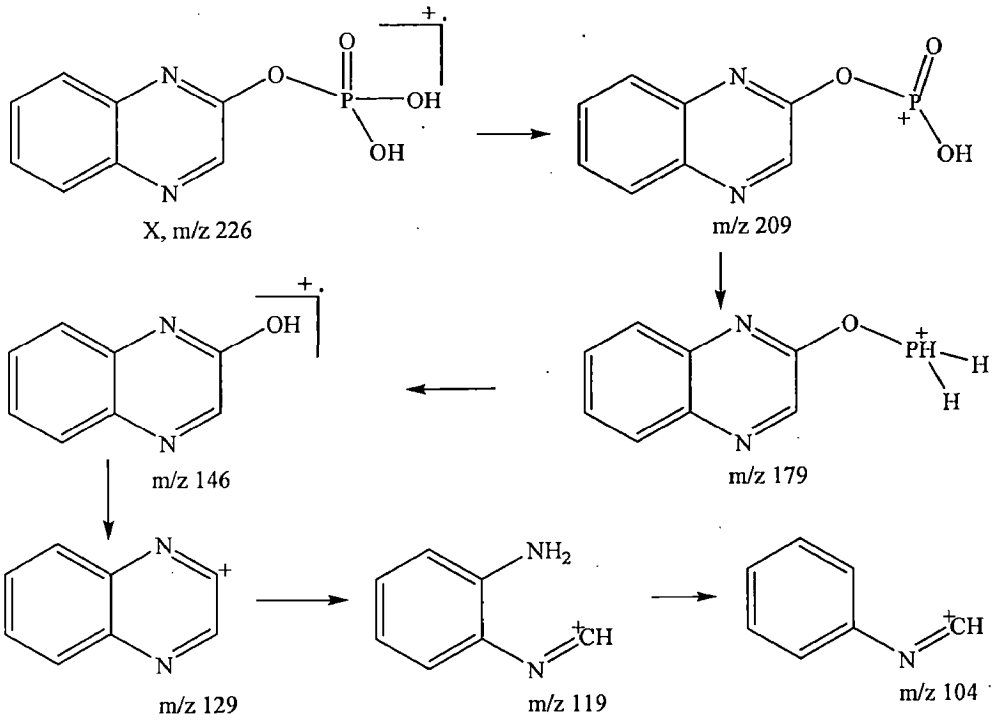


The GC-MS of sample withdrawn on 78th day shows V, VII and VIII along with a new product with a molecular ion peak at m/z 114. On the basis of parent ion peak, it is tentatively identified as trihydroxythiophosphoric acid (IX).

3.3.5.2 Degradation products of quinalphos in water at pH 6.0

Samples were withdrawn on 20th, 40th , 60th and 80th day. The GC-MS of the 20th day water sample shows the formation of VI and VIII, whereas the 40th day water sample shows the formation of II, V and VIII. Water samples collected on 60th day shows the

formation of II, III and a new product with molecular ion peak at m/z 226 and fragment ion peaks at m/z 209, 179, 146, 129, 119 and 104 (Fig. 3.15). This is identified as dihydroxy quinalphos oxon (X).

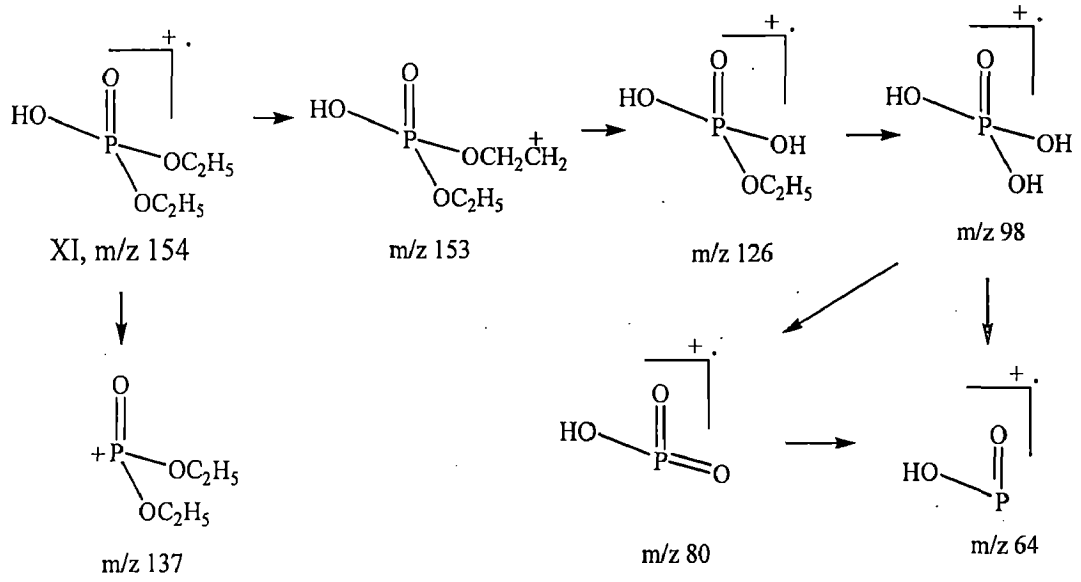


The water sample collected after 80th day shows the formation of II, III, V, VI, VII, VIII and IX.

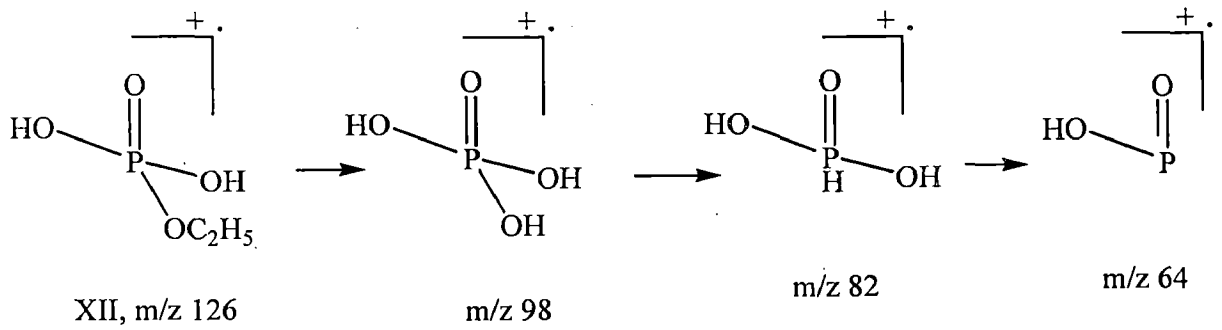
3.3.5.3 Degradation products of quinalphos in soil at pH 8.1

The samples were collected on 9th, 18th, 27th, 36th day. The mass spectrum of the soil sample collected on 9th and 18th day show the presence of II, III and V. The sample collected on 27th day shows the formation of X in addition to II, III and V.

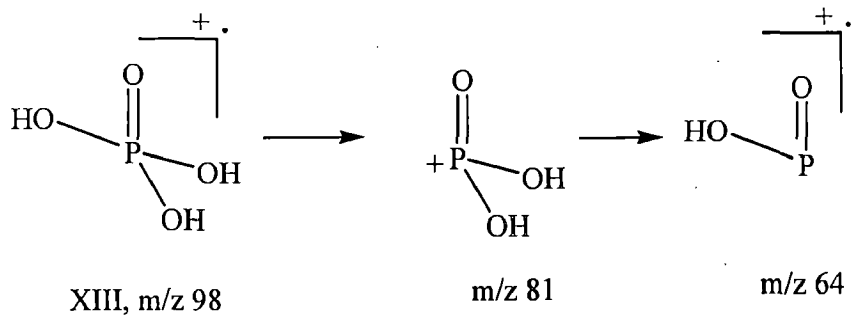
The GC-MS of sample collected on 36th day shows the formation of II, III, V, VII and three new products. One of these new products has a molecular ion peak at m/z 154 and fragment ion peaks at m/z 153, 137, 126, 137, 126, 98, 80 and 64. It is tentatively assigned the structure of diethyl phosphoric acid (XI, m/z 154) (Fig. 3.16).



The mass spectrum of the second product has a molecular ion peak at m/z 126 and fragment ion peaks at m/z 98, 82 and 64 (Fig. 3.17). It is tentatively identified as monoethyl phosphoric acid (XII).

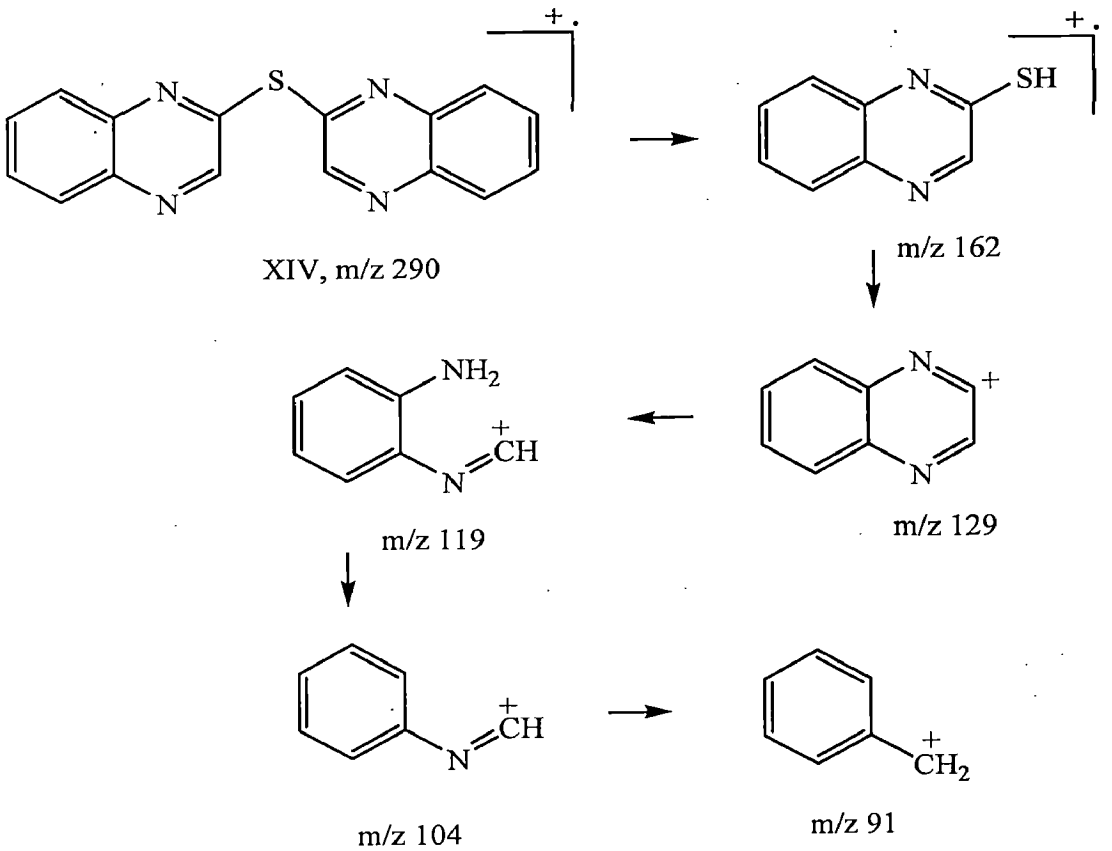


The mass spectrum of third product shows a molecular ion peak at m/z 98 and fragments ion peaks at m/z 81 and 64 (Fig. 3.18). Based on the mass spectrum it is assigned as phosphoric acid (XIII).

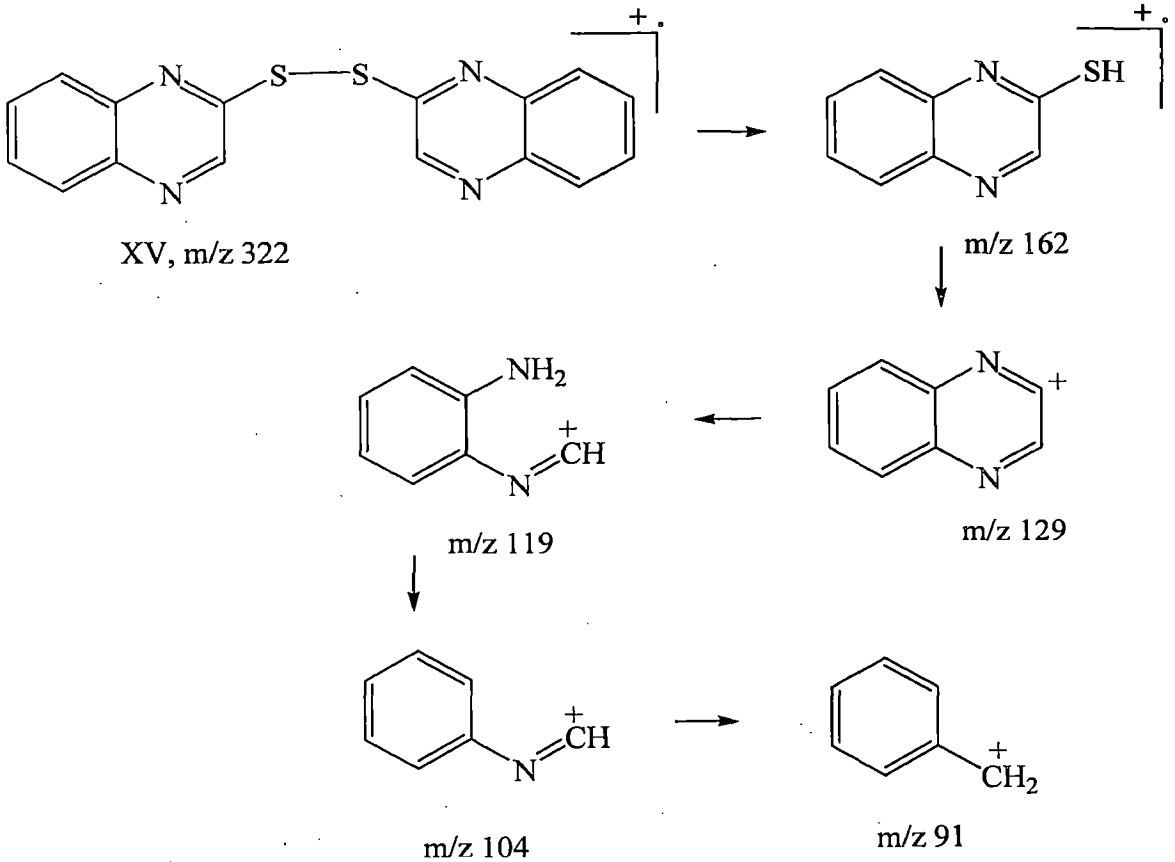


3.3.5.4 Degradation products of quinalphos in soil at pH 5.1

Soil samples were collected on 6th, 18th, 26th, 53rd and 78th day. The 6th day mass spectrum shows no degradation only molecular ion peak of quinalphos. Traces of quinalphos oxon (II) have been observed in 18th day sample. The sample collected on 26th day shows the formation of II, III, VI, VII and X , whereas the sample collected on 53rd day shows the presence of II, IV, V, VII, VIII and IX. The sample collected on 78th day shows the formation of degradation products V, VII, VIII, IX, XI, XII and XIII along with two new products. The first product has a molecular ion peak at m/z 290 along with fragment ion peaks at m/z 162, 129, 119, 104 and 91. The product has been identified as monosulphide dimer (XIV) (Fig. 3.19).



The second product has a molecular ion peak at m/z 322 along with the fragment ion peaks at m/z 162, 129, 119, 104 and 91. Based on the mass spectrum, it is tentatively identified as disulphide dimer (XV, m/z 322) (Fig. 3.20).



The products XIV and XV are formed by the dimerization of quinoxaline-2-thiol (VII).

3.3.5.5 Degradation products of quinalphos on radish leaf

Radish leaves applied with quinalphos were collected on 2nd , 4th , 6th ,8th ,10th and 12th day. The leaf sample collected on 2nd day shows the formation of II, III and VI, whereas sample collected on 4th day shows the presence of II, III, V and VI. The 6th day sample shows the formation of XI in addition to II, III, V and VI. Only the molecular ion peaks of II, III and V have been observed in 8th day sample whereas the 10th day sample shows the presence of II, III, V and VIII. The degradation products II, III, IV, V, VII, VIII, IX, XI, XII and XIII have been identified in 12th day sample.

3.3.5.6 Degradation products of quinalphos on radish root

Radish roots spotted with quinalphos were collected on 2^{nd}, 4^{th} , 6^{th} ,8^{th} ,10^{th} and 12^{th} day. The sample collected on 2^{nd} day shows the formation of II, III, IV and VII. The 4^{th} day sample shows the molecular ion peaks of II, III and V only. The peaks of metabolites III, V and VII are identified in 6^{th} day sample. The 8^{th} and 10^{th} day samples show the presence of II, III, V, VI and VII, whereas the formation of V, VII, VIII, IX, XI and XIII is indicated in 12^{th} day sample.

3.3.5.7 Degradation products of quinalphos on tomato fruit

Tomato fruits spotted with quinalphos were collected on 2^{nd}, 4^{th} , 6^{th} ,8^{th} ,10^{th} and 12^{th} day. The degradation products identified on 2^{nd} , 4^{th} ,6^{th} and 8^{th} day are II, III, V and VI whereas on 10^{th} and 12^{th} day, in addition to II, III, V and VI, product (VII) has been identified.

The degradation products identified can be rationalized as originating by P=S oxidation to P=O (II), isomerization (IV), O-dealkylation (III, VI and X), thiono thiolo rearrangement (IV), dimerization (XIV and XV) and hydrolysis (V, VII, VIII and XI). The pathways seem to be complex and different metabolites were observed with the change in the matrix. Hydrolysis of quinalphos can occur *via* bimolecular nucleophilic substitution reaction (SN_2) at the phosphorus or at the aliphatic carbon of methylene of the ethoxy group.

On the basis of metabolites obtained it appears that the metabolite formation in water and soil at both the pH is initiated by hydrolysis which occurs at different bonds. In the acidic range the rupture of O-C bond takes place resulting into the formation of desethyl quinalphos (VI). At pH 8.0, the cleavage of P-O bond results in the formation of 2-hydroxy quinoxaline (V). Both the products have also been detected as impurities by Sanyal and Dureja [28] in technical quinalphos on storage.

Dimerized products were identified in the acidic soil only on 78th day. Probably soil microorganisms in acidic environment facilitate the dimerization reaction. In case of water, hydrolysis is favoured both in acidic and alkaline pH but the oxidized products appear late in water at acidic pH. Formation of isoquinalphos is observed only in water (pH 8.0), soil (pH 5.1) and radish leaf and root. Most probably it is hydrolysed to quinoxaline-2- thiol (VII) in other systems of the matrices. Isomerised products appear early in soil of acidic pH as compared to the soil of alkaline pH.

From the half lives of pesticide summarized in Table 3.1, it can be concluded that the decay of quinalphos is faster with the increase in temperature, pH and organic content in the investigated range. The decay profiles in all these cases follow a first order kinetics. From the field studies it is apparent that the pesticide is highly unstable in the presence of plant juices/enzymes. The decay patterns in the plants under the field conditions are similar to that in water and soil under laboratory conditions. The half lives in radish leaf and root and tomato fruit are nearly of the same order but this similarity cannot be necessarily extended to different crops. The half lives observed in the plants in the field condition are lower than those observed in water and soil under the controlled laboratory conditions. In the case of field conditions one cannot ignore the long exposure of plants to daylight/sunshine which may contribute towards the photodegradation at the surface. This would amount to the fact that the laboratory data cannot be necessarily extended to the field studies. However, it may provide a guideline to the persistence behaviour of the pesticide. It may not be very logical to compare the persistence of quinalphos with other well known pesticides because most of the data cited on the subject are conditional. If we compare the stability of quinalphos with other well known pesticides it is more stable than the carbamates namely mancozeb ($t_{1/2}$ = 1-3 days at pH 7.0 at 25 ^{0}C) [45] and carbofuran ($t_{1/2}$ = 4 days on root) [44] and with the two well known organophosphorus pesticides malathion ($t_{1/2}$ = 5.4 days at pH 6.3 in soil at 20 ^{0}C) and methyl parathion ($t_{1/2}$ = 6.2 days at

pH 6.3 in soil at 20 ^{0}C) [40]. The byproducts identified would be highly useful for providing the data bank of metabolites for forensic and epidemiological investigations. The results further indicate that the metabolites, 2-hydroxy quinoxaline and oxon, which are more toxic than the parent compound, persist for a longer time. This study cannot be rated as complete in itself but is definitely a pointer to predict the fate of quinalphos under laboratory and field conditions and the trends in the results are more or less in agreement with the earlier studies.

The contents of this chapter have been published in journal "**Chemosphere**".

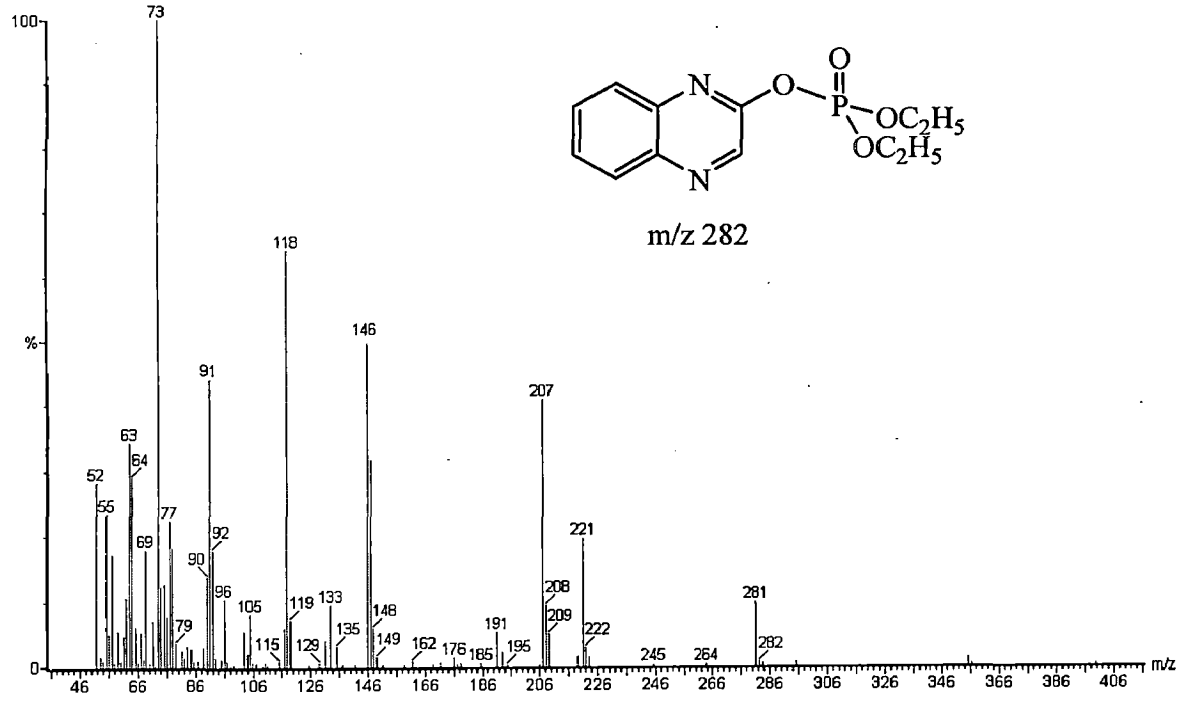


Figure 3.8: Mass spectrum of quinalphos oxon (II)

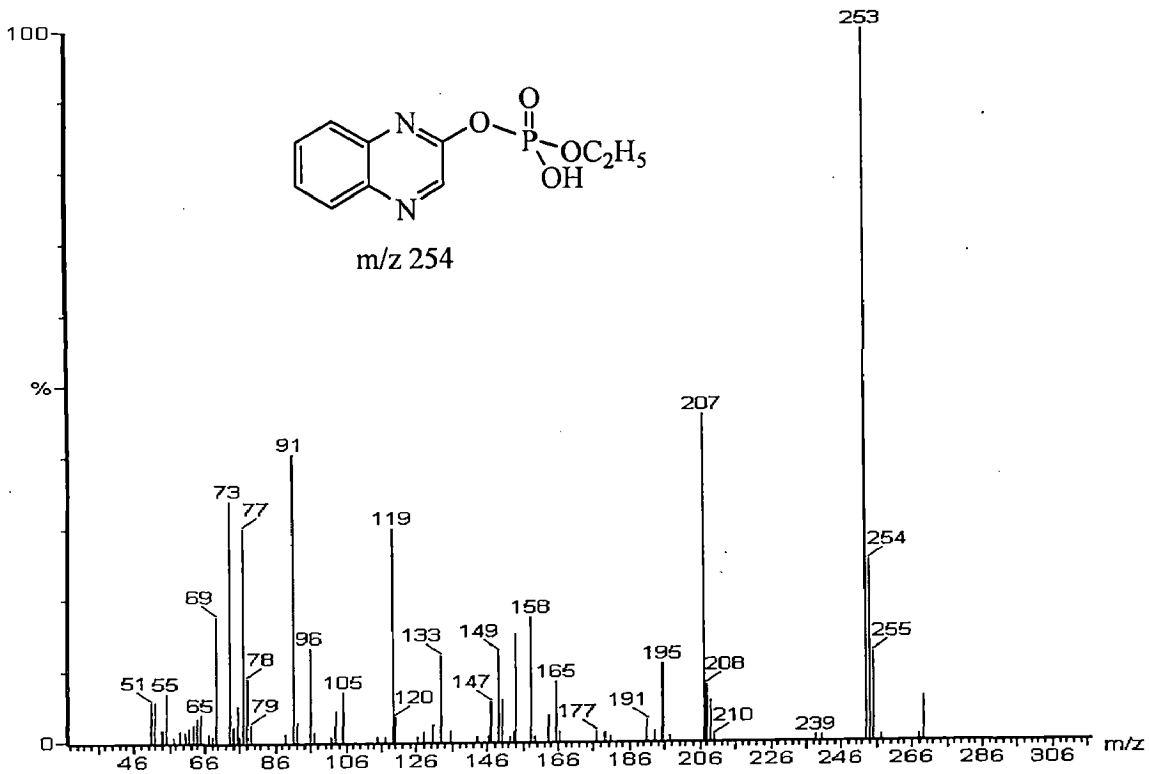


Figure 3.9: Mass spectrum of O-ethyl-O-quinoxalin-2-yl phosphoric acid (III)

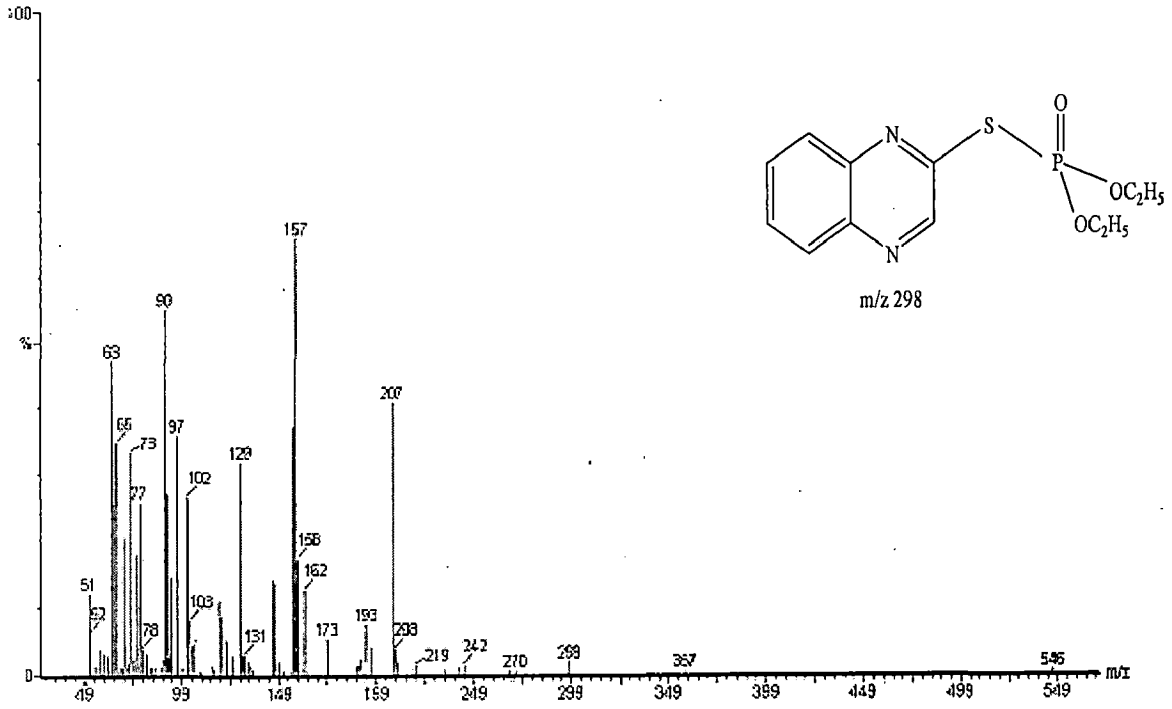


Figure 3.10: Mass spectrum of isoquinalphos (IV)

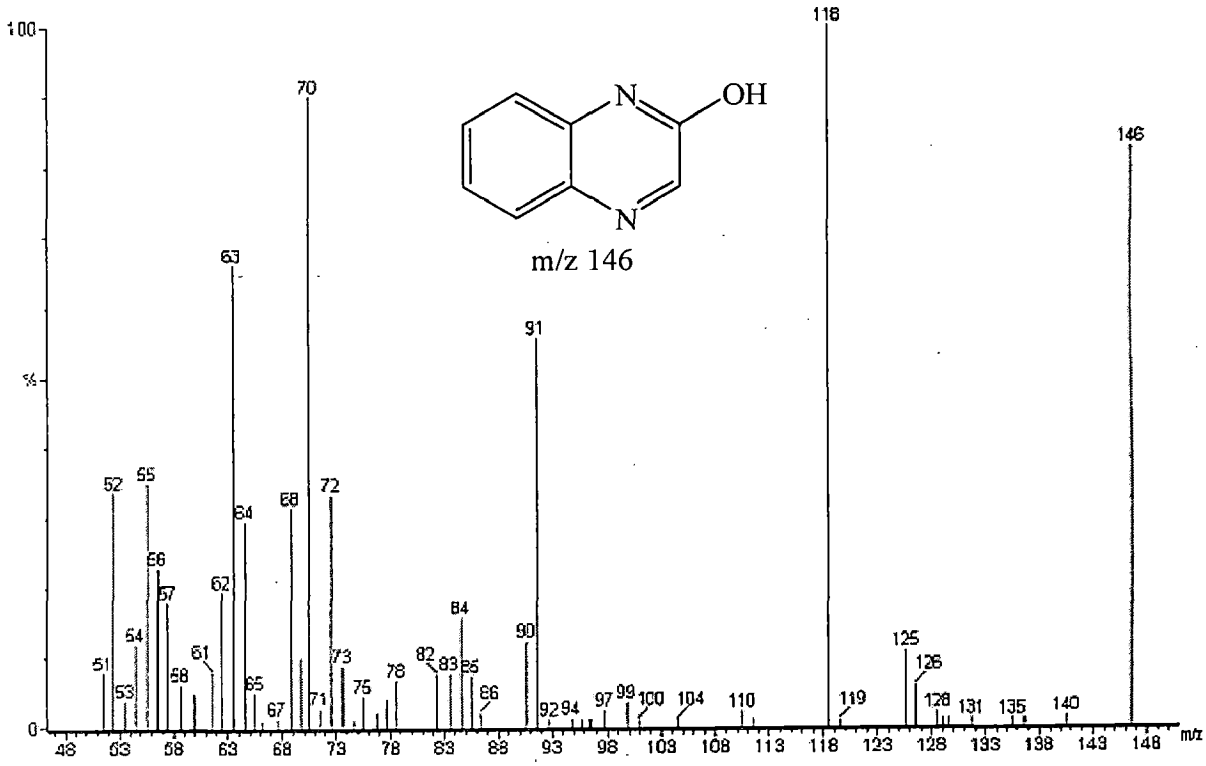


Figure 3.11: Mass spectrum of 2-hydroxy quinoxaline (V)

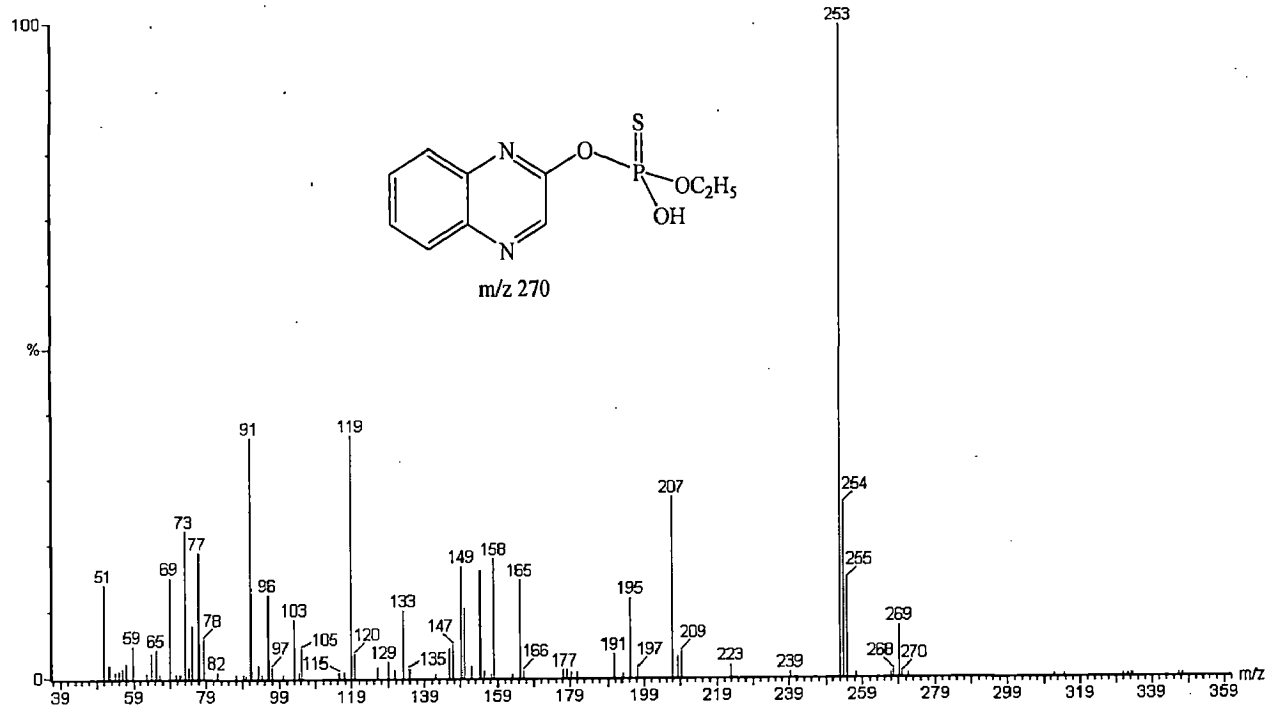


Figure 3.12: Mass spectrum of desethyl quinalphos (VI)

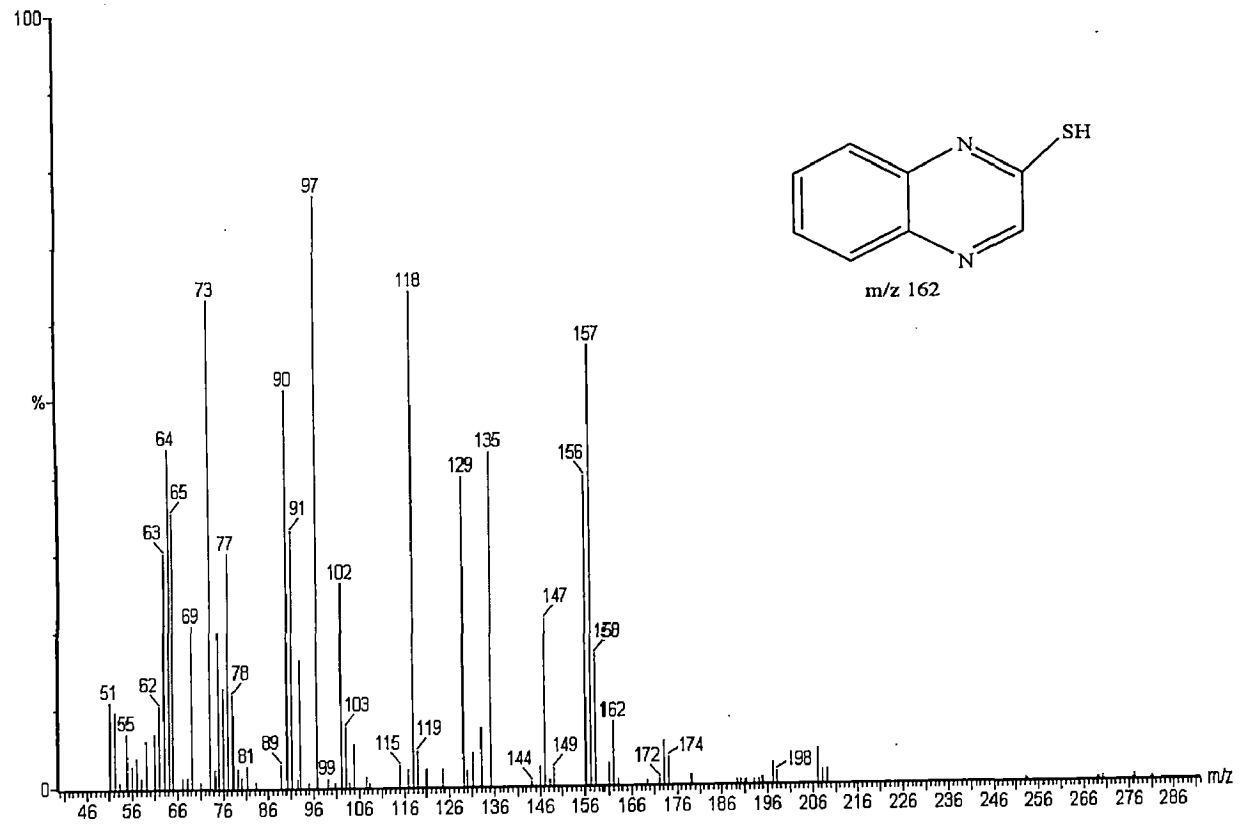


Figure 3.13: Mass spectrum of desethyl quinalphos (VII)

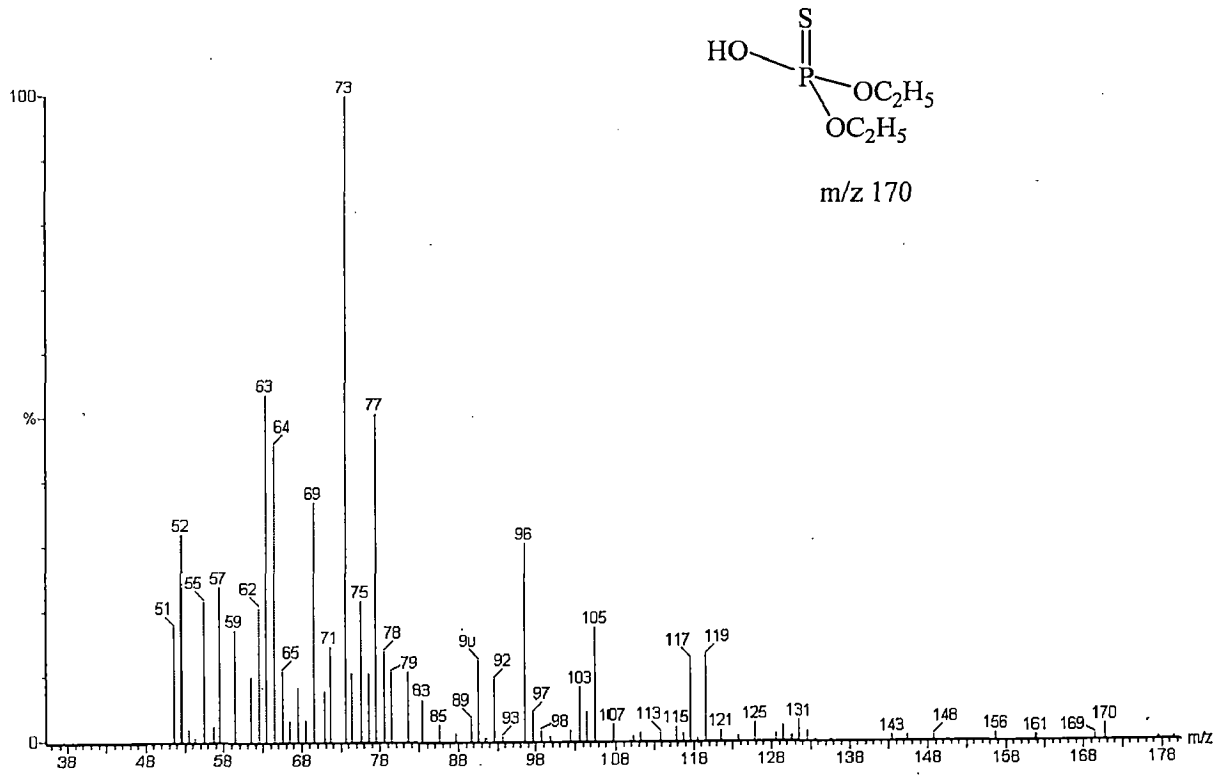


Figure 3.14: Mass spectrum of diethyl thiophosphoric acid (VIII)

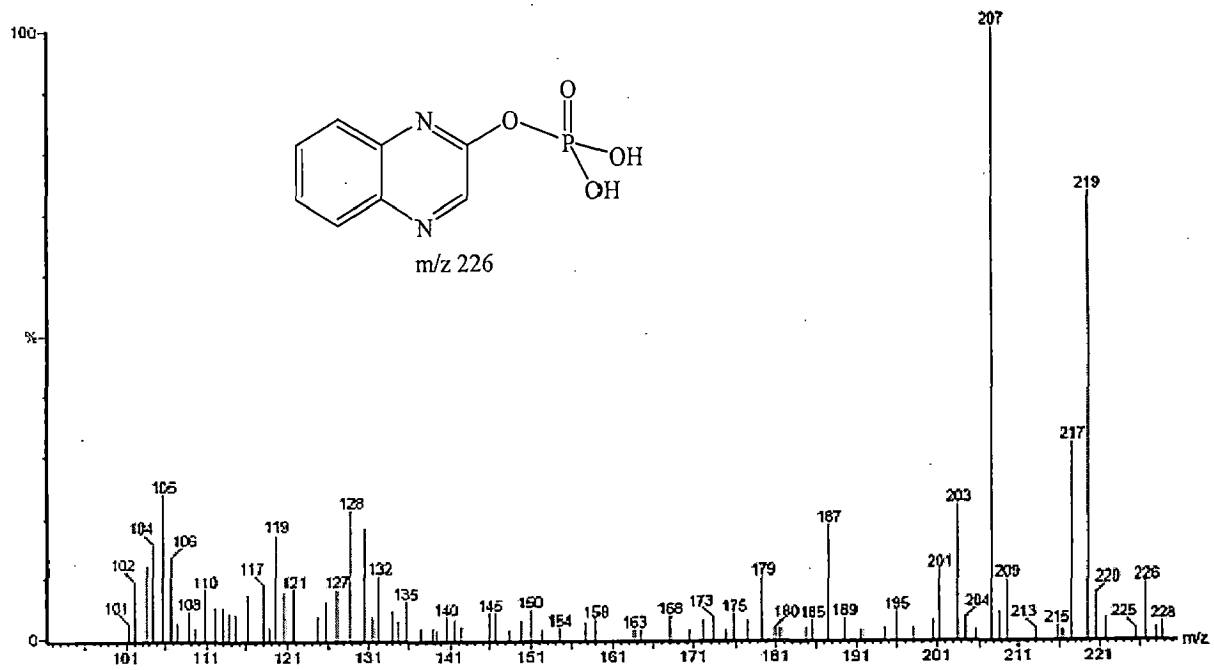


Figure 3.15: Mass spectrum of dihydroxy quinalphos oxon (X)

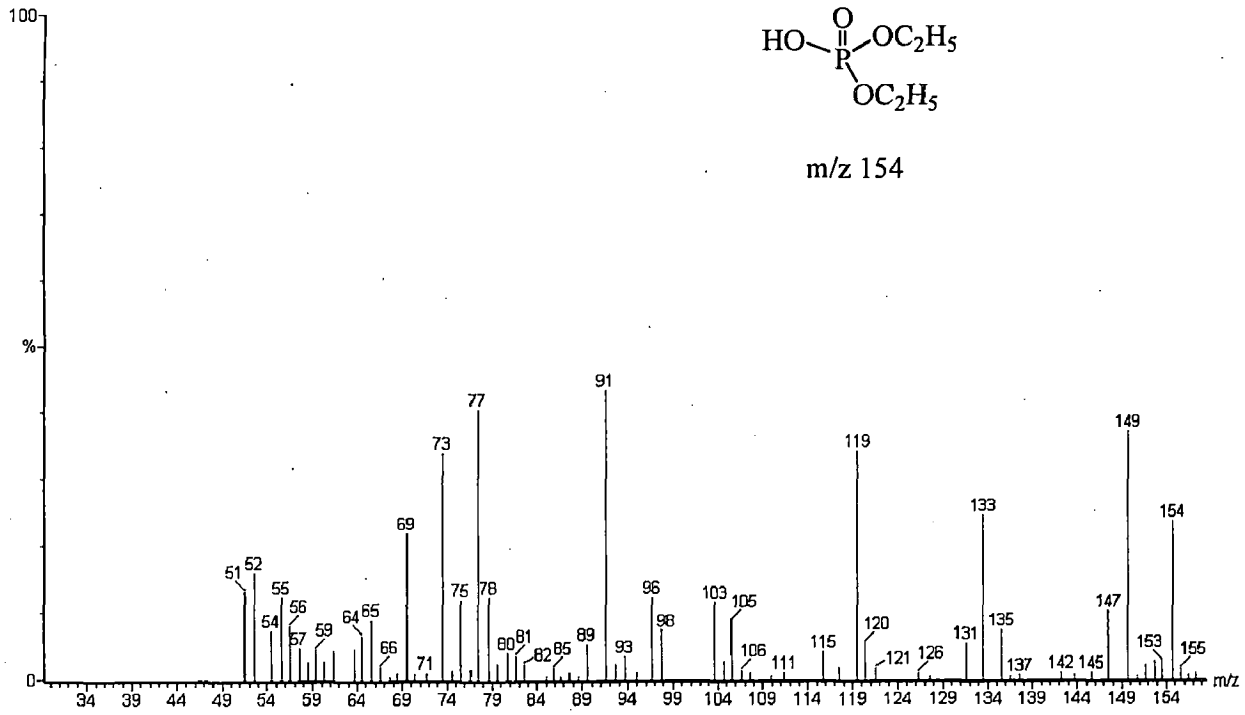


Figure 3.16: Mass spectrum of diethyl phosphoric acid (XI)

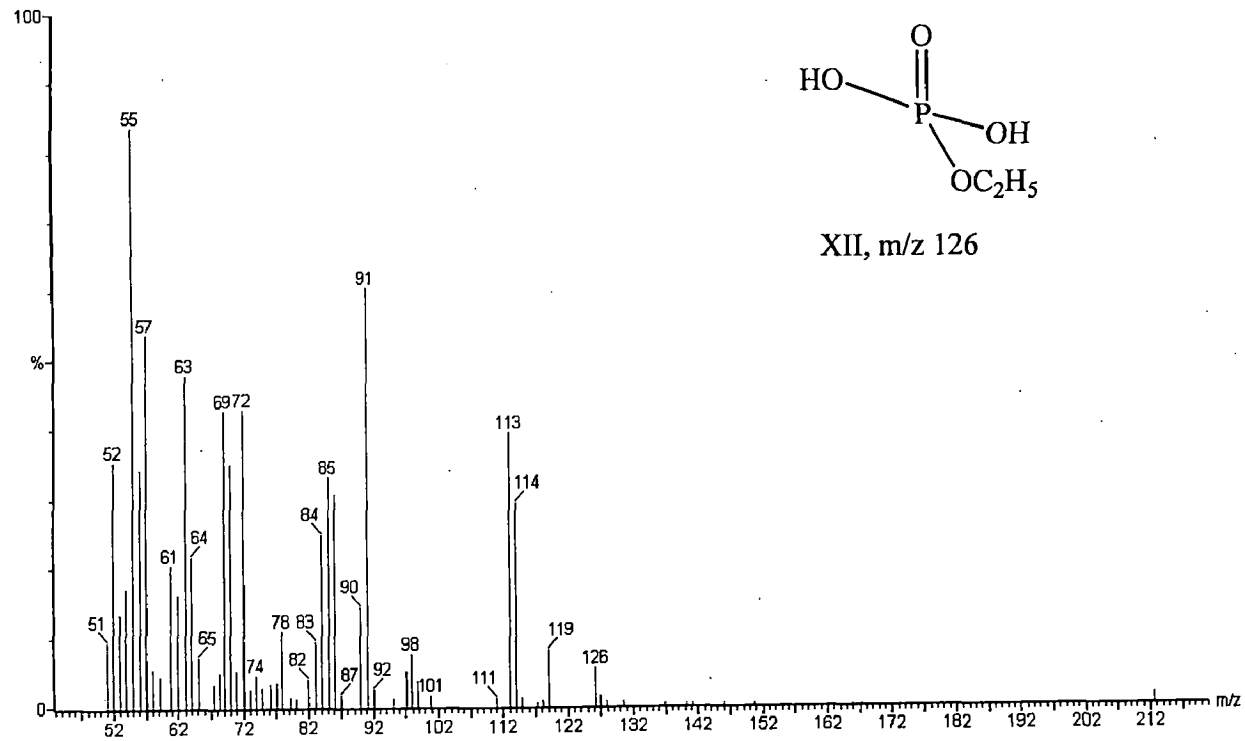


Figure 3.17: Mass spectrum of monoethyl phosphoric acid (XII)

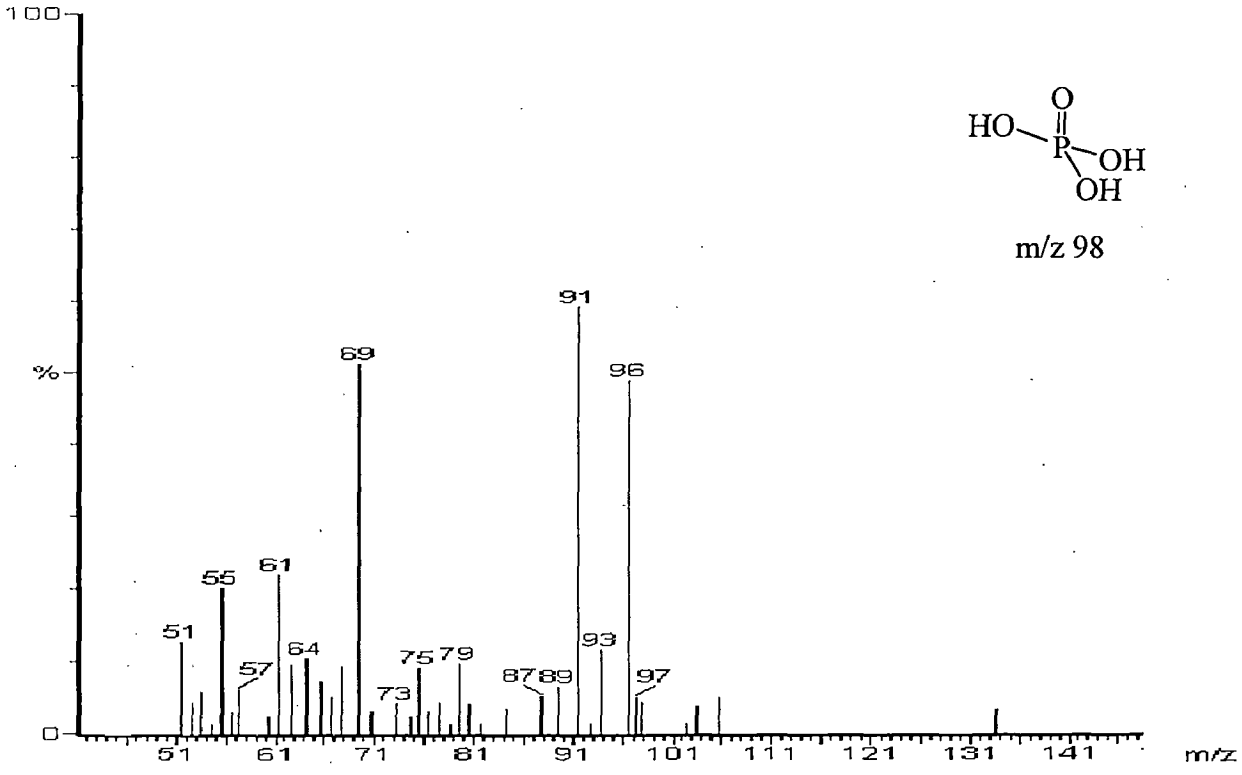


Figure 3.18: Mass spectrum of phosphoric acid (XIII)

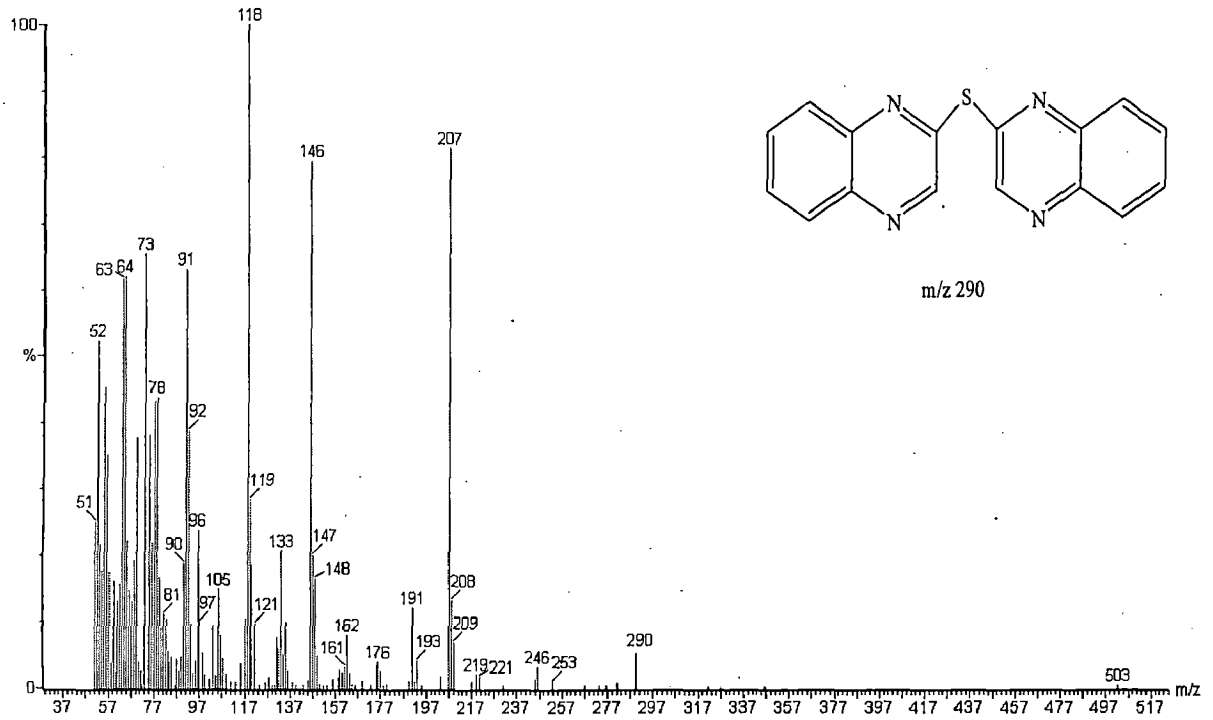


Figure 3.19: Mass spectrum of monosulphide dimer (XIV)

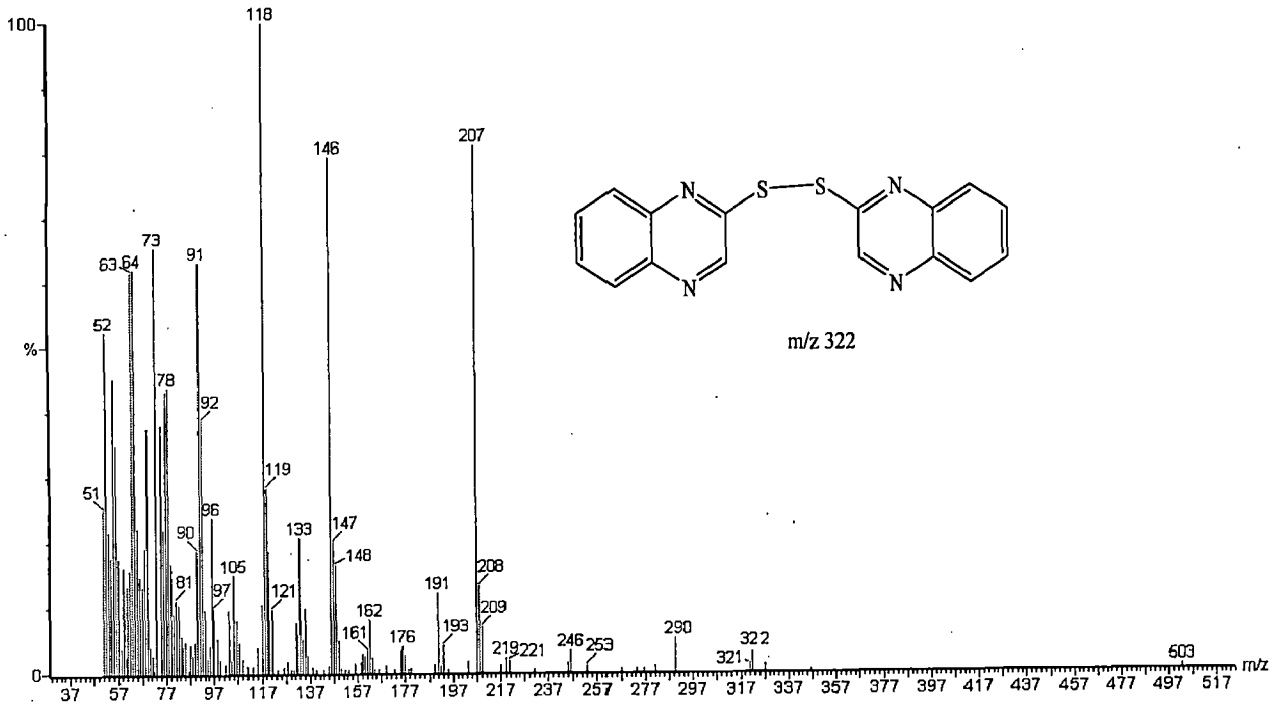


Figure 3.20: Mass spectrum of disulphide dimer (XV)

3.4 References

[1]. Kookana, R. S., Baskaran, S., Naidu, R., "Pesticide fate and behaviour in Australian soils in relation to contamination and management of soil and water: a review", Aust. J. Soil Res. **36**, 715 (1998).

[2]. Paris, D. F., Steen, W. C., Baughman, G. L. and Barnett, J. T. Jr., "Second-order model to predict microbial degradation of organic compounds in natural waters", Appl. Environ. Microbiol. **41**, 603 (1981).

[3]. Glotfelty, D. E., Seiber, J. N. and Liljedahl, L. A., "Pesticides in fog", Nature **325**, 602 (1987).

[4]. Glotfelty, D. W., Majewski, M. S. and Seiber, J. N., "Distribution of several organophosphorus insecticides and their oxygen analogues in a foggy atmosphere", Environ. Sci. Technol. **24**, 353 (1990).

[5]. Seiber, J. N., Wilson, B. W. and McChesney, M. M., "Air and fog deposition residues of four organophosphate insecticides used on dormant orchards in the san Joaquin valley, California", Environ. Sci. Technol. **27**, 2236 (1993).

[6]. Seiber, J. N. and Woodrow J. E., "Transport and fate of pesticides in fog in California's Central Valley", In: Agrochemical Fate and Movement, ACS Symposium Series, **751**, p. 323 (2000).

[7]. Ragnarsdottir, K. V., "Environmental fate and toxicology of organophosphate pesticides", J. Geol. Soc. London **157**, 859 (2000).

[8]. Liu, B., McConnell, L. L. and Torrents, A., "Hydrolysis of chlorpyrifos in natural waters of the Chesapeake Bay". Chemosphere **44**, 1315 (2001).

[9]. Hebert, V. R. and Miller, G. C., "Depth dependence of direct and indirect photolysis on soil surfaces", J. Agric. Food Chem. **38**, 913 (1990).

[10]. Hultgren, R. P., Hudson, R. J. M. and Sims, G. K., "Effects of soil pH and soil water content on prosulfuron dissipation", J. Agric. Food Chem.**50**, 3236 (2002).

[11]. Das, A. C., Chakravarty, A., Sen, G., Sukul, P. and Mukherjee, D., "A comparative study on the dissipation and microbial metabolism of organophosphate and carbamates insecticides in orchaqualf and fluvaquent soils of West Bengal", Chemosphere **58**, 579 (2005).

[12]. Reddy, P. S. and Ghewande, M. P., "Major insect pests of groundnut and their management", Pesticides **20**, 52 (1986).

[13]. Jena, M., Dani, R. C. and Rajamani, S., "Effectiveness of insecticides against rice gundhi bug", Oryza **27**, 96 (1990).

[14]. Armes, N. J., Jadhav, D. R., Bond, G. S. and King, A. B. S., "Insecticide resistance in *Helicoverpa armigera* in South India", Pestic. Sci. **34**, 355 (1992).

[15]. Schmidt, K. J., "Structure and activity of some phosphates and phosphonates in the series of azanaphthols", Proceedings of 2nd Int. IUPAC Con. Pest. Chem. (Ed. Tahori, A.S.). Gordon and Breach, New York, **1**, 365 (1972).

[16]. Vig, K., Singh, D. K. and Sharma, P. K., "Endosulfan and quinalphos residues and toxicity to soil microarthropods after repeated applications in a field investigation", J. Environ. Sci. Health B **41**, 681 (2006).

[17]. Ying, S., "Ovicidal activities of some new insecticides", Kunchong Xuebao **25**, 289 (1982).

[18]. Satpute, N. S., Deshmukh, S. D., Rao, N. G. V., Tikar, S .N., Moharil, M. P. and Nimbalkar, S. A., "Temperature-dependent variation in toxicity of insecticides against *Earias vitella* (Lepidoptera: Noctuidae)", J. Econ. Entomol. **100**, 357 (2007).

[19]. Galli, R., Rich, H. W. and Scholtz, R., "Toxicity of organophosphate insecticides and their metabolites to the water flea *Daphnia magna*, the microtox test and an acetylcholinesterase inhibition test", Aquat. Toxicol. **30**, 259 (1994).

[20]. Goncalves, C., Dimou, A., Sakkas, V., Alpendurada, M. F. and Albanis, T. A., "Photolytic degradation of quinalphos in natural waters and on soil matrices under simulated solar irradiation", Chemosphere **64**, 1375 (2006).

[21]. Megharaj, M., Venkateswarlu, K. and Rao, A. S., "Metabolism of monocrotophos and quinalphos by algae isolated from soil", Bull. Environ. Contam. Toxicol. **39**, 251(1987).

[22]. Rangaswamy, V. and Venkateswarlu, K., "Degradation of selected insecticides by bacteria isolated from soil", Bull. Environ. Contain. Toxicol. **49**, 797 (1992).

[23]. Cabras, P., Garau, V. L., Angioni, A., Farris, G. A., Budroni, M. and Spanedda, L., "Interaction during fermentation between pesticides and oenological yeasts producing H_2S and SO_2", Appl. Microbiol. Biotechnol. **43**, 370 (1995).

[24]. Pusino, A., Gessa, C. and Kozlowski, H., "Catalytic hydrolysis of quinalphos on homoionic clays", Pestic. Sci. **24**, 1 (1988)

[25]. Pusino, A., Gessa, C. and Frigerto, A., "Photolysis of quinalphos in ethanolic solution", Pestic. Sci. **26**, 193 (1989).

[26]. Banerjee, K. and Dureja, P., "Phototransformation of quinalphos on clay surfaces", Toxicol. Environ. Chem. **68**, 475 (1998).

[27]. Chukwudebe, A., March, R. B., Othman, M. and Fukuto, T. R., "Formation of trialkylphosphorothioate esters from organophosphorous insecticides after exposure to either ultraviolet light or sunlight", J. Agric. Food Chem. **37**, 539 (1989).

[28]. Sanyal, A. and Dureja, P., "Isolation and identification of impurities in technical quinalphos", J. Agric. Food Chem. **40**, 2013 (1992).

[29]. Prasad, C. U., Raju, K. V., Swaminathan, K. M., Rao, K. G. R., Rao, D. S. and Murty, Y. S., "Qualitative and quantitative assessment of wastewaters from an organophosphate pesticide manufacturing industry", J. Environ. Sci. Eng. **27**, 244 (1985).

[30]. Mansour, M., Feicht, E. A., Behechti, A., Schramm, K. W. and Kettrup, A., "Determination photostability of selected agrochemicals in water and soil", Chemosphere **39**, 575 (1999).

[31]. Gopal, M., Mukherjee, I., Prasad, D. and Yaduraju, N. T., "Soil solarization: technique for decontamination of an organophosphorus pesticide from soil and nematode control", Bull. Environ. Contam. Toxicol. **64**, 40 (2000).

[32]. Zhang, Z., Nie, G., Gao, L., Wu, C., Wang, G. and Chen, B., "Residue and dynamic degradation studies of quinalphos.cntdot.fenvalerate 12.5% EC in wheat and soil", Nongyao **47**, 912 (2008).

[33]. Babu, G. V. A. K., Reddy, B. R., Narasimha, G. and Sethunathan, N., "Persistence of quinalphos and occurrence of its primary metabolite in soils", Bull. Environ. Contam. Toxicol. **60**, 724 (1998).

[34]. Menon, P. and Gopal, M., "Dissipation of ^{14}C carbaryl and quinalphos in soil under a groundnut crop (*Arachis hypogaea L.)* in semi-arid India", Chemosphere **53**, 1023 (2003).

[35]. Dureja, P., Walia, S. and Mukerjee, S. K., "Multiphase photodegradation of quinalphos", Pestic. Sci. **22**, 287 (1988).

[36]. Gajbhiye, V. T., Agnihotri, N. P., Sirohi, P. S., George, T. and Gupta, R. K., "Effect of formulation on the persistence and degradation of quinalphos on tomato, *Lycopersicon esculentus Mill*", J. Entomol. Res. **19**, 13 (1995).

[37]. Murthy, K. S. R. K., Rao, B. N., Lakshminarayana, K., Rao, B. H., Krishna, M. and Azam, K. M., "Residues of quinalphos on rice", Pestic. Environ. Proc. 4 (1983).

[38]. Awasthi, M. D. and Anand, L., Fate of quinalphos and monocrotophos residues on bitter gourd fruits", J. Food Sci. Tech. Mys. **21**, 113 (1984).

[39]. Nazer, I. K., "Analysis for quinalphos residues on and in lemon fruits". J. Agr. Entomol. **3**, 304 (1986).

[40]. Kaur, I., Mathur, R. P. and Tandon, S. N., "Parameters affecting the decay of pesticide-a study by high performance liquid chromatography", Biomed. Chromatogr. **11**, 22 (1997).

[41]. Kolbe, A. and Bernasch, A., Stock, M., Schutte, H. R. and Dedek, W., "Persistence of the insecticide dimethoate in three different soils under laboratory conditions", Bull. Environ. Contam. Toxicol. **46**, 492 (1991).

[42]. Greenhalgh, R., Dhawan, K. L. and Weinberger, P., "Hydrolysis of fenitrothion in model and natural aquatic systems", J. Agric. Food Chem. **28**, 102 (1980).

[43]. Eneji, I. S., "The behaviour of the organophosphorus pesticide, pirimiphos-methyl in soil/water systems", M. Sc. Thesis, Queen's University, Canada, (2000).

[44]. Menzie, C. M., "Metabolism of pesticides: an update", Washington, D.C.:U.S. Fish and Wildlife Service, p. 486 (1974).

[45]. U. S. Environmental Protection Agency, "Pesticide fact sheet: mancozeb", Office of Pesticides and Toxic Substances, Washington, DC, USA, p. 4 (1987).

CHAPTER 4

DECAY PROFILES AND METABOLIC PATHWAYS OF THIRAM IN WATER, SOIL AND PLANTS

4.1 Introduction

The release of the pesticides into the environment may follow a complex series of events which can transport them into the different segments of the biosphere. There is a chain in which the pesticides, whether sprayed on the plants or applied directly to the soil/water bodies for pest/weed control, may eventually end up in the human beings. These agrochemicals may accumulate, metabolise or interact with the different environmental matrices. The presence and the persistence of the pesticides in water and soil affects the innocent biota and cause unprecedented damage to the ecology of the area. The deleterious effects, to a large extent, will depend upon the decay rate and the toxicity of the pesticides and their metabolites. Degradation process usually leads to the formation of less harmful breakdown products but in some instances can produce more toxic products. In the cases where the metabolites are more toxic than the parent the extent of persistence of the pesticide may not be the sole parameter of significance towards toxicity.

The persistence and the ultimate fate of pesticides in the food, soil, water and air are affected by the interrelated properties such as chemical characteristics of the parent moiety, its volatility, solubility, susceptibility to UV light, hydrolysis, adsorbility and chemical rearrangement etc. The weather conditions like temperature, humidity, wind velocity and rain or snow exert a considerable influence on the exposed pesticide. The fate of a pesticide in a particular matrix also depends on the characteristics of the medium. Say in the case of soil it is generally decided by the pH, moisture, organic content, clay and the presence of microbial activity. However, in the case of water the decay profile may generally be affected by the pH, presence of organics, inorganics and microbes. The above statements clearly indicate that the decay pathways of any pesticide are not easy to predict.

The decay of the pesticide residues on plants is an intriguing phenomenon. After the application of the pesticide on the crops, a major part of the spotted pesticide enters into the plant tissues and is affected by the inner constituents of the plants. The remaining part results into a physical binding to the plant surface. The strength of the bonds depends on the chemical properties of the pesticide, its concentration, nature of the plant and characteristics of the leaf surfaces (e.g. hairy, waxy, cuticle type). The pesticide on the surface is affected by physical loss, microbial activity and the photochemical degradation. The loss due to vaporization from the surface reaches the atmosphere. Pesticide vapours that drift through the air may be hazardous to plants, humans and animals. Diazinon carbamate is one of the most commonly detected insecticides in air, rain and fog [1].

The absorbed pesticide residues may be broken down or remain inside the plant and be released back into the environment when the plant decays. From the ecological point of view, the decay of the pesticide in the plant assumes a greater significance as it is transported to the different parts of the plant such as root, stem, foliage and fruit which directly or indirectly enter the human system. In this context it may be important to know that with the passage of time as to how much remains in the different parts of the plant.

It has already been mentioned in chapter I that carbamates as agrochemicals are next in the importance and usage to the organophosphorus compounds. On screening the available information from the various sources they are known to be less hazardous but their toxicity to various forms of biota in environment cannot be overlooked. They are the irreversible inhibitor of AChE enzyme. The frequent use of carbamates and potent threat to non-target population makes it imperative to study their decay profiles and metabolic pathways in different types of the ecosystems.

A survey of the Northern region of India indicated that amongst the important carbamate

pesticides, thiram (tetramethyl thiuram disulphide) is quite a popular pesticide. It is a well known nonsystemic DTCs fungicide. Compared to the other carbamates a comprehensive degradation study on thiram has not been done. Thus to investigate the degradation profile of thiram and comment on its persistence and pattern of decay is desired. The use of thiram in India as a fungicide is increasing and it is listed as one of the priority chemicals to be tested for carcinogenicity and mutagenicity in experimental animals by International Agency for Research on Cancer [2]. Another important source of thiram for environmental contamination is the degradation of the two widely used ethylene bisdithiocarbamate fungicides, ferbam and ziram [3].

The toxicity of thiram is well documented. Thiram is considered to have low short term toxicity. Thiram is slightly toxic by ingestion and inhalation and moderately toxic by dermal absorption [4]. Human chronic exposure may cause headache, nausea, diarrhea and other gastrointestinal problems [5]. Among many toxic effects of thiram to the exposed workers and test animals, hepatotoxicity and focal necrosis are of great concern [6]. A number of reports are available on toxicology and biomonitoring of thiram in biota. Biomonitoring of exposure of thiram has been accomplished by determining the principal metabolites, dimethyl dithiocarbamate and carbon disulfide. These compounds have been shown to inhibit hepatic microsomal enzymes [7]. Hepatic cytochrome P450 isozymes mediated the metabolism of thiram in rats [8]. Polar metabolites of thiram and its conjugates were identified by Gay et al. [9] in the urine of the rats after dosing them with thiram. Norris [10] has used HPLC for the identification of metabolites of thiram in the urine of rats. They found that the proportions of some of the metabolites depend on the dosage level and the time after dosing. A number of *in vitro* short term assays including the Ames test and assays on mammalian cell systems have shown genotoxic activity of thiram [11-14]. The metabolites of thiram are reported to have

toxicological concern. Among them, dimethyldithiocarbamic acid (DMDTC) and carbon disulfide are highly toxic compared to the parent compound. They are responsible for the observed hepatotoxicity [8].

Apart from toxicological investigations, studies have also been conducted on the degradation of thiram and its byproducts under various conditions. A major portion of these investigations focuses on photodegradation of the pesticide. Some useful information is also available on microbial and oxidative degradation and decay in other matrices. Thakare and Bhave [15] carried out a study on the photocatalytic degradation of thiram under visible light irradiation. They found that 150 minutes are required for the complete degradation of thiram. The end products of photocatalytic degradation of thiram are carbon dioxide, nitrate and sulphate. Haque and Muneer [16] investigated the photocatalysed degradation of the thiram in aqueous suspension of titanium dioxide as a function of irradiation time under a variety of conditions. The photocatalyst Degussa P25 was found to be more efficient as compared to other photocatalysts tested. The efficiency of degradation was found to be slower under sunlight as compared to the artificial light source. Niitsuma et al. [17] reported the degradation of thiram by ozone treatment with or without UV radiation. Thiram was rapidly degraded in a water solution when irradiated with UV light. More than 94% of the pesticide was destroyed within 20 minutes. Kaneco et al. [18] investigated the photocatalytic degradation of thiram in aqueous solution in the presence of titanium oxide. They found that the photocatalytic degradation of thiram follows a pseudo-first order kinetics and depends upon various factors such as photocatalyst load, initial substrate concentration, temperature, pH, and sunlight intensity and illumination time. Crank and Mursyidi [19] identified a number of products by UV photolysis, photo-oxidation, and visible photosensitized oxidation of thiram due to C-S and S-S bond fissions. Thiram has been reported to undergo degradation during water

purification using sodium hypochlorite [20]. Kodama et al. [21] studied the oxidative degradation pathway of thiram in tap water processed by oxidation with sodium hypochlorite. On the basis of the results, it was concluded that the oxidation of thiram with sodium hypochlorite initially produced an intermediate dimethylthiocarbamoyl dimethylcarbamoyl disulfide which finally degraded to bis(dimethylcarbamoyl) disulfide, trisulfide and dimethylamine. A study on the screening and the characterization of special effective bacteria for degrading thiram in the water body was presented by Gao et al. [22]. Among the three bacteria investigated, TM1 was found to have the highest ability to degrade the pesticide. It is reported that thiram under acidic conditions and in biological media produced dimethyl dithiocarbamate and carbamic acid [7]. Electrochemical investigation on the reactivity of thiram have also been carried out in order to predict the fate of the pesticide in the environment [23, 24]. The degradation of thiram is complex and the exact reaction mechanism is still not very well understood.

It may be important to point out here that some studies have been conducted on the persistence of thiram in different matrices, namely water, soil and plants. It was observed that high organic matter content increases chemical/microbial degradation of this pesticide [25, 26]. Czarnik et al. [27] assessed the effects of growth conditions on the dynamics of thiram disappearance in lettuce. The studies revealed that the rate of degradation of thiram is enhanced by watering, with increasing temperature and also depends on lettuce growth rate. A field study conducted on residue dynamics of thiram in rice paddy and paddy soil indicated that it decays within one week and thus confirmed that thiram belongs to the category of nonpersistent pesticides [28]. Norris et al. [29] have conducted a study on the hydrolysis of thiram and concluded that its half life varies from 6.9 hours to 68.5 days in the pH range 5-9. It has been reported that thiram degrades faster in the field than in the greenhouse, and as a

wettable powder than as an emulsion [30]. The major metabolites of thiram in the soil are copper dimethyldithiocarbamate, dimethylamine and carbon disulfide. In acidic soil thiram undergoes degradation by a microbial action or by hydrolysis [25].

The above literature report clearly points out that the degradation of thiram has been investigated in a variety of conditions. The conditions range from exposure to UV light to the presence of various compounds and microbes. The matrices chosen for the study have also been varied. In spite of all this a coherent study on the decay of the pesticide under the controlled laboratory conditions and comparing the data with the field conditions is missing. Needless to say that some metabolites in different systems have been identified but that study also does not seem to be comprehensive.

Keeping this in mind a systematic study was planned on the decay and identification of metabolites of thiram in water, soil and plants. In the case of water the effect of temperature, pH and organic content (humic acid) was investigated. For soil it was only possible to observe the effect of pH. The plants chosen for the study are radish and tomato which are frequently used in salads. In all the above matrices the degradation products were identified after different time intervals. Studies on the plants were carried out in conditions which were near to field conditions. As discussed in chapter III the plants were exposed to sun light during day time and at sunset they were placed under a tin shade.

4.2 Experimental

The details and the operating conditions of HPLC and LC-MS used for the decay study and identification of metabolites are cited in chapter II.

4.2.1 Persistence studies

Laboratory experiments on the degradation of thiram were conducted using deionised water and soil as described in chapter III (Section 3.2.1). The water samples were spiked with

20 μg mL^{-1} of thiram solution in methanol and those of soil with 100 μg mL^{-1}. They were stored at 28±2 ^{0}C unless mentioned otherwise. The decay was followed for about a period of one month in water and soil. The plants were grown in Roorkee soil contained in clay pots. Precautions were taken to avoid the pesticide loss as described in chapter III (Section 3.2.1). To investigate the decay profile of thiram on different parts of the plant, the leaves and roots of radish and tomato fruits of four-week old plants were spotted with a known amount (100 μg mL^{-1}) of the pesticide. While planning the field experiments on plants it was observed that for the recovery of remaining pesticide when thiram is triturated with plant material it decays almost instantaneously. Similar observation on carbamates with fruit juices has been reported earlier [31-33]. Therefore, the homogenization of the plant material with the pesticide was avoided (As discussed in chapter II section 2.2.6.4). The leaves, fruits and the roots were carefully spotted with a known amount of the pesticide on the surface and subsequently at certain interval of time the particular part was picked up and thoroughly washed with the solvent to recover the remaining pesticide. The details of washing are given in chapter II (Section 2.2.6.4). The decay was followed by determining the residual pesticide on the particular part namely, leaf, root and fruit separately for thirty days between March and April. The minimum and the maximum temperatures, average humidity and wind velocity were 19±3 - 36±3 ^{0}C, 47.6±9% and 4±3 km h^{-1}, respectively. In the case of all the three matrices the limit of detection was found to be 1 μg mL^{-1}. Control and blank were run wherever necessary. The values reported are the average of triplicate runs. Standard deviation of the concentration based on the three observations at 50 mg kg^{-1} in water, soil and on plant was 0.73, 1.01 and 4.85. The schemes applied for the extraction of thiram from water, soil and plants are given in chapter II as Flowsheets 2.1, 2.3 and 2.5.

For the identification of metabolites, the water (pH 6.0 and 8.0), soil (pH 5.1 and 8.1) and the different parts of the plants were spiked with 1000 μg mL^{-1}-of thiram. At periodic intervals, duplicate samples of water and soil were removed for extraction and analysed by LC-MS. As thiram decays instantaneously on contact with plant juices, the identification of its degradation products in plants was carried out in two different samples, the one is the surface washout and the other crushed sample. For surface washout sample the spotted part of the plant was washed by dip and shake method with appropriate solvent and subjected to LC-MS for identification of degradation products. For observing the metabolites in crushed plant sample, the spotted part of the plant was chopped, homogenized and extracted with solvent. The extract was cleaned on a column containing florisil and anhydrous sodium sulphate. Finally the extract was concentrated and analysed by LC-MS for the identification of degradation products.

4.3 Results and Discussion

The half lives and rate constants for the decay of thiram under varying conditions are given in Table 4.1.

4.3.1 Effect of temperature

The effect of temperature (10, 20 and 30 ^{0}C) on the decay of thiram in deionised water (pH 6.3) is shown in Figure 4.1. The results suggest that the rate of decay increases with the increase in temperature and the $t_{1/2}$ varies from 12.1 days to 5.0 days. Remya and Lin [34] also observed that the carbofuran, a carbamate pesticide, decays faster with the increase in the temperature. There is an exponential decrease in the concentration of thiram with time. The data plotted on the natural log scale gives a straight line indicating that the process of disappearance of the pesticide follows a first order kinetics.

Table 4.1: Half lives of thiram pesticide under different conditions

Matrices	Conditions	$t_{1/2}$ (d)	k (d^{-1})
	Temperature (pH: 6.3)		
Water	10 ^{0}C	12.1	0.057
	20 ^{0}C	7.8	0.089
	30 ^{0}C	5.0	0.139
	pH (Temperature: 28±2 ^{0}C)		
	5.0	28.1	0.025
	6 .0	11.3	0.061
	7.0	5.7	0.122
	8.0	2.4	0.289
	Organic content		
	Pesticide: Humic acid		
	(pH: 8.0, Temperature: 28±2 ^{0}C)		
	1:0.1	1.0	0.693
	1:1	0.5	1.386
	1:100	0.3	2.310
Soil	Uttarkashi (pH: 5.1)	7.7	0.090
	Roorkee (pH: 6.8)	5.4	0.128
	Sriganganagar (pH: 8.1)	4.6	0.151
Plants	Tomato fruit	10.3	0.067
	Radish leaf	11.3	0.061
	Radish root	5.8	0.120

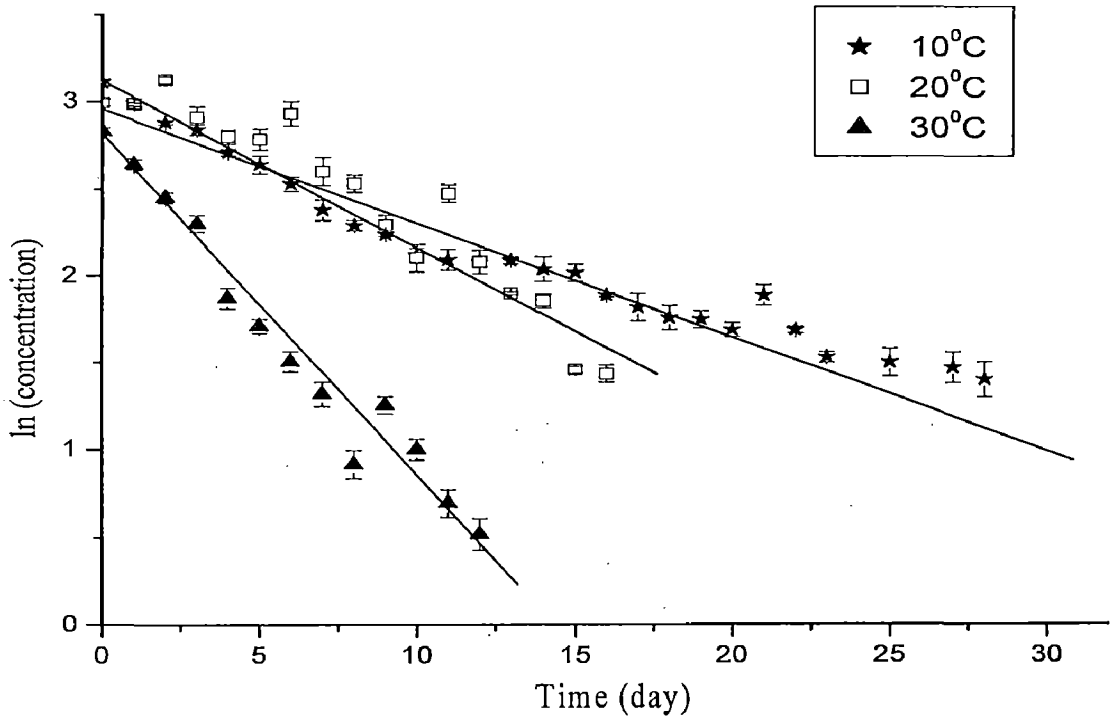


Figure 4.1: Decay of thiram in water at different temperatures (pH: 6.3)

4.3.2 Effect of pH

In order to examine the effect of pH on the rate of degradation of thiram, decay studies were carried out at pH 5.0, 6.0, 7.0 and 8.0 in water at 28±2 ^{0}C (Fig. 4.2). The half life of thiram is shorter than quinalphos. Therefore, it was planned to extend the studies to a more acidic range by including pH 5.0. The results of the similar study conducted on soil at pH 5.1, 6.8 and 8.1 are depicted in Figure 4.3. In both the cases the data plotted on a natural log scale give straight lines indicating that the degradation of the pesticide obeys the first order rate equation. It is apparent from the results (Table 4.1) that both in water and soil the decay rate increases with the increasing pH. The results of the effect of pH both in water and soil fall in line with the observations made by earlier workers on malathion and methyl parathion [35, 36].

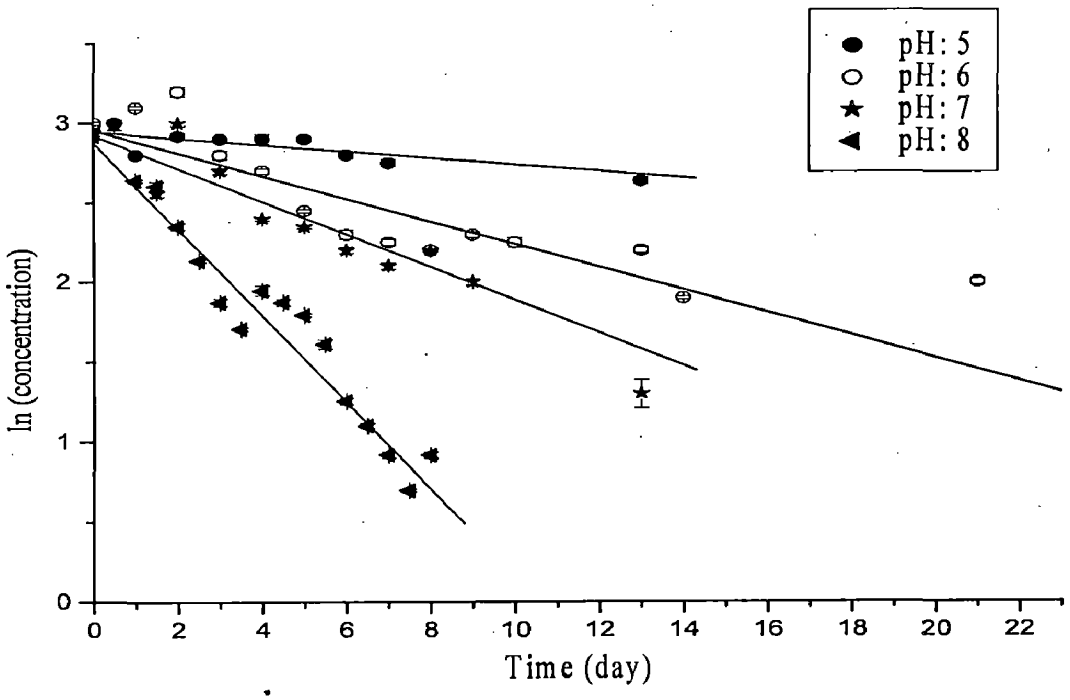


Figure 4.2: Decay of thiram in water at different pH (Temperature: 28±2 ^{0}C)

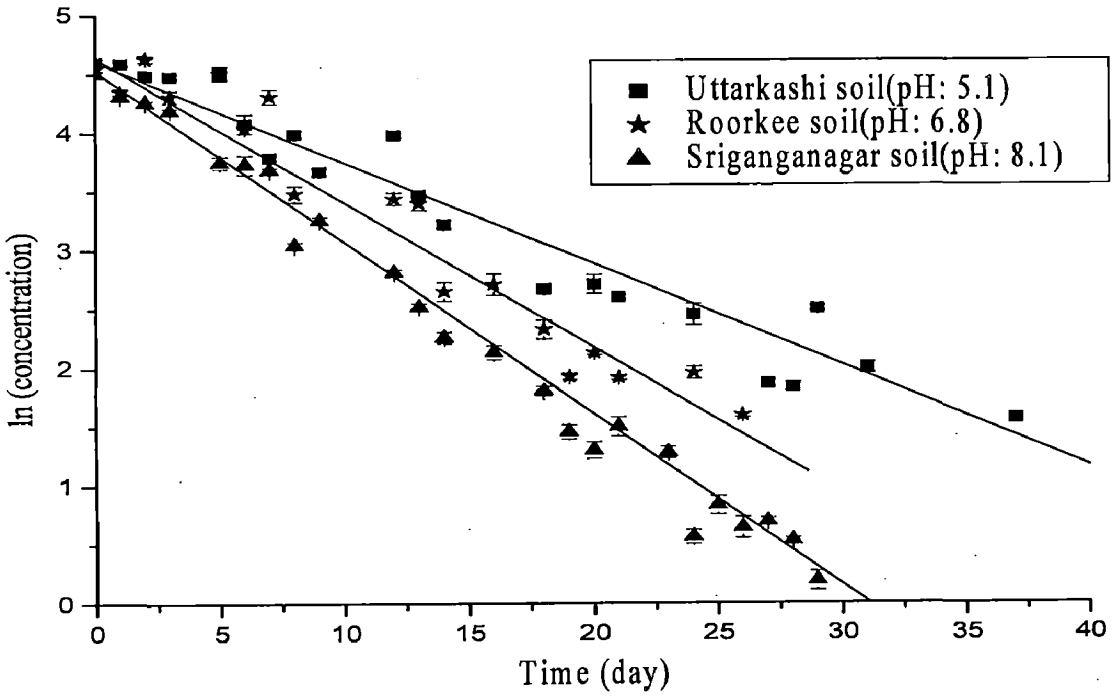
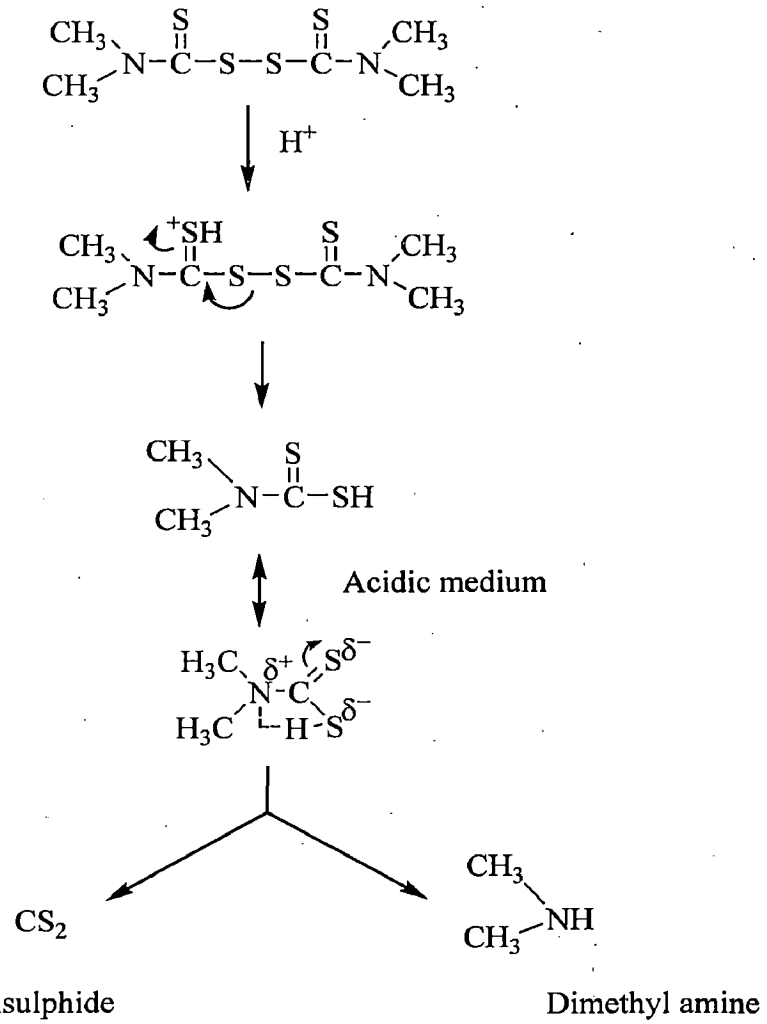
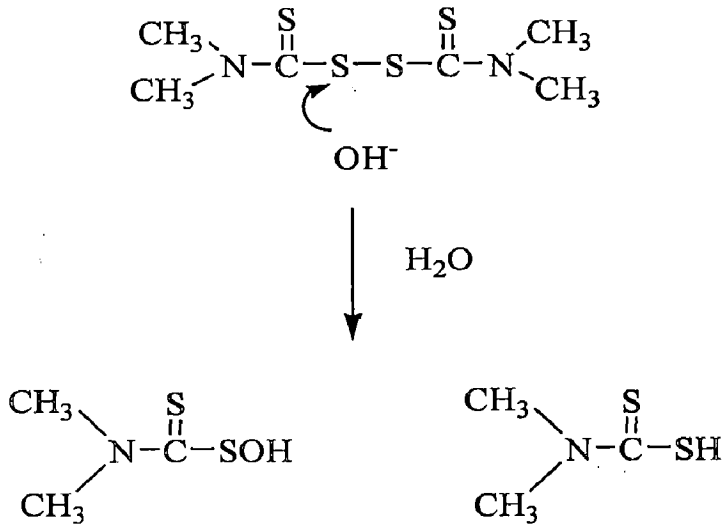


Figure 4.3: Decay of thiram in soils of different pH (Temperature: 28±2 ^{0}C)

In alkaline condition, cleavage of S-S bond takes place by the attack of OH^- ion on one of the sulphur atom while in acidic condition the protonation of S atom of C=S takes place. In both the conditions rupture of S-S bond occurs resulting in the formation of DMDTC. In thiram moiety S-S bond is the weakest bond and more prone to hydrolysis. The probable mechanisms are depicted below:-



In acidic medium



In alkaline medium

The faster kinetics in alkaline medium may be attributed to the fact that with the increase in OH^- ion concentration the probability of rupture of S-S bond in the moiety of thiram increases [18]. Dimethyldithiocarbamate thus formed is stable in alkaline medium [37]. In the acidic medium protonation of the dimethyldithiocarbamate takes place resulting into an intramolecular hydrogen bond between N and one of the S atoms. Repulsion between the fractional positive charge developed on N and C leads to cleavage of N-C bond forming dialkyl amine and CS_2 [38].

On comparing the $t_{1/2}$ of thiram in deionised water and soil at pH 5 and 8 it is observed that in acidic pH the persistence of thiram in water is almost four times more than that in soil of a similar pH. But in alkaline pH the persistence of thiram in water is half as compared to the soil of a similar pH. The data further indicate that the effect of pH is more discernible in the case of deionised water than soil thus pointing out that the decay of pesticide in the soil is very complex and may be governed by the different factors namely moisture, organic content and microbial activity other than H^+ ion concentration.

4.3.3 Effect of humic acid

The effect of humic acid concentration on the decay profile of thiram in water at pH 8.0 is shown in Figure 4.4. The studies indicate that the presence of humic acid increases the rate of decay (Table 4.1) as it acts as a reducing agent. The results thus reveal that higher the organic content the lower is the persistence of thiram.

4.3.4 Effect of environmental conditions

The decay behaviour of thiram on tomato (fruit) and different parts of radish (leaf and root) has been investigated (Fig. 4.5). The decay studies on thiram in the field conditions were planned for a month. But in the case of tomato fruit and radish root the studies could not be carried out for a month because it was difficult to keep the tomato fruit and radish root intact for a longer time in normal conditions. The half life of the pesticide on tomato fruit and radish leaf is almost the same but a faster decay rate is observed in the case of radish root. A similar decay rate on tomato and radish leaf can be explained by the fact that after the pesticide is sprayed on them, the solvent gets evaporated and the pesticide remains reasonably in a dry state under similar environmental conditions. It may be important to point out that the faster decay at the root is due to the availability of some moisture in the soil with which it comes in contact. Moreover, here the contribution of the microbial activity of the soil cannot be ignored. Similar trend of decay on leaves and roots has been observed in the case of quinalphos and another carbamate, carbofuran [39].

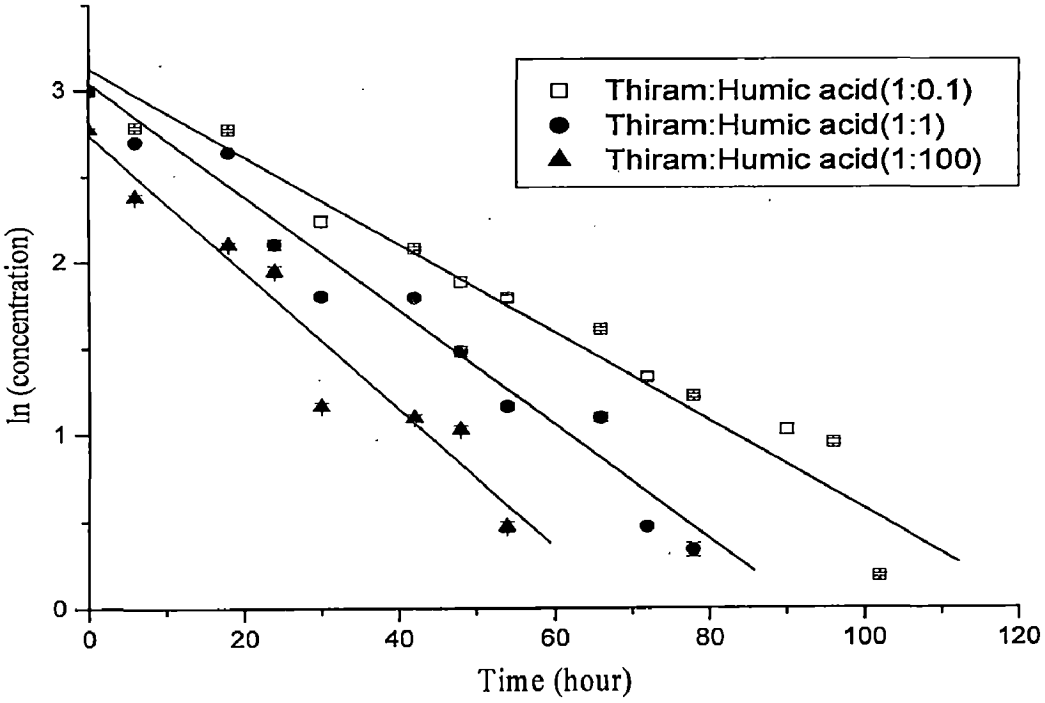


Figure 4.4: Decay of thiram in water at different humic acid concentrations (pH: 8.0, Temperature: 28±2 ^{0}C)

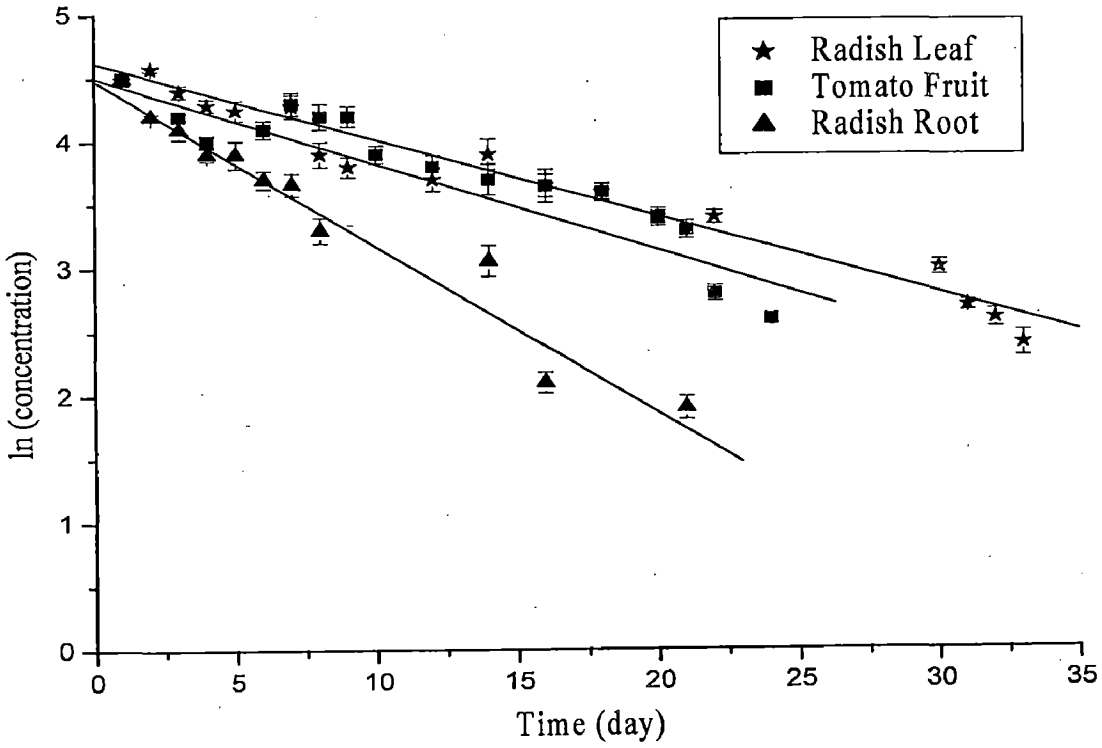


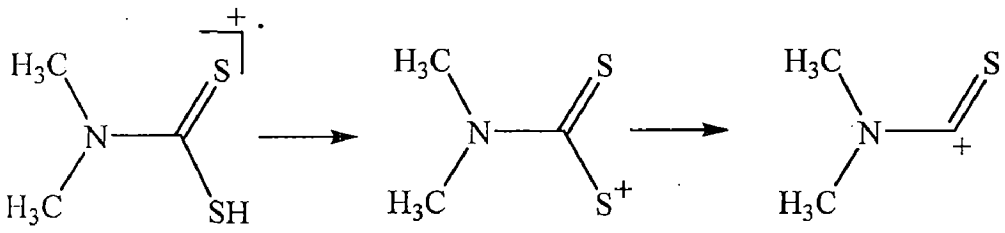
Figure 4.5: Decay of thiram on different parts of the plants

4.3.5 Identification of Metabolites

The metabolites of thiram were identified in water (pH 6.0 and 8.0), soil (pH 5.1 and 8.1) and on/in plants. All the spiked samples with thiram were kept under the ambient conditions and withdrawn at different time intervals, extracted and analysed by LC-MS for the identification of the degradation products. In the present study we observed that M^+ peaks are prominent and $([M+H]^+$ peaks are either very small or absent. The criterion of selecting the days for withdrawing the sample was based on the half lives in different matrices. The proposed metabolic pathways of thiram in three different matrices are depicted in Figure 4.6. This is based on the identification of different metabolites as discussed in the following text. Fragmentation pathways of various metabolites proposed in the following text are based on mass spectrometric fragmentations peaks.

4.3.5.1 Identification of degradation products in water at pH 8.0

The degradation of thiram is fast in alkaline medium as compared to that in acidic medium. Thus, the water samples were collected on 2nd, 7th and 10th day. The LC-MS analysis of sample, withdrawn on 2nd day of storage, shows the formation of two products which were subjected to further MS. The mass spectrum of first product has a molecular ion peaks at m/z 121 along with a fragment ion peak at m/z 88 (Fig. 4.7). It has been tentatively identified as dimethyl dithiocarbamate (II) formed due to hydrolysis (cleavage of S-S bond).



II, m/z 121 m/z 120 m/z 88

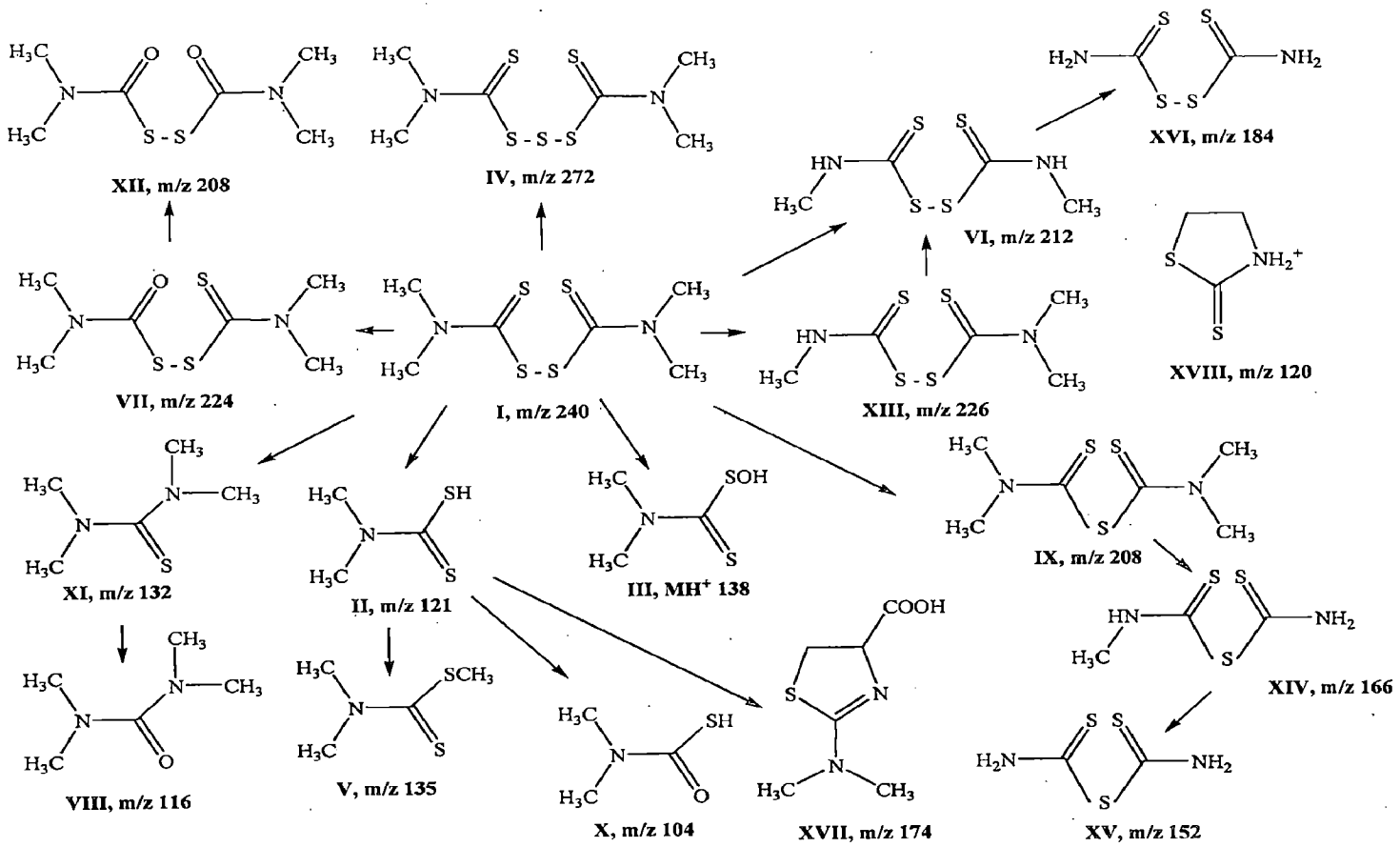
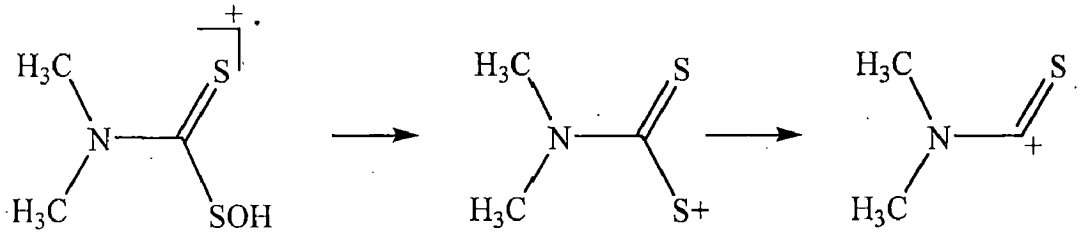


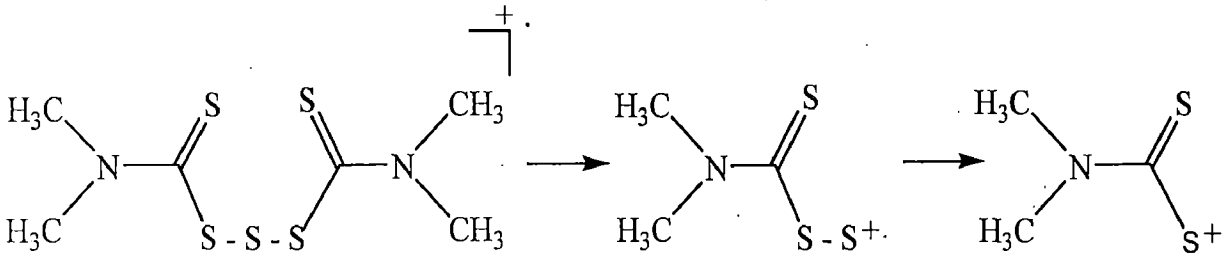
Figure 4.6: Degradation products of thiram identified in water, soil and plants by LC-MS

The second product has a molecular ion peak at m/z 138 (MH+) with fragment ion peaks at m/z 120 and 88 (Fig. 4.8). Based on the mass spectrum the product is tentatively identified as dimethyl dithiocarbamoylsulfenic acid (III).



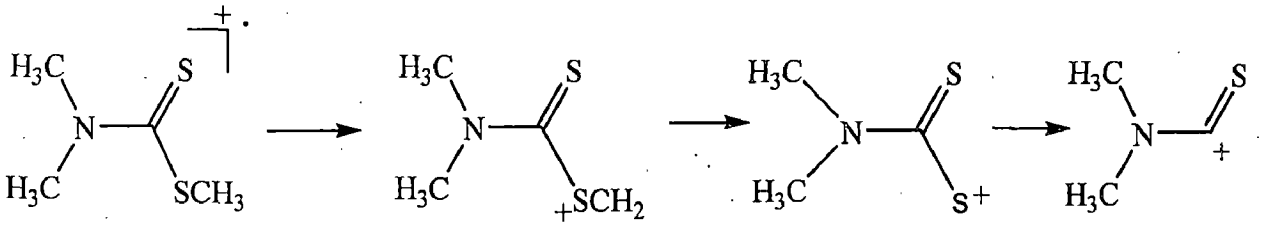
III, MH+ 138 m/z 120 m/z 88

The water sample withdrawn after 7th day when analyzed by LC-MS shows the formation of two more products with molecular ion peaks at m/z 272 and 135 in addition to II and III. The first product with a molecular ion peak at m/z 272 and fragment ion peaks at m/z 120 and 152 is identified as bis (dimethyl dithiocarbamoyl) trisulphide (IV) (Fig. 4.9).



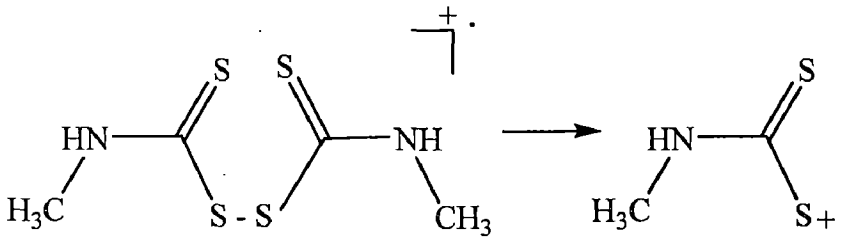
IV, m/z 272 m/z 152 m/z 120

Another product with a molecular ion peak at m/z 135 and fragment ion peaks at m/z 134, 120 and 88, has been tentatively identified as methyl dimethyldithiocarbamate (V) (Fig. 4.10).



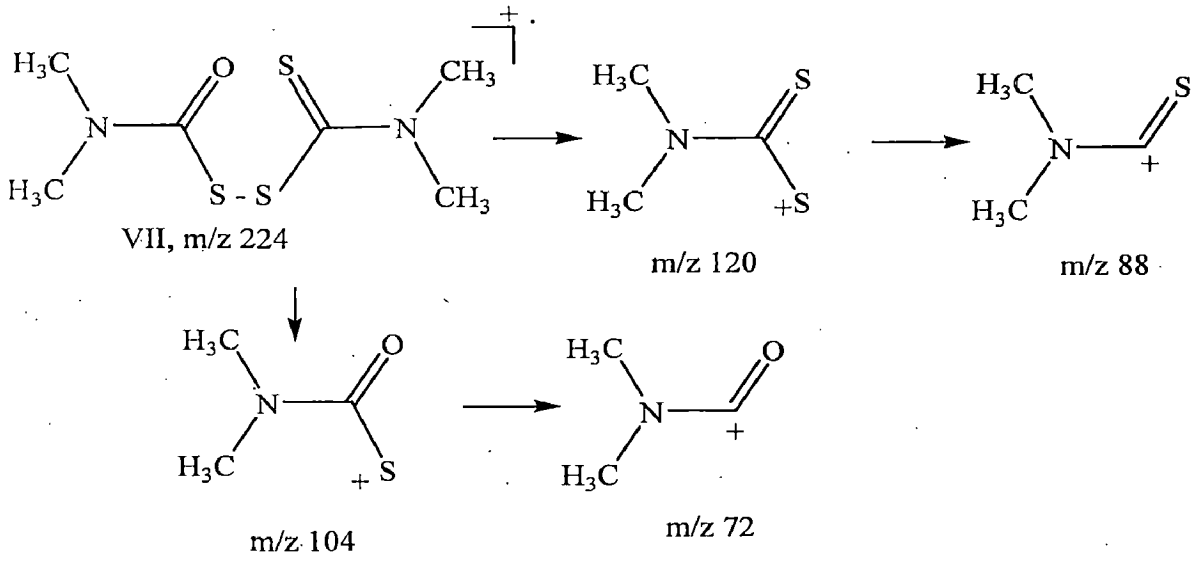
V, m/z135 m/z 134 m/z 120 m/z 88

The 10th day sample, besides II, III, IV and V, shows six new products. The first product has a molecular ion peak at m/z 212 and a fragment ion peak at 106 (Fig. 4.11). This has been identified as bis (methyl dithiocarbamoyl) disulphide (VI) formed by N-dealkylation.

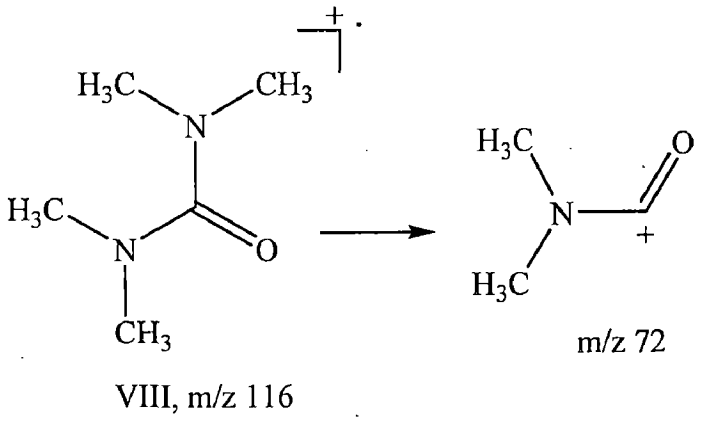


VI, m/z 212 m/z 106

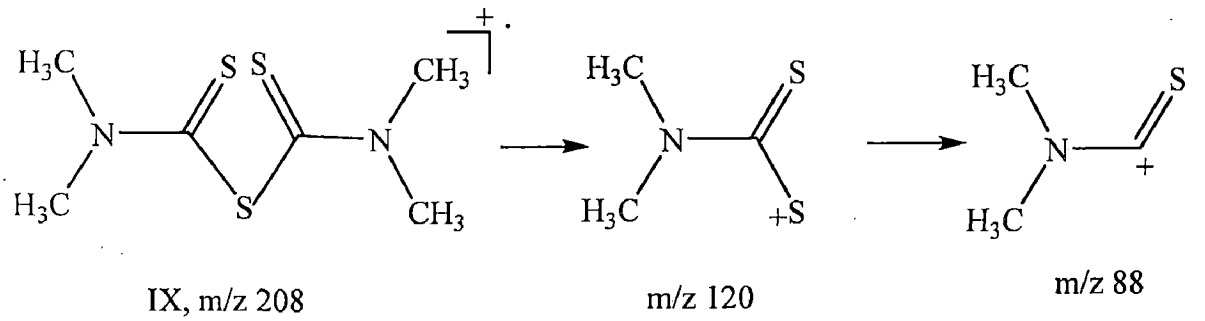
The second product with a molecular ion peak at m/z 224 and fragment ion peaks at 120, 104, 88 and 72, is supposed to be identified as N,N-dimethyl carbamoyl-N,N-dimethyl thiocarbamoyl disulphide (VII) (Fig. 4.12). This product may be formed by oxidation of C=S to C=O.



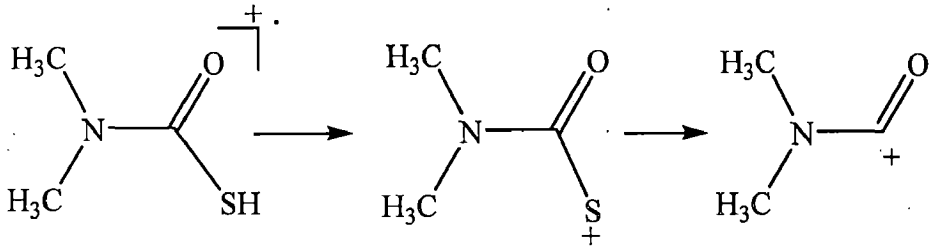
The third product has a molecular ion peak at m/z 116 and a fragment ion peak at m/z 72 (Fig. 4.13). This has been identified as 1, 1, 3, 3-tetramethylurea (VIII, TMU).



The fourth product has a molecular ion peak at m/z 208 and fragment ion peaks at 120 and 88 (Fig. 4.14). It can be proposed as tetramethylthiuram monosulfide (IX, TMTM).

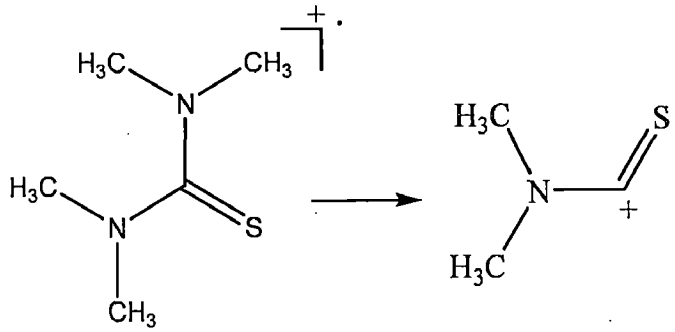


The fifth product has a molecular ion peak at m/z 104 with a fragment ion peak at m/z 72. (Fig. 4.15) This has been identified as dimethylcarbamo (thioperoxic) acid (X).



X, m/z 105 m/z 104 m/z 72

The sixth product has a molecular ion peak at m/z 132 and a fragment ion peak at 88. This can be attributed to the structure of 1,1,3,3-tetramethyl-2-thiourea (**XI,** TMTU).

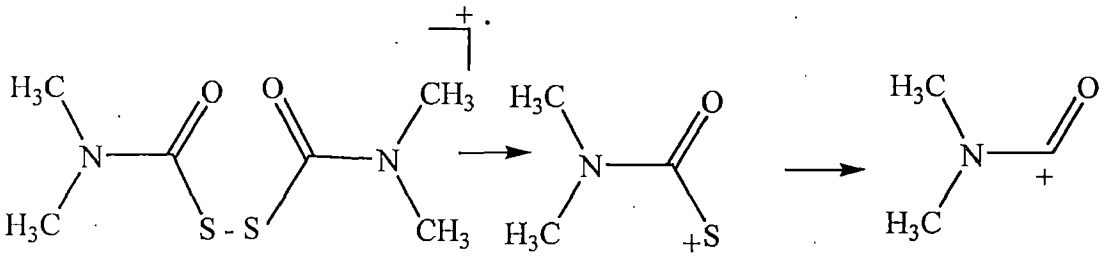


XI, m/z 132 m/z 88

4.3.5.2 Identification of degradation products in water at pH 6.0

The degradation of thiram was also studied in water at pH 6.0. It is slower in acidic medium than in the alkaline medium. The samples were withdrawn on 2^{nd}, 7^{th}, 11^{th}, 21^{st} and 30^{th} day. The samples withdrawn on 2^{nd} and 7^{th} day shows the presence of thiram only which indicates towards the persistence of thiram in acidic conditions. The sample withdrawn on 11^{th} day shows the formation of two products II and IV. The 21^{st} day sample shows the presence of VI and VII. The 30^{th} day sample, besides VI, VII, VIII, IX, X and XI, shows a new product

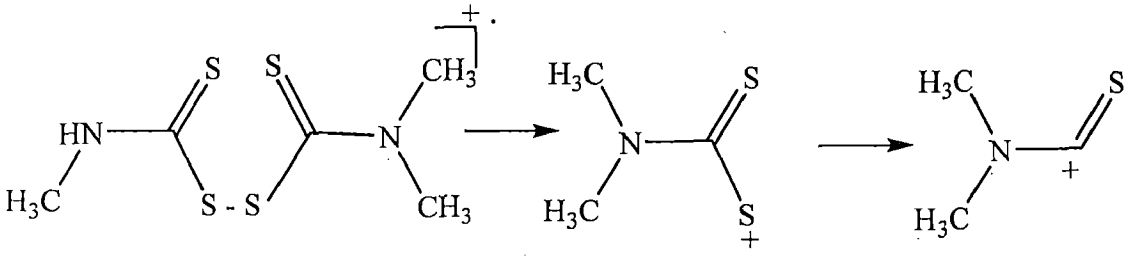
with a molecular ion peak at m/z 208 along with fragment ion peaks at m/z 104 and 72 (Fig. 4.16). It is proposed as bis(dimethyl carbamoyl) disulphide (XII).



XII, m/z 208 m/z 104 m/z 72

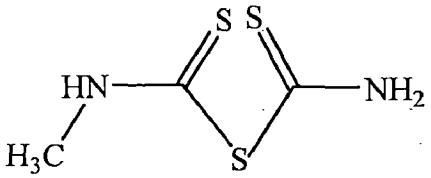
4.3.5.3 Identification of degradation products in soil (pH 8.1)

The soil spiked with thiram was incubated at the room temperature and the samples collected on 5th, 10th and 15th day were analyzed for the identification of degradation products. The 5th day sample shows the formation of II as the major product and VI as the minor product. It also exhibits a new product with a molecular ion peak at m/z 227 and fragment ion peaks at m/z 120 and 88 (Fig. 4.17). It was tentatively identified as N-methyl-N,N-dimethyl dithiocarbamoyl-disulphide (XIII) formed due to N-dealkylation.

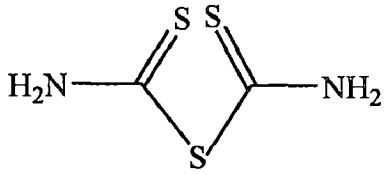


XIII, m/z 226 m/z 120 m/z 88

The soil sample withdrawn on 10th day, besides the presence of II, IV, VI, VII and XII, shows the presence of two new products with molecular ion peaks at m/z 166 and m/z 152. The former is identified as N-methyl-amino-dithiocarbamoyl sulphide (XIV) and the latter as bis (thiocarbamoyl) sulphide (XV).

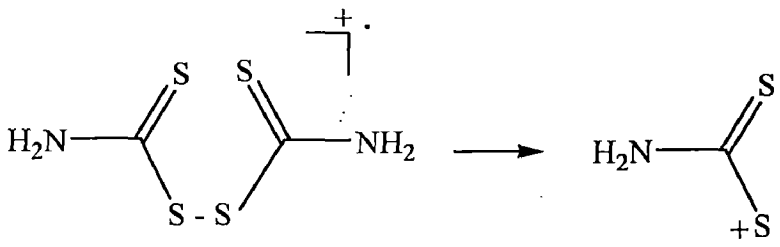


XIV, m/z 166 (M^+)



XV, m/z 152 (M^+)

The 15th day sample, besides VIII, IX, X and XI, shows a new product with a molecular ion peak at m/z 184 and a fragment ion peak at m/z 92 (Fig. 4.18). It is tentatively identified as bis (dithiocarbamoyl) disulphide (XVI).



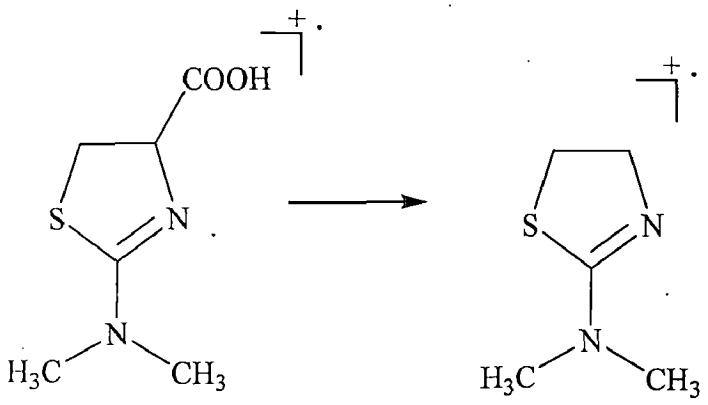
XVI, m/z 184 m/z 92

4.3.5.4 Identification of degradation products in soil (pH 5.1)

Samples were taken on 8th, 16th and 24th days from the soil spiked with thiram. The 8th day sample shows the formation of II, IV and XV. The 16th day sample shows the presence of XII and XIV. The 24th day sample shows the formation of only XVI.

4.3.5.5 Identification of degradation products on leaf surface

The samples of radish leaves were collected on 11th and 33rd day after spotting. The 11th day crushed sample shows the formation of IV, XIV, XV, XVI and a new cyclized product. This new product has a molecular ion peak at m/z 174 and a fragment ion peak at m/z 130 (Fig. 4.19). Based on the mass spectrum, it is tentatively identified as 2(N,N-dimethyl amino)thiazoline carboxylic acid (XVII).



XVII, m/z 174 m/z 130

The 33rd day crushed sample shows the formation of only one major product which is proposed to be XVI. The 11th day sample of radish leaf obtained by surface washout in a suitable solvent shows the formation of II, IV, XIII, XIV, XV, XVI and XVII. The 33rd day sample shows the presence of only II.

4.3.5.6 Identification of degradation products on radish root

The samples of radish root were collected on 5th and 15th day after spotting the pesticide. Both the crushed and washed samples were processed. The 5th day crushed sample of radish root shows the formation of II mainly, whereas 15th day sample shows the formation of a new product along with IV, VI, XII and XVII. The mass spectrum of the new product shows a molecular ion peak at m/z 120 (Fig. 4.20). A prominent peak at m/z 120 is obtained in the case of dimethyl dithiocarbamate (II) also (Fig. 4.7). But the fragmentation pattern of the two is different. Based on this the new product is proposed to be 2-thioxo-4-thiazolidine (XVIII). The 5th day washout sample of radish root shows the formation of only IV and the 15th day sample shows the formation of XII and XIV.

4.3.5.7 Identification of degradation products on tomato fruit

Tomato fruits were spotted with a solution of thiram at the fruiting stage and the samples were collected on 10th and 30th day after spotting. The 10th and 30th day crushed samples of

tomato show the formation of II, IV, XIV, XV, XVI and XVII. The 10^{th} day washout sample of tomato fruits exhibits the formation of IV, XV and XVI while the 30^{th} day sample shows peaks due to II and XV only.

The identified degradation products can be rationalized as originating by hydrolysis (II, III), oxidation(VII, VIII, X, XII), N-dealkylation (VI, XIII, XIV, XV, XVI), S-methylation (V), sulfuration (IV), desulfuration (IX), cyclization (XVII, XVIII) and photodegradation (XI). The degradation product dimethyl dithiocarbamate (II) may be formed due to cleavage of disulphide bond (S-S) of thiram. Thiram and its metabolites undergo oxidation of C=S to C=O to give products such as VII, VIII, X and XII. Thiram can also undergo cleavage at C-S bond to form N,N-dimethyl dithiocarbamoyl moiety and N,N-dimethyl thiocarbamoyl moiety with the release of a sulphur atom [37]. These two moieties combine together to form bis(dimethyl dithiocarbamoyl) sulphide (IX), which further undergoes N-dealkylation to give XIV and XV. The product III, formed by conjugation of N, N-dimethyl dithiocarbamoyl moiety with hydroxyl radical, has been identified only in water at the pH 8.0. Product (XI) is formed by conjugation of N,N-dimethyl thiocarbamoyl and dimethyl amine moieties, obtained by photodegradation of thiram(I) [37].

On the basis of the metabolites obtained it appears that the metabolite formation in each matrix is initiated by hydrolysis which occurs at S-S bond. The cleavage of S-S bond results in the formation of dimethyl dithiocarbamate (II). It is interesting to note that product II is observed at initial stages and persists upto the end in the alkaline conditions. In the acidic medium cleavage of N-C bond of the dimethyl dithiocarbamate takes place resulting into the formation of dimethyl amine and CS_2. Dimethyl dithiocarbamate and CS_2 are mainly responsible for the hepatotoxicity of thiram [8].

In the soil sample of pH 8.1 dealkylated products can be noticed after first half life itself and observed upto third half life. In water sample of pH 8.0 the peak of dealkylated product appears only after third half life (Fig. 4.21). These results suggest that N-dealkylation is not so prominent in water and is favoured in soil. A perusal of data further indicates that oxidation products are observed mainly in water and soil but are mostly absent in plants. The exception is XII which appears in root and that too at a certain point. This suggests that in water and soil hydrolysis is followed by oxidation and dealkylation but in plants oxons are unstable. On a similar line cyclized metabolites are observed only in plants i.e., radish leaf and root and tomato fruit. This suggests that cyclization is favoured in the presence of plant juices or enzymes.

The data on half lives of pesticide summarized in Table 4.1 suggests that the decay of thiram is faster with the increase in temperature, pH and organic content in the investigated range. The decay profiles in all these cases follow a first order kinetics. From the field studies, it is apparent that the pesticide is more stable in the absence of water. It may not be very logical to compare the persistence of thiram with other well known pesticides because most of the data cited on the subject is conditional. However, a fairly good idea about the degree of persistence of the pesticide can be had from the range of half lives under different controlled laboratory conditions. In this regard thiram is more stable than the other carbamates namely mancozeb ($t_{1/2}$=1-3 days at pH 7.0 at 25 ^{0}C) [40] and carbofuran ($t_{1/2}$= 4 days on root) [39]. If one compares the thiram with the two well known organophosphorus pesticides, malathion ($t_{1/2}$=5.4 days at pH 6.3 in soil at 20 ^{0}C) and methyl parathion ($t_{1/2}$=6.2 days at pH 6.3 in soil at 20 ^{0}C) [36] the average half life of thiram is slightly more. A perusal of data thus indicates that the decay of thiram depends upon the nature of medium and the environmental conditions. Therefore large scale variations are observed in the data on the decay profile. For any

meaningful interpretation of results it is important to generate a database on the decay pattern of pesticide under controlled and field conditions simultaneously as the laboratory results cannot be necessarily extrapolated to the field conditions.

During the process of identification of degradation products of thiram in water, soil and plants (radish leaves, roots and tomato fruits) a number of metabolites are identified at different time intervals. The metabolic pathways seem to be complex, consisting of hydrolysis, oxidation, dealkylation, sulfuration, desulfuration, cyclization and photodegradation processes. The pattern of metabolite formation both in water and soil is more or less similar but the cyclized products appear in plants only. The persistence and the nature of the metabolites formed is not the same in different matrices because of different chemical environment present there. The identification of different metabolites in these matrices can act as a data bank for forensic science experts for the identification of thiram as a source of poisoning. Of the different metabolites formed, dimethyl dithiocarbamate and oxons seem to persist for a longer time period and are reported to be highly toxic [8, 36]. The metabolites 2(N,N-dimethyl amino)thiazoline carboxylic acid and 2-thioxo-4-thiazolidine identified in radish leaf and root and tomato fruit are also reported to be toxic and have been identified in the rat metabolism also [41]. The said metabolites independently or synergistically enhance the toxicity of the pesticide. The information on toxicity of many other metabolites formed is not available. The possibility of their contribution towards the toxicity cannot be ignored. These studies reflect that the pesticide thiram can leave its imprints of toxicity even after its substantial decay over a long period of time.

The contents of this chapter have been published or accepted in the form of two research papers in the journals **"Biomedical Chromatography"** and **"Journal of Environmental Science and Health Part B"**.

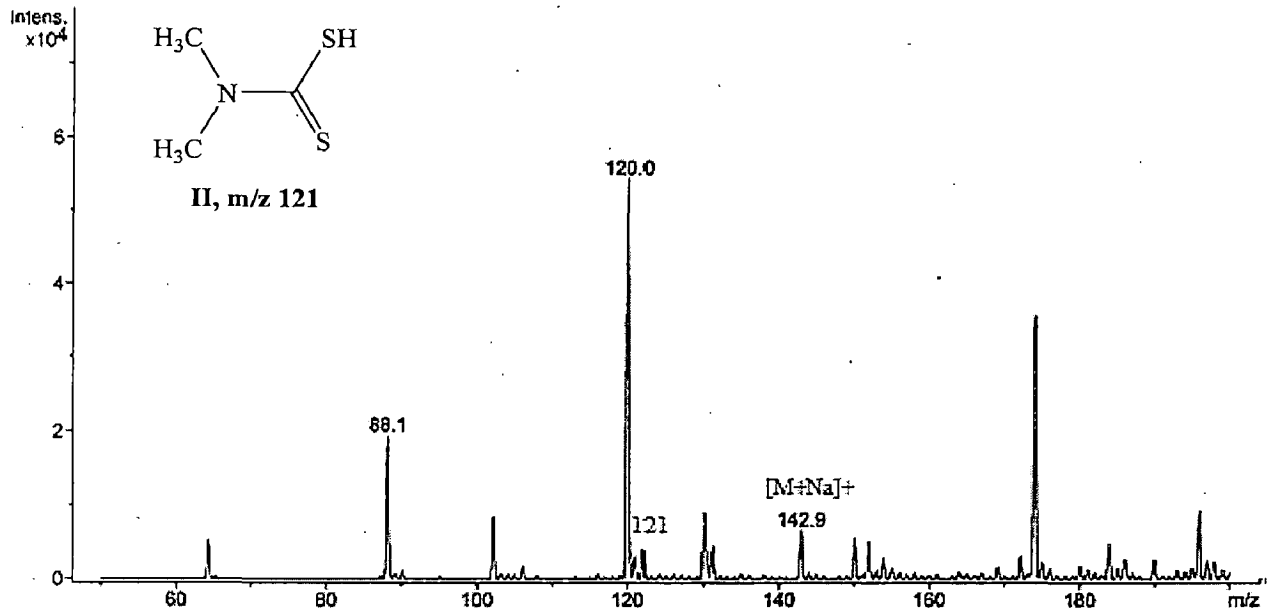


Figure 4.7: Mass spectrum of dimethyl dithiocarbamate (II)

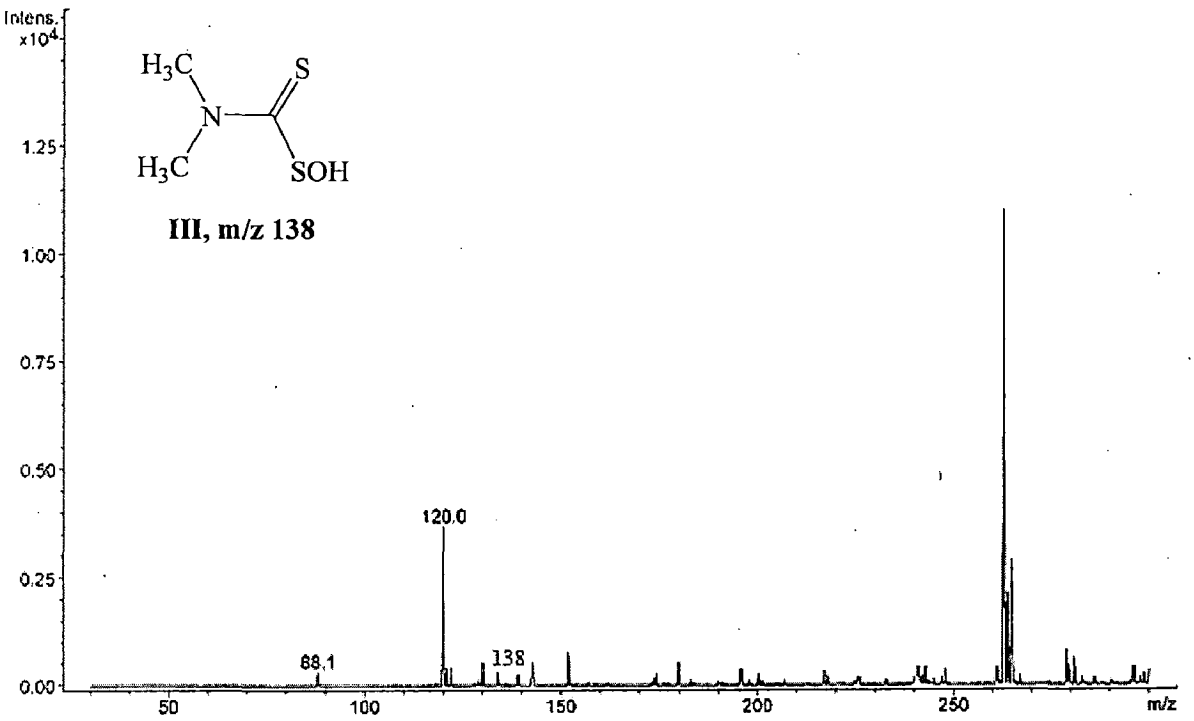


Figure 4.8: Mass spectrum of dimethyl dithiocarbamoylsulfenic acid (III)

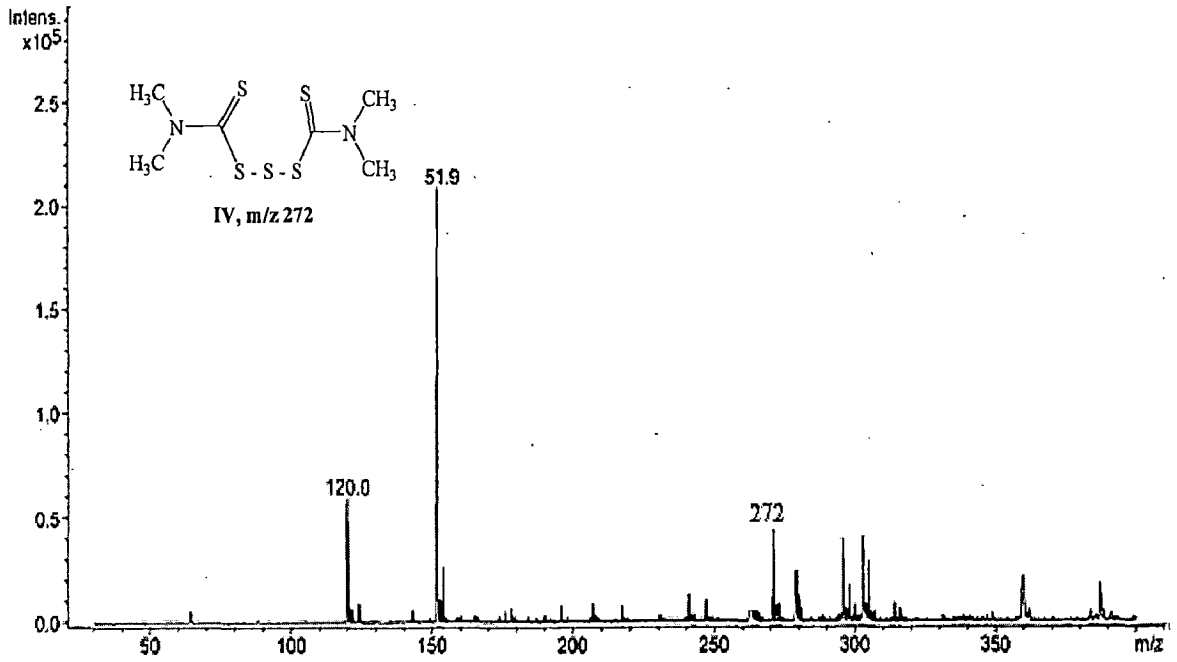


Figure 4.9: Mass spectrum of bis (dimethyl dithiocarbamoyl) trisulphide (IV)

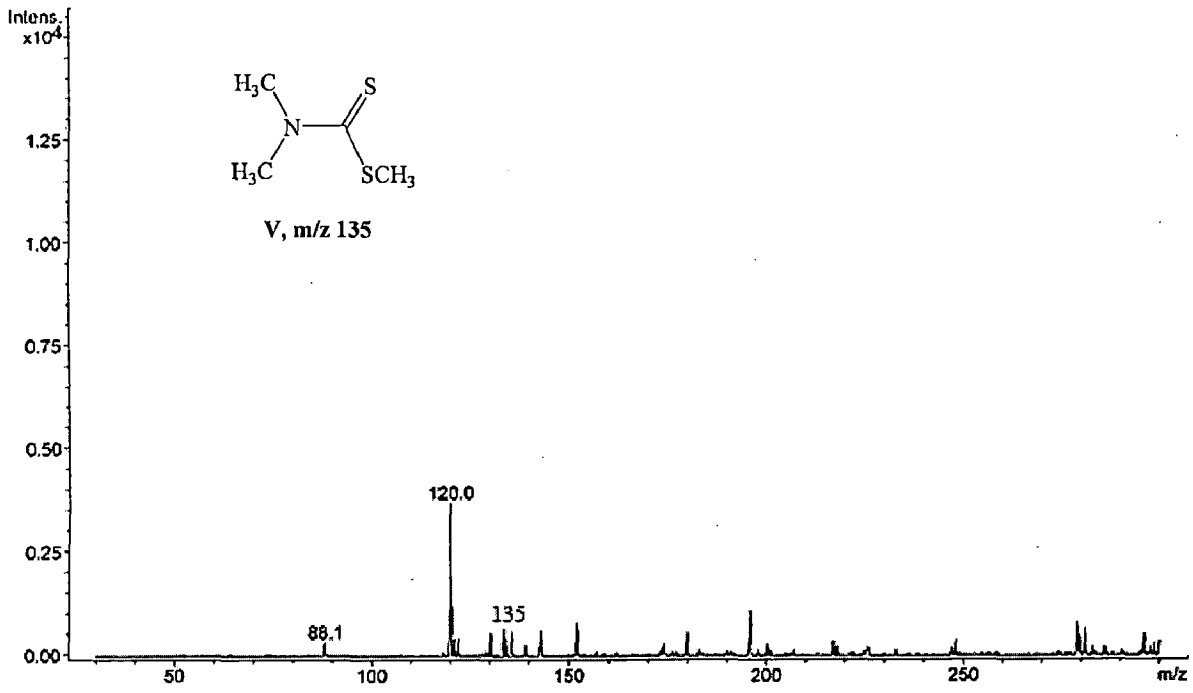


Figure 4.10: Mass spectrum of methyl dimethyldithiocarbamate (V)

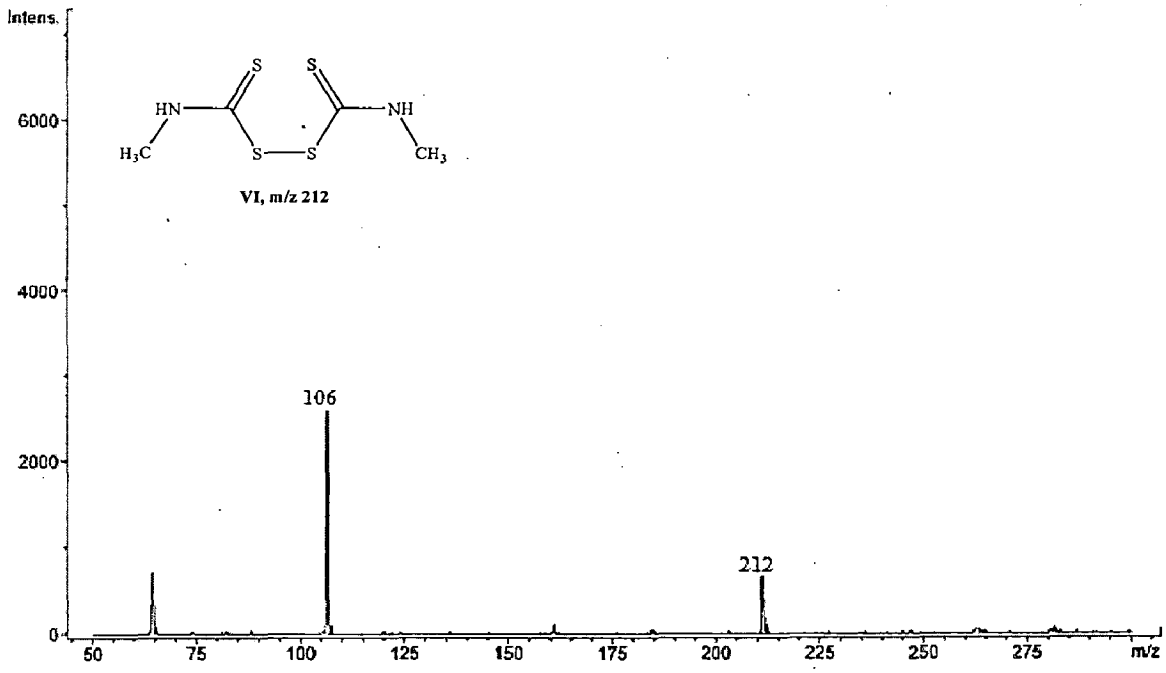


Figure 4.11: Mass spectrum of bis (methyl dithiocarbamoyl) disulphide (VI)

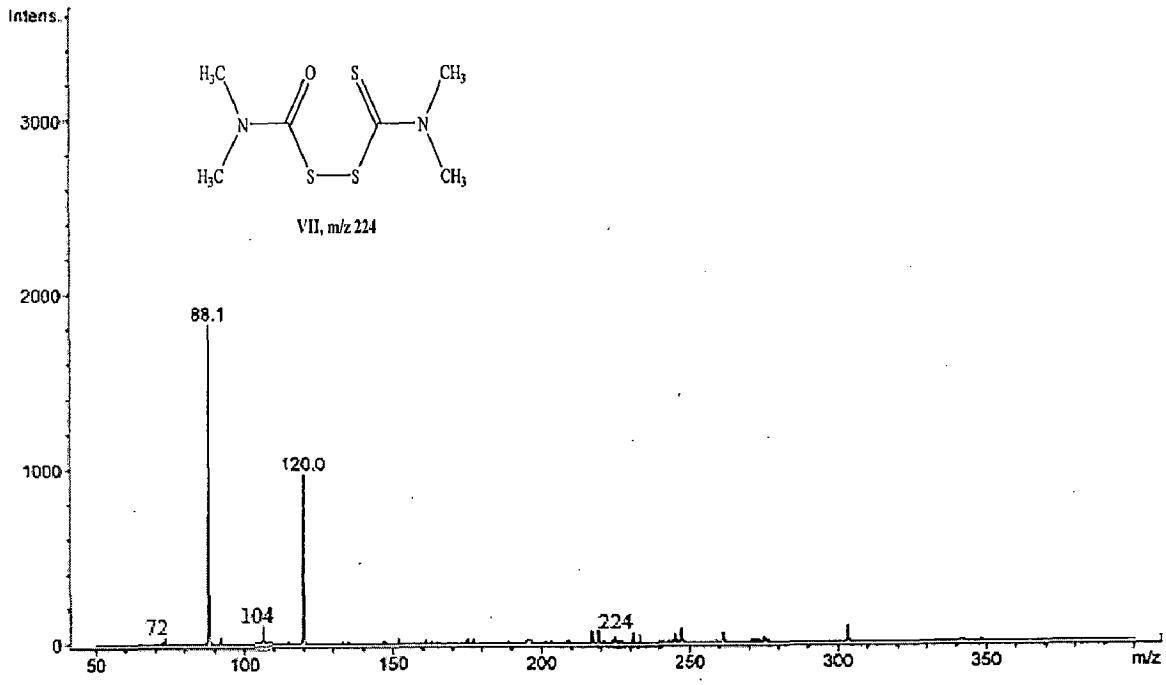


Figure 4.12: Mass spectrum of N, N-dimethyl carbamoyl-N, N-dimethyl thiocarbamoyl disulphide (VII)

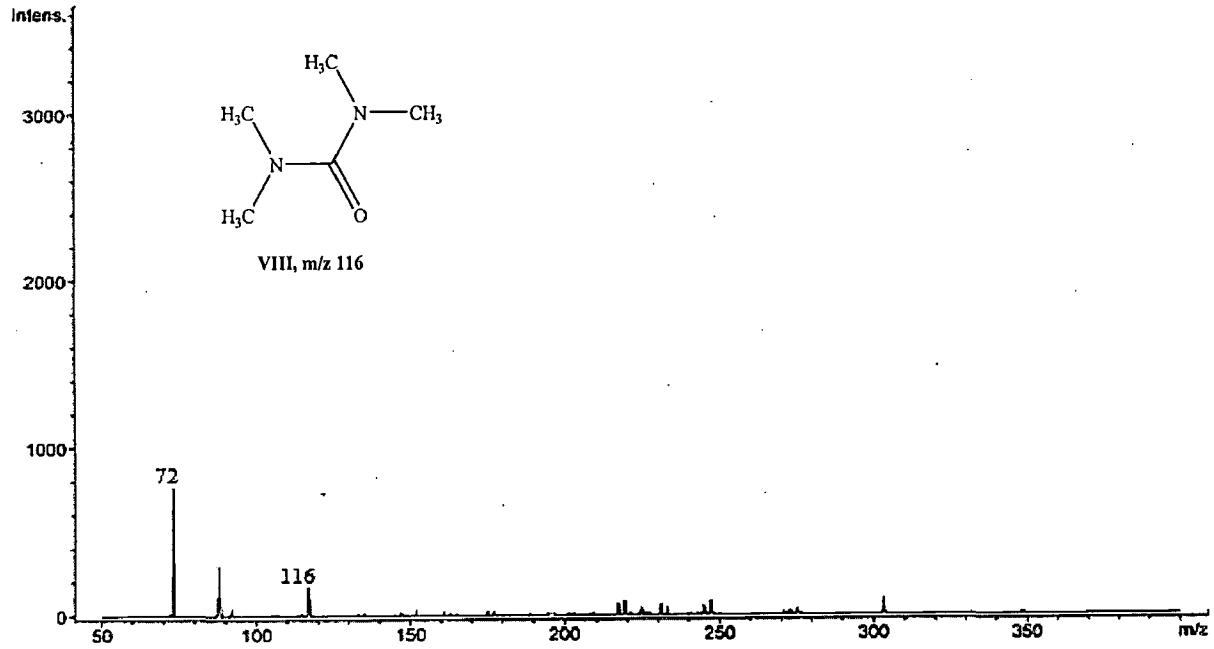


Figure 4.13: Mass spectrum of 1, 1, 3, 3-tetramethylurea (VIII)

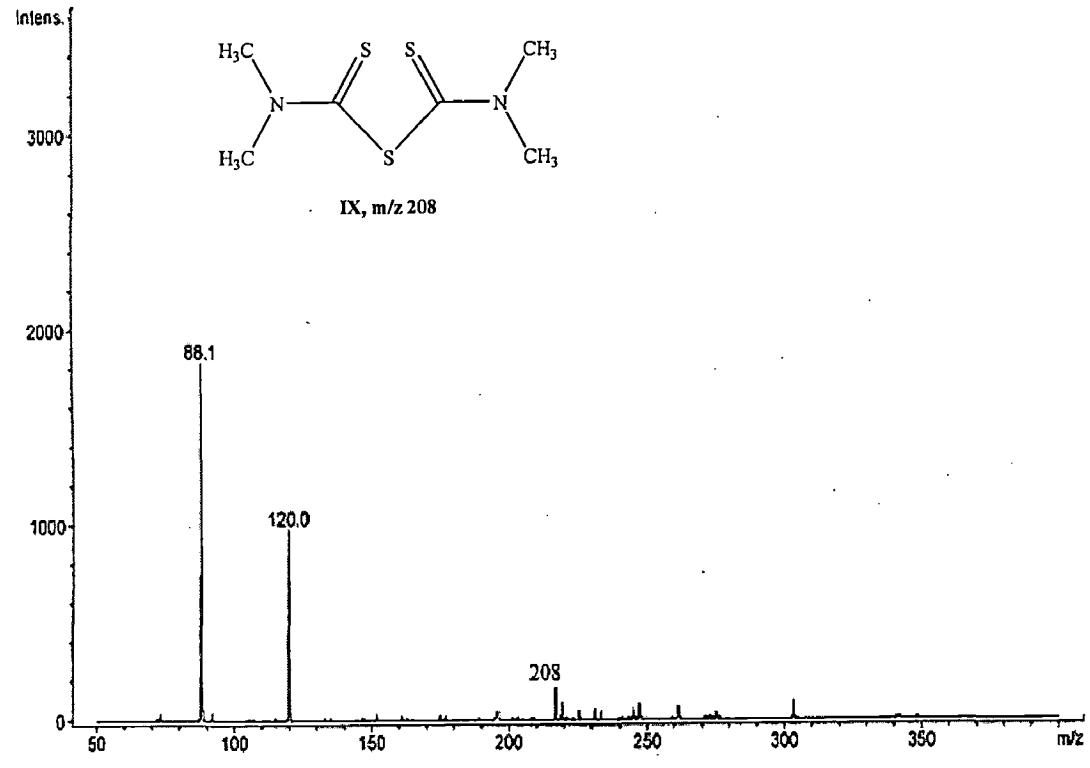


Figure 4.14: Mass spectrum of tetramethylthiuram monosulfide (IX)

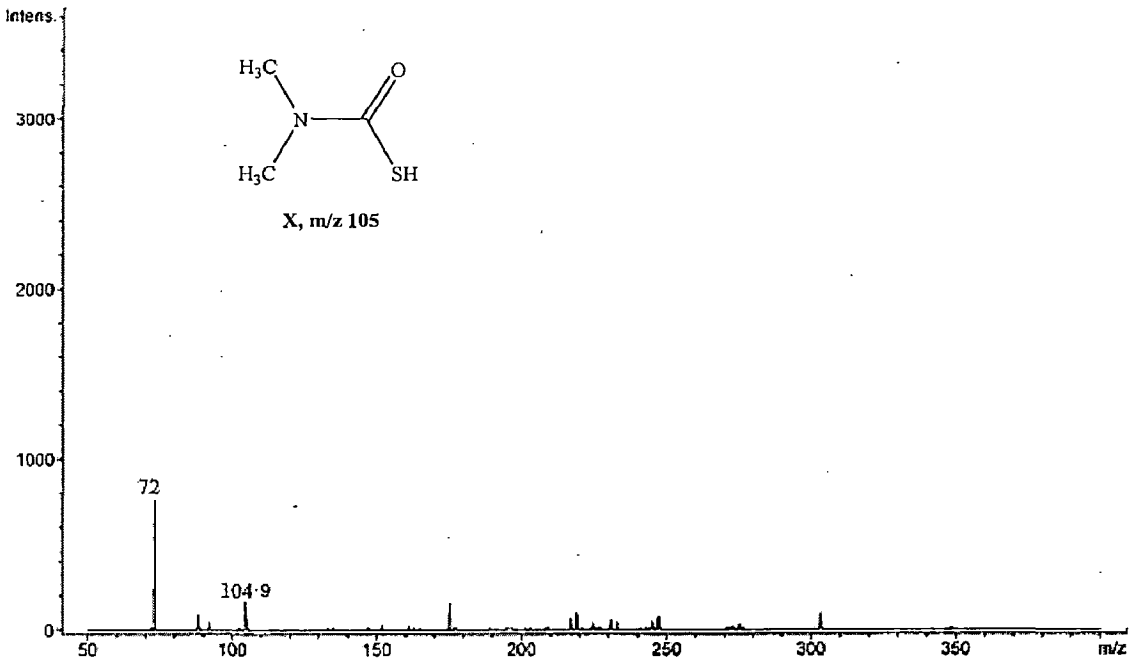


Figure 4.15: Mass spectrum of dimethylcarbamo (thioperoxic) acid (X)

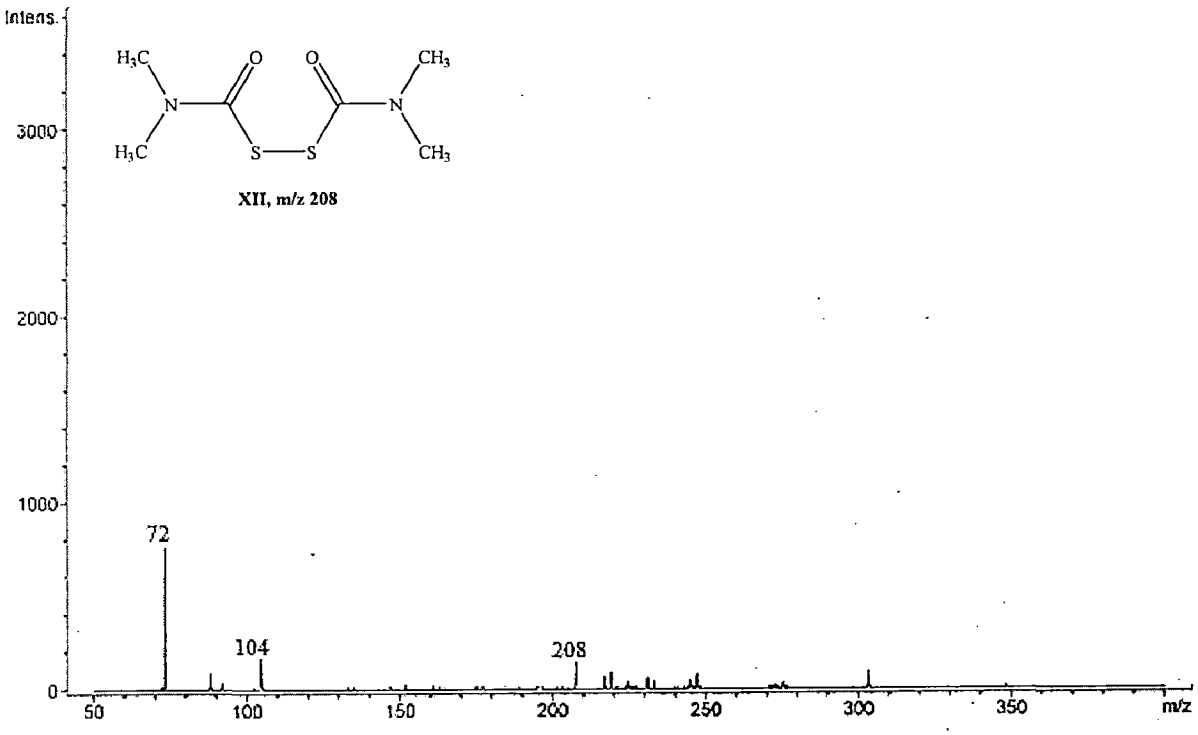


Figure 4.16: Mass spectrum of bis(dimethyl carbamoyl) disulphide (XII)

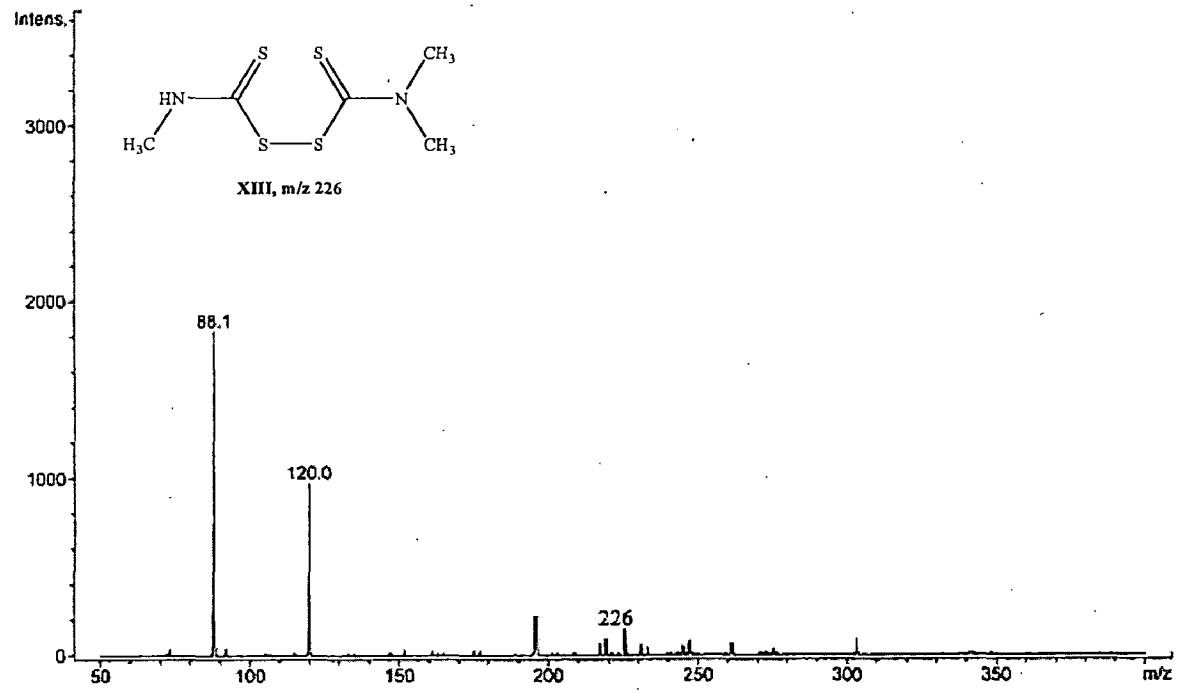


Figure 4.17: Mass spectrum of N-methyl-N, N-dimethyl dithiocarbamoyl-disulphide (XIII)

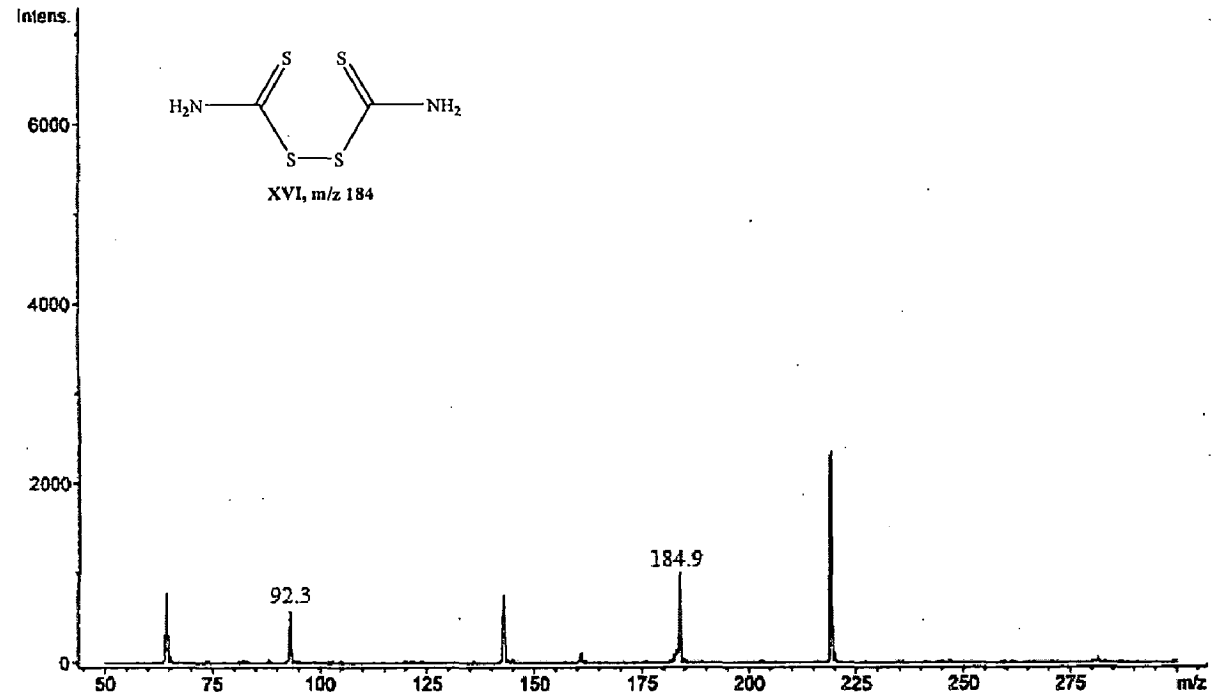


Figure 4.18: Mass spectrum of bis (dithiocarbamoyl) disulphide (XVI)

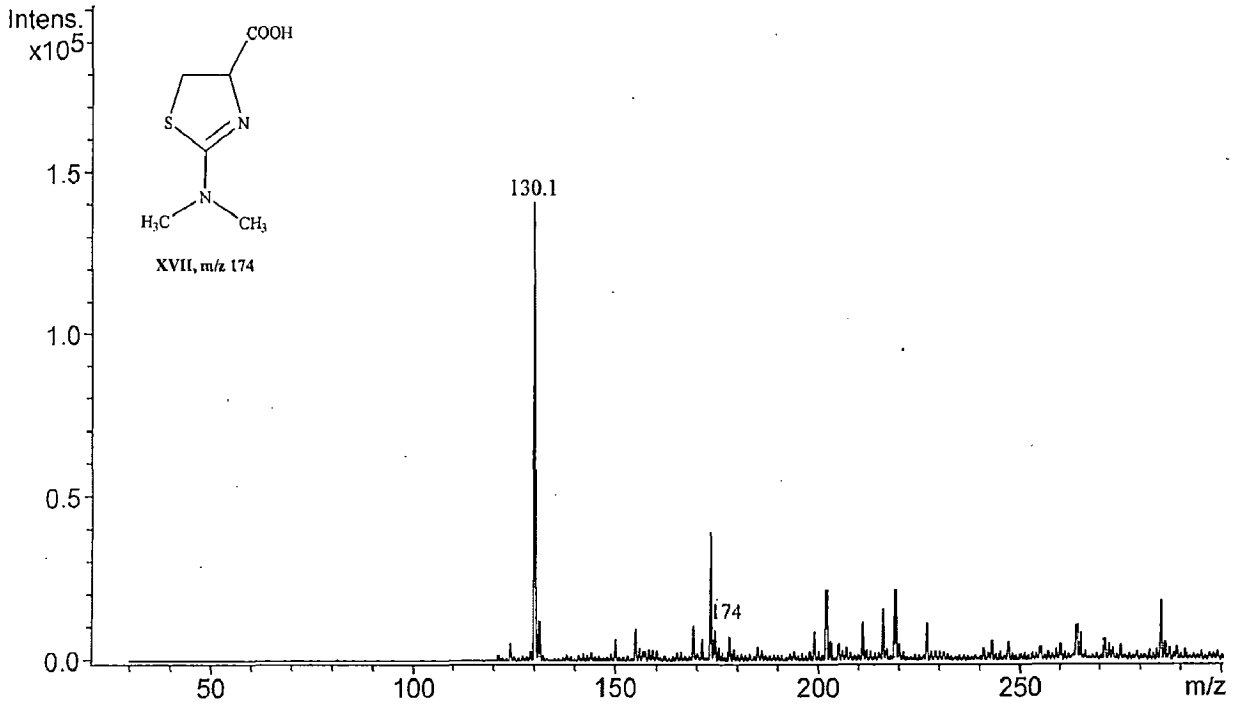


Figure 4.19: Mass spectrum of 2(N, N-dimethyl amino)thiazoline carboxylic acid (XVII).

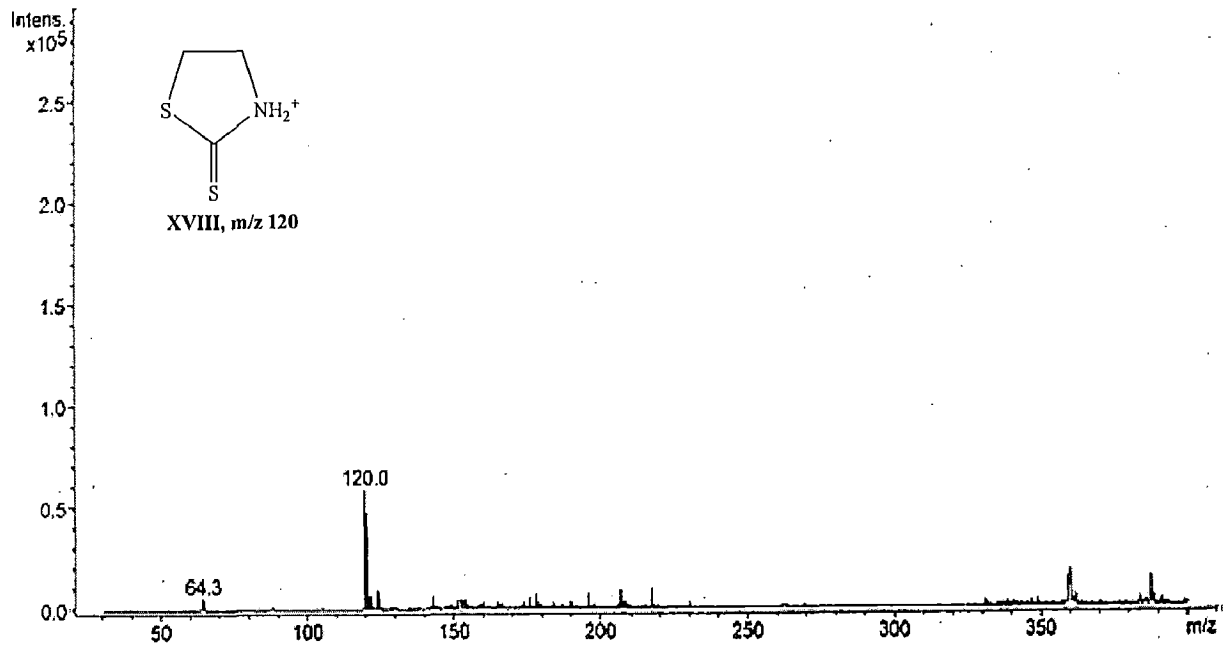


Figure 4.20: Mass spectrum of 2-thioxo-4-thiazolidine (XVIII).

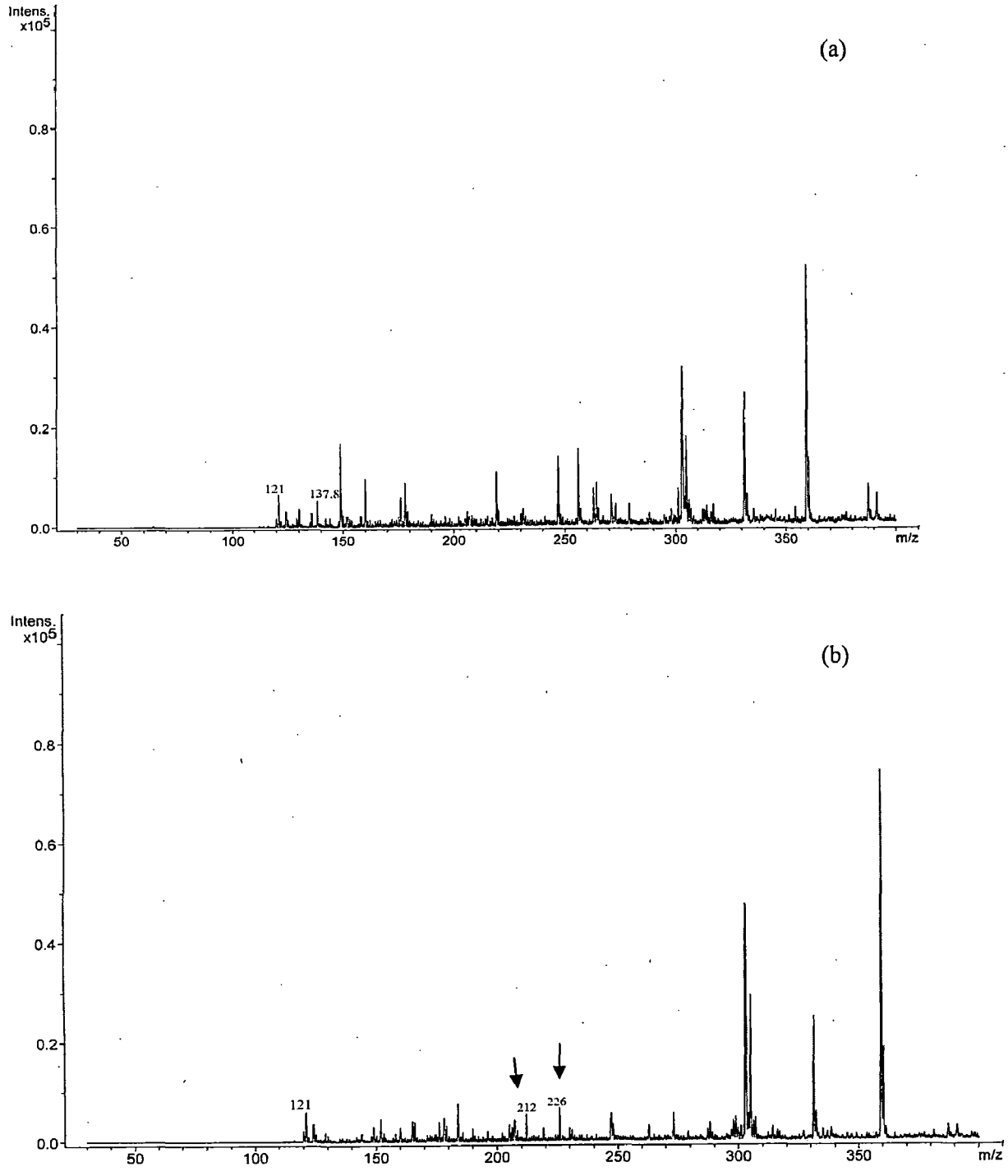


Figure 4.21: Mass spectra of thiram in (a) water (pH 8.0) and (b) soil (pH 8.1) after 1st half life (arrows indicate the peaks due to dealkylated products)

4.4 References

[1]. Ezemonye, L. I. N., Ikpesu, T. O. and Ilechie, I., "Distribution of diazinon in water, sediment and fish from warri river, niger delta Nigeria", Jordan Journal of Biological Sciences (JJBS) **1**, 77 (2008).

[2]. IARC working group, "Thiram. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans", **53**, 403 (1991).

[3]. Sharma, V. K., Aulakh, J. S. and Malik, A. K., "Thiram: degradation, application and analytical methods", J. Environ. Monit. **5**, 717 (2003).

[4]. Fernandez, C., Reviejo, A. J., Polo, L. M. and Pingarron, J. M., "HPLC-Electrochemical detection with graphite-poly (tetrafluoroethylene) electrode: determination of the fungicides thiram and disulfiram", Talanta **43**, 1341(1996).

[5]. U.S. National Library of Medicine, "Hazardous substances data bank", Bethesda, MD, **4** (1995).

[6]. Maita, K., Tsuda, S. and Shirasu Y., "Chronic toxicity studies with thiram in Wistar rats and beagle dogs", Fund. Appl. Toxicol. **16**, 667 (1991).

[7]. Elskens, M. T., and Penninckx, M. J., "Thiram and dimethyldithiocarbamic acid interconversion in Saccharomyces cerevisiae: a possible metabolic pathway under the control of the glutathione redox cycle", Appl. Environ. Microbiol. **63**, 2857 (1997).

[8]. Dalvi, P. S., Wilder-Kofie, T., Mares, B., Dalvi, R. R. and Billup, L. H., "Toxicological implications of the metabolism of thiram, dimethyldithiocarbamate and carbon disulfide mediated by hepatic cytochrome P450 isozymes in rats", Pest. Biochem. Physiol. **74**, 85 (2003).

[9]. Gay, M. H., Norris, K. J., Nomeir, A. A., Markham, P. and McManus, J. P., "Metabolism of orally administered ^{14}C-thiram in rats", Project 8767, 8833, 8839, 8926A, 9049.

Biotek, Inc. Analytical Development Corp. Arthur D. Little, Inc. Uniroyal Chemical Co Inc., Unpublished. (1992).

[10]. Norris, K. J., "Identification and comparison of urinary metabolite profiles of 14C-thiram in subchronicvs single oral dose in rats", Project 1113. Analytical Development Corporation (ADC), USA, Unpublished. (1991).

[11]. Franekic, J., Bratulic, N., Pavlica, M. and Papes, D., "Genotoxicity of dithiocarbamates and their metabolites", Mutat. Res. **325**, 65 (1994).

[12]. Agrawal, R.C., Shukla, Y. and Mehrotra, N. K., "Assessment of mutagenic potential of thiram", Food Chem. Toxicol. **35**, 523 (1997).

[13]. Ardito, G., Bigatti, P. and Lamberti, L., "Increased frequencies of sister chromatid exchanges and micronuclei in *'in vitro'* lymphocyte cultures treated with the fungicides thiram and ziram", Boll. Soc. Ital. Biol. Sper. **73**, 1 (1997).

[14]. Cereser, C., Boget, S., Parvaz, P. and Revol, A., "Thiram-induced cytotoxicity is accompanied by a rapid and drastic oxidation of reduced glutathione with consecutive lipid peroxidation and cell death", Toxicology **163**, 153 (2001).

[15]. Thakare, S. R. and Bhave, N. S., "Photocatalytic degradation of thiram (fungicides) under visible light irradiation", E- J. Chem. **2**, 62 (2005).

[16]. Haque, M. M. and Muneer, M., "Photocatalysed degradation of a fungicide, thiram in aqueous suspension of titanium dioxide", Indian J. Chem. Technol. **12**, 68 (2005).

[17]. Niitsuma, T., Suzuki, K., Hachiya, E. and Takemoto, Y., "Degradation of six pesticides by ozone with ultraviolet radiation", Kankyou Kagaku. **3**, 350 (1993).

[18]. Kaneco, S., Li, N., Itoh, K., Katsumata, H., Suzuki, T. and Ohta, K., "Titanium dioxide mediated solar photocatalytic degradation of thiram in aqueous solution: Kinetics and mineralization", Chem. Eng. J. **148**, 50 (2009).

[19]. Crank, G. and Mursyidi, A., "Photochemistry of some organosulfur pesticides", J. Photochem. Photobiol. Chem. **68**, 289 (1992).

[20]. Ohto, M., Yamamoto, A., Matsunaga, A., Takayanagi, N., Kemmei T., Saito, Y., Mizukami, E., "Chemical changes of pesticides used for golf links in chlorinated water", KankyouKagaku. **3**, 59 (1993).

[21]. Kodama, S., Yamamoto, A., Ohto, M. and Matsunaga, A., "Major degradation pathway of thiuram in tap water processed by oxidation with sodium hypochlorite", J. Agric. Food Chem. **47**, 2914 (1999).

[22]. Gao, S., Song, Y., Wang, L. and Jiang, B., "Study on the screening and characterization of special effective bacteria of degrading thiuram in the water body", Diqiu Kexue Ban. **36**, 455 (2006).

[23]. Priyantha, N. and Weliwegamage, S., "Amperometric method for trace level determination of the fungicide, thiram", J. Natl. Sci. Found. Sri Lanka, **33**, 17 (2005).

[24]. Priyantha, N. and Weliwegamage, S., "Interaction of thiram with glassy carbon electrode surfaces under applied potentials conditions", Int. J. Electrochem. Sci. **31,** 25 (2008).

[25]. Howard, P. H., "Handbook of environmental fate and exposure data for organic chemicals: Pesticides", Lewis Publishers, Chelsea, MI, p.4 (1989).

[26]. U.S. Department of Agriculture, Soil Conservation Service. SCS/ARS/CES Pesticide Properties Database: Version 2.0 (Summary), Syracuse, NY, (1990).

[27]. Czarnik, W., Dabrowski, J., Sadlo, S., Pelz, A., Srzedzinska, A. and Pilecka, D.,"Effects of growth conditions on the dynamics of mancozeb and thiuram disappearance in lettuce", Materialy Sesji Naukowej Instytutu Ochrony Roslin (Poznan) **20**, 307 (1980).

[28]. Li, H., Yang, R., Jiang, W. and Wang, Z., "Residue dynamics of thiram in rice and paddy soil", Nongyao Kexue Yu Guanli **30**, 26 (2009).

[29]. Norris, K. J., Wilkes, L. C., McManus, J. P. and Putterman, G.J., "Hydrolysis of thiram in three buffers", Book of Abstracts, 211th ACS National Meeting, New Orleans, LA, March 24-28 (1996).

[30]. Vyas, S. C. and Nene, Y. L., "Degradation of thiram of treated seed in storage", JNKVV Research 7, 181(1973).

[31]. Vuik, J., Van Dinter, R. and De Vos, R. H., "Improved sample pretreatment of the carbon disulfide evolution method for the determination of dithiocarbamate residues in lettuce", J. Agric. Food Chem. **40**, 604(1992).

[32]. Malik, A. K., Seydel, B. S. and Faubel, W., "Capillary electrophoretic determination of disodium ethylene bisdithiocarbamate (nabam) and sodium diethyldithiocarbamate (NADDC)", Int. J. Environ. Anal. Chem. 7, 51 (1999).

[33]. Heise, S., Weber, H. and Aldder, L., "Reasons for the decomposition of the fungicide thiram during preparation of fruit and vegetable samples and consequences for residue analysis", Fresenius. J. Anal. Chem. **366**, 851 (2000).

[34]. Remya, N. and Lin, J. G., "Carbofuran degradation by the application of MW-assisted H_2O_2 process", J. Environ. Sci. Health B. **46**, 350 (2011).

[35]. Miles, C. J., Takashima, S., "Fate of malathion and O, O, S-Trimethyl phosphorothioate by-product in Hawaiian soil and water", Arch. Environ. Contam. Toxicol. **20**, 325 (1991).

[36]. Kaur, I., Mathur, R. P. and Tandon, S. N., "Parameters affecting the decay of pesticide- a study by high performance liquid chromatography", Biomed. Chromatogr. **11**, 22 (1997).

[37]. Roberts, T. R. and Hutson, D. H., "Metabolic pathways of agrochemicals: herbicide and plant growth Part1", Royal Society of Chemistry, London, Great Britain, p. 849 (1998).

[38]. Joris, S. J., Aspila, K. I. and Chakrabarti, C. L., "Decomposition of monoalkyl dithiocarbamates", Ana. Chem. **42**, 647 (1970).

[39]. Menzie, C. M., "Metabolism of Pesticides; an update", Washington, D.C.:U.S. fish and wildlife service. 486 (1974).

[40]. U. S. Environmental Protection Agency, "Pesticide fact sheet: mancozeb", Office of Pesticides and Toxic Substances, Washington, DC, USA, p. 4 (1987).

CHAPTER 5

IN VITRO AND *IN VIVO* STUDIES ON DEGRADATION OF QUINALPHOS IN RATS

5.1 Introduction

The *in vitro* and *in vivo* identification of the pesticides and their metabolites in the biological fluids provides an important biomarker of the exposure risk of the pesticides to the population and the occupational workers. The toxicity of a pesticide basically depends upon its chemical nature and persistence in the specified environment. The toxicity of its decay products is equally significant. It is a known fact that some times the metabolites are more toxic than the parent compound. Therefore, before accepting a pesticide for use its metabolites should be properly identified and their toxicity evaluated. The formation of the metabolites is susceptible to the nature of the medium. The information on the decay products can also be of great value in tracing the pathways of the pesticide. Besides the use as agrochemicals the pesticides are the known homicidal and suicidal agents because of their easy availability. According to a WHO report published in 1990 [1] worldwide about three million people annually consume these agrochemicals resulting into 2,20,000 deaths. About 99% of these deaths occur in the developing countries. Later, in 2007 Gunnell et al. [2] reported that there are 3,00,000 deaths annually by intentional pesticide poisoing in Asia. The exact data on the incidence of poisoning in India are not officially available. It is estimated that about 39% of the total cases of poisoning occur in India and nearly 10,000 people die every year. The various monitoring programmes need to fulfil the obligation to know as to what is the behaviour of a pesticide in the human system and what are the effects of its byproducts? In the absence of reliable information on the decay products sometimes problems are encountered by forensic experts to identify the source of poisoning. The requirement of generating a data bank of metabolites for forensic and epidemiological investigations is well known. By identifying the metabolites in the excreta or stomach wash it may be easier to detect the original pesticide,

even if it has decayed substantially.

Organophosphates have a prominent position as agrochemicals and are easily available in the market without any restriction. Their use as a poisoning agent has become a common practice [3, 4]. As observed respiratory failure is the most common complication of the poisoning. In the third world countries like India because of social and ethical reasons and delayed response of concerned vigilance authorities, there is a time lapse and invariably the causative pesticide may be available only in the traces in the system. It may only be possible to identify the original pesticide with the help of byproducts formed with the passage of time.

Various organophosphates pesticides are reported to be the common resource of poisoning in India [5-7]. Kumar et al. [8] have published a noteworthy review on the management of common poisoning sources in India. They focused on two types of poisoning in India, one with agrochemicals and the other with pharmacological agents i.e. drugs. Palimar and coworkers [9] have investigated more than 150 cases of organophoshorous poisoning in Karnataka (India) from 2001 to 2002. According to them most of the cases were of suicide. Methyl parathion was the common compound consumed (37.9%) while quinalphos contributed 7%. Esquivel-Senties et al. [10] evaluated the effects of diethyldithiophosphate, a metabolite of organophosphorous pesticide, on the human CD4+ T lymphocytes and found that it induces changes in the activation status of the human lymphocytes by modulating the interleukin 2 receptor signal transduction pathway. The research group of Salazar-Arredondo [11] studied the sperm chromatin alteration and DNA damage in the human spermatozoa by methyl parathion, chlorpyrifos, diazinon and their oxon metabolites. They suggested that oxons are 15 percentage to 10 times more toxic to sperm DNA than their corresponding parent compounds. Eyer along with coworkers [12] reported extreme variability in the formation of

chlorpyrifos oxon in patients poisoned by chlorpyrifos and found that difference in the clinical outcomes and the response to antidotes in patients with an acute poisoning may occur due to inter-individual variability in the metabolism. Bicker et al. [13] investigated a case of acute human chlorpyrifos poisoning and identified the bioactivated intermediate chlorpyrifos oxon and other fifteen metabolites of chlorpyrifos. The three distinct biotransformation routes of chlorpyrifos are also proposed. The study further gives a detailed view on the biotransformation of chlorpyrifos and adds novel aspects to the human metabolism of this xenobiotic at a high dosage. The toxicity of organophosphate insecticides, thiometon and disulfoton and their metabolites to the water flea (*Daphnia magna)* was estimated by Galli et al. [14]. The results showed that some of their degradation products (PO analogs) are stronger inhibitors of AChE than the parent compounds. They also concluded that *D. magna* is less sensitive to the degradation products lacking the sulfur containing side chain and the low molecular weight sulfur compounds.

The wide spread agricultural usage of the organophosphorus pesticides and their potential mammalian toxicity dictate to carry out a study on the identification of their metabolites. A number of studies on the biomonitoring of metabolites of some of the important organophosphate pesticides are reported in the literature. Drevenker et al. [15] observed the presence of chlorpyrifos metabolites in the serum and urine of poisoned persons. The total urinary diethylphosphorus metabolites in chlorpyrifos poisoned persons were excreted with an average elimination half time of around 6 hours in the faster and of 80 hours in the slower elimination phase. The same group of workers [16] also reported the presence of dialkylphosphorus metabolites of organophosphorus pesticides in human blood at a microlevel thereby confirmed the human poisoning with organophosphorus pesticides. Tsatsakis et al.

[17] detected dialkyl phosphates in human hair while biomonitoring the exposure to organophosphate pesticides. They observed significant differences in the levels of the total dialkyl phosphates amongst the exposed and non-exposed groups. Timchalk et al. [18] compared the pharmacokinetics of the organophosphate insecticide chlorpyrifos and its major metabolites diethylphosphate (DEP), diethylthiophosphate (DETP) and 3,5,6 trichloro-3-pyridinol (TCPy) in the rat. All the three metabolites are well absorbed with peak blood concentrations being attained between 1 and 3 hours after dosing. The distribution of metabolites in the urine follows the order: TCPy > DETP > DEP. Caceres et al. [19] evaluated the toxicity of fenamiphos and its metabolites to cladoceran (*Daphnia carinata)* showing the influence of microbial degradation in natural waters. The study suggested that the organophosphate pesticides are highly toxic to fresh water invertebrates and therefore pollution with these compounds may adversely affect the natural ecosystems. They [20] also studied the toxicity and transformation of fenamiphos and its metabolites by two microalgae, *Pseudokirchneriella subcapitata* and *chlorococcum species*, with the finding that the fenamiphosphenols are more toxic to the algae. The need to consider the transformation products in ecological risk assessment of fenamiphos was also highlighted. Vega et al. [21] investigated the genotoxicity and genotoxic mechanisms of diethylthiophosphate and diethyldithiophosphate. They found that both induce DNA damage only in hepatic cell lines when activated by further biotransformation via Cytochrome P450. Vasilic and coworkers [22] studied the diethylphosphorus metabolites in serum and urine of persons poisoned by phosalone . The workers found that diethylphosphorus metabolites are more sensitive indicator of exposure as phosalone is rapidly hydrolysed and eliminated from the body in serum samples. In another similar study the same group of researchers [23] showed the presence of

dimethylphosphorus metabolites in serum and urine of persons poisoned by malathion or thiometon. The decrease in the concentration of single and total dimethylphosphorus metabolites is biphased with a fast initial rate followed by a slow rate. The total metabolite elimination half times are 4.1 and 4.7 hours in the initial phase and 53.3 and 69.3 days in the later slower elimination phase.

From the literature cited above it is apparent that information is available on the biomonitoring of a few organophosphate pesticides and their metabolites. In the case of quinalphos focus has been mainly on its toxicological aspects. These studies have been mainly on birds, rats and fish. Anam and Maitra [24] suggested that the increased blood glucose level and decreased hepatic glycogen concentration in the quinalphos-exposed parakeets may be related to the degree of pesticide induced inhibition of AChE activity in brain and or pancreas of the concerned birds. The sublethal effect of quinalphos on selected blood parameters of *Labeo rohita* was investigated by Das with Mukherjee [25] and observed a decreasing pattern in the serum protein level and haemoglobin percentage while elevation in blood glucose level. Debnath and coworkers [26] carried out a study on the effect of quinalphos formulation in Sprague-Dawley albino rats and found that it induces damage to the testicular tissues and antioxidant defence systems. The effects are more pronounced at the low doses than at the higher doses, indicating that some physiological defence mechanisms (an endogenous antioxidant enzyme defence system) are in operation at higher doses. This indicates that quinalphos causes damage and degeneration of the testicular tissues due to free-radical-mediated lipid peroxidation. Dikshith et al. [27] planned a study on the effects of repeated oral administration of quinalphos to the malegoat (*Capra hircus).* The study suggested that although quinalphos in low concentrations does not produce discernible cellular

changes, but it induces highly significant enzymatic and haematological changes in the goat. The role of cytochrome P450 in quinalphos toxicity and effect on hepatic and brain antioxidant enzymes in rats was investigated by Dwivedi et al. [28]. A significant decrease in the body, brain and liver weights is observed. From the above study it was inferred that the toxicity of quinalphos may be due to the parent compound or its metabolite(s) produced prior to P450 oxidation. Gupta et al. [29] evaluated the effect of the quinalphos exposure on the developing blood brain barrier (BBB). It is evident from the study that quinalphos exposure during the early postnatal period causes significant impairment in the development and maturation of the BBB that may have adverse consequences on the normal brain functioning with long-term neurotoxic effects. The toxicity of quinalphos to soil microarthropods in cotton fields was evaluated by Vig and her research group [30]. A decrease in the population of acarina is observed. Ray et al. [31] treated the Wistar rats with quinalphos and suggested that quinalphos may exert a suppressive effect on the functional activity of accessory sex glands by decreasing testicular testosterone production following inhibition of pituitary gonadotrophins release. Riediger and coworkers [32] studied the toxicity of the quinalphos metabolite, 2-hydroxyquinoxaline, in respect of growth inhibition, induction of oxidative stress, and genotoxicity in test organisms. 2-hydroxyquinoxaline photocatalytically destroys antioxidant vitamins and biogenic amines *in vitro* in several small aquatic organisms and causes mutagenicity in *Salmonella typhimurium*. They found 2-hydroxyquinoxaline to be a source of secondary quinalphos toxicity which deserves further attention. The respiratory responses and behavioural anomalies of the carp (*Cyprinus carpio)* under quinalphos intoxication in sublethal doses was investigated by Chebbi and David [33]. The concentration causing an acute toxicity is found to be 7.5 μL/L. The caudal bending is the chief

morphological alteration observed during the exposure tenures. Sarkar et al. [34] evaluated the effects of chronic sublethal doses (7–14 mg kg^{-1}) of quinalphos in adult male rats and concluded that quinalphos decreases fertility in adult male rats by affecting the pituitary gonadotrophins directly. Shukla and his research group [35] conducted long-term animal bioassays for the evaluation of the complete carcinogenic, tumour-initiating and tumour-promoting potential of quinalphos. The studies revealed that it has only a tumour-initiating potential at a dose of 10 mg kg^{-1} b.wt. in the two-stage mouse skin model of carcinogenesis.

The phototoxicity of quinalphos *in vitro* and *in vivo* under sunlight was investigated by Srivastava and associates [36]. An oral dose of quinalphos (5 mg kg^{-1} b.wt.) to the mouse did not produce any change in tail and skin sensitization test but produced ear swelling under sunlight. A significant amount of activated oxygen species (AOS) is also produced. The production of AOS is both concentration and irradiation time dependent. Srivastava and Raizada [37] assessed the no observed effect level (NOEL) of quinalphos in pregnant rats. The AChE activity in the brain and red blood cells is also significantly inhibited at 3 and 4.5 mg $kg^{-1}day^{-1}$ quinalphos. Hence, 2 mg kg^{-1} body weight of quinalphos could be considered as NOEL on the foetal and maternal toxicity in rats. Vairamuthu and Thanikachalam [38] observed some severe effects of quinalphos on blood and brain esterase activity in chickens which increases with the increase in quinalphos dosage. Further, blood, plasma, brain and erythrocyte cholinesterase activity is inhibited. Praveen et al. [39] carried out a quantitative densitometric determination of quinalphos in postmortem blood by HPTLC in a suicidal case. Vasilic et al. [40] studied the urinary excretion rates of diethyl phosphate and diethyl phosphorothioate and changes in blood cholinesterase activities in persons poisoned by the quinalphos. The excretion of metabolites follows a biphasic reaction kinetics. In the fast

excretion phase the half lives of urinary metabolites range between 5.5-14.2 hours while in the slow excretion phase the half lives range from 66.5 to 127.9 hours.

It is apparent from the preceding discussion that a number of studies have been carried out on the biochemical effects of various organophosphate pesticides and their metabolites on different types of animals. Some reports are available on the poisoning cases also. However, only a limited information exists on the phamacokinetics of some of the organophosphate pesticides. But no systematic *in vitro* and *in vivo* studies appear to have been on pharmacokinetics and identification of metabolites of quinalphos. Such studies have their own importance because they reflect the fate of pesticide when it is ingested directly.

The present investigation are planned to study the *in vitro* and *in vivo* pharmacokinetics of quinalphos. Studies were conducted *in vitro* by simulating digestive system conditions in the laboratory and *in vivo* by administering quinalphos to wistar albino rats, a species supposed to be similar to humans. At different time intervals the parent compound and the different metabolites formed were analysed in simulated *in vitro* and blood and urine samples. The simulated studies indicate a time- dependent degradation of the pesticide in the gastric and intestinal phases. The analysis of various samples collected at different time intervals from the *in vitro* and *in vivo* is able to suggest the possible pathways of the pharmacokinetics of quinalphos.

5.2 Experimental

For *in vitro* and *in vivo* decay studies Waters 2489 HPLC system (Waters Corporation, USA) equipped with a UV detector was employed. A linear plot (r^2=0.99) was obtained in the concentration range 0.1– 500 μg mL^{-1}. The system was operated under the conditions specified in chapter II .The details of GC-MS and its operating conditions are same as cited in

chapter II. Schematic representation of the gastric and intestinal digestion of *in vitro* samples and extraction procedure for the pesticide from *in vivo* samples are also cited in chapter II as Flowsheets 2.6 and 2.7, respectively. Triplicate samples were analysed at 0, 0.5, 1 and 2 hours intervals for gastric and intestinal digestion. All the animals were dosed with 5 mg kg^{-1} b.wt. quinalphos in DMSO suspended in PBS by oral gavaging and sacrificed after 0.5, 1, 2, 3, 6, 9, 12, 18 and 24 hours. It is important to note that all the rats were starved for 12 hours before dosing with quinalphos.

The simulated, urine and the blood serum samples collected at different time points were stored immediately at −40 ^{0}C till further processing. The decay profile of the pesticide was followed in different lyophilized samples of simulated digestion, blood serum and urine collected after different time intervals. The decay was measured for a maximum of two hours in simulated gastric and intestinal phases and twenty four hours in rats.

At periodic intervals triplicate samples were removed and analysed by HPLC for the determination of residual quinalphos. The limit of detection for HPLC was 0.1 μg mL^{-1}. The standard deviation (n=3) for the measurement of concentration at 200 μg mL^{-1} in simulated and biological samples was 1.0 and 1.9, respectively. For the identification of metabolites by GC-MS duplicate samples were withdrawn at periodic intervals.

5.3 Results and Discussion

5.3.1 Pharmacokinetics of quinalphos in simulated *in vitro* samples

Studies on the degradation of quinalphos in simulated gastric (pH 2.0) and intestinal phase (pH 7.0) were carried out at 37±0.5 °C at different time intervals (0, 0.5, 1 and 2 hours). Simultaneously blank studies were carried out in deionised water adjusted at pH 2.0 and pH 7.0. The data plotted on a natural log scale give straight lines indicating that the degradation of

the pesticide follows a first order kinetics (Fig. 5.1). The rate of degradation is faster in gastric ($t_{1/2}$ = 4.0 hours) and intestinal ($t_{1/2}$ = 5.0 hours) phases than that in the blank solution at pH 2.0 ($t_{1/2}$ = 5.7 hours) and at pH 7.0 ($t_{1/2}$ = 13.9 hours). It is apparent from the results that the presence of pepsin and pancreatin enzymes is leading to a faster decay. Similar observations have been reported earlier while studying the effect of the gastric [41] and intestinal [42] juices on the stability of drugs. Control studies further suggest that the degradation of quinalphos is faster in the acidic conditions.

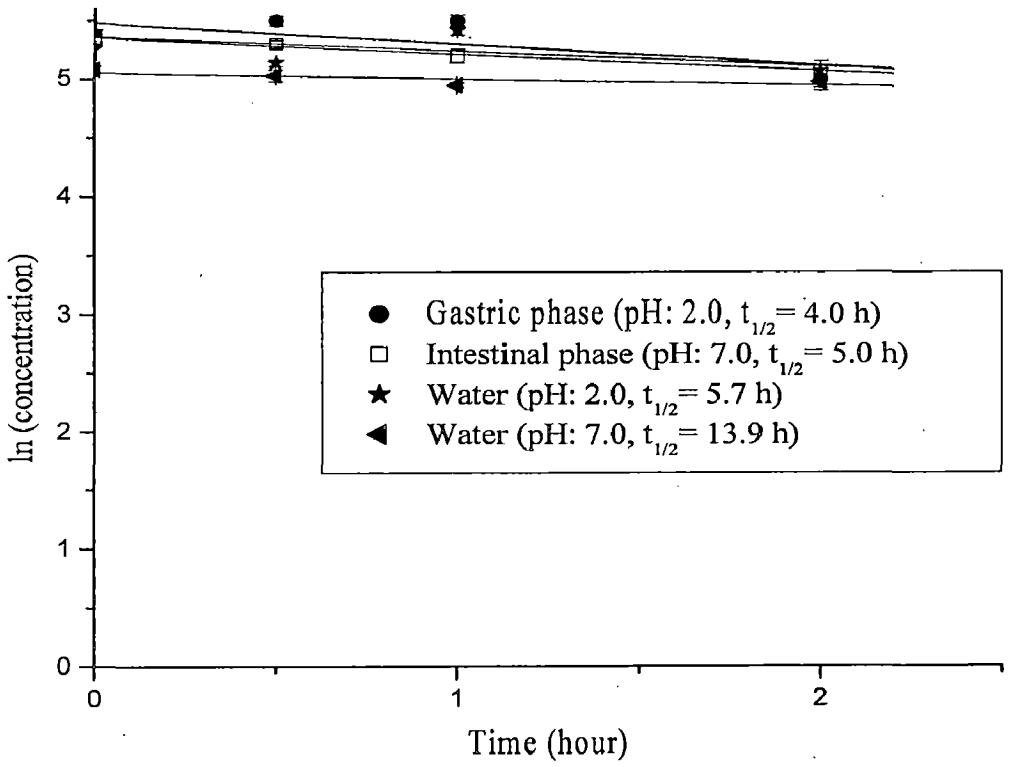


Figure 5.1: Persistence of quinalphos in simulated gastric and intestinal phases

5.3.2 Pharmacokinetics of quinalphos in Wistar albino rats

All the animals survived a single acute oral dose (5 mg kg^{-1}) of quinalphos The persistence of quinalphos for 24 hours in the blood and urine is presented in Figure 5.2 (A) and 5.2 (B), respectively. The profiles of concentration observed in the biological fluids suggest that quinalphos is rapidly absorbed and reaches a peak concentration in 2 hours after dosing and thereafter it is metabolized with a half life of 3.8 and 4.0 hours in the blood and urine, respectively. Similar trend of attaining a maximum value followed by a decay has been reported by Timchalk et al. [43] for chlorpyrifos in blood and urine of rats and humans.

5.3.3 Identification of metabolites of quinalphos

The possible degradation products of quinalphos identified under simulated gastric and intestinal conditions and in the blood serum and urine are shown in Figure 5.3. The metabolic pathways are proposed. The formation of the different metabolites is discussed in the following subheadings. In *in vitro* studies the samples were withdrawn at 0.5, 1 and 2 hours intervals, whereas in *in vivo* studies the blood and urine samples were collected at intervals of 0.5, 1, 2, 3, 6, 9, 12, 18 and 24 hours. After processing, the samples were analysed by the GC-MS for the identification of metabolites. **It is pertinent to mention here that a number of metabolites obtained in *in vitro* and *in vivo* studies are same as those obtained in the case of water, soil and plants. Therefore the formation of only those metabolites is discussed in this chapter which appeared for the first time in *in vitro* and *in vivo* studies.** Fragmentation pathways of various metabolites proposed in the following text are based on mass spectrometric fragmentations peaks.

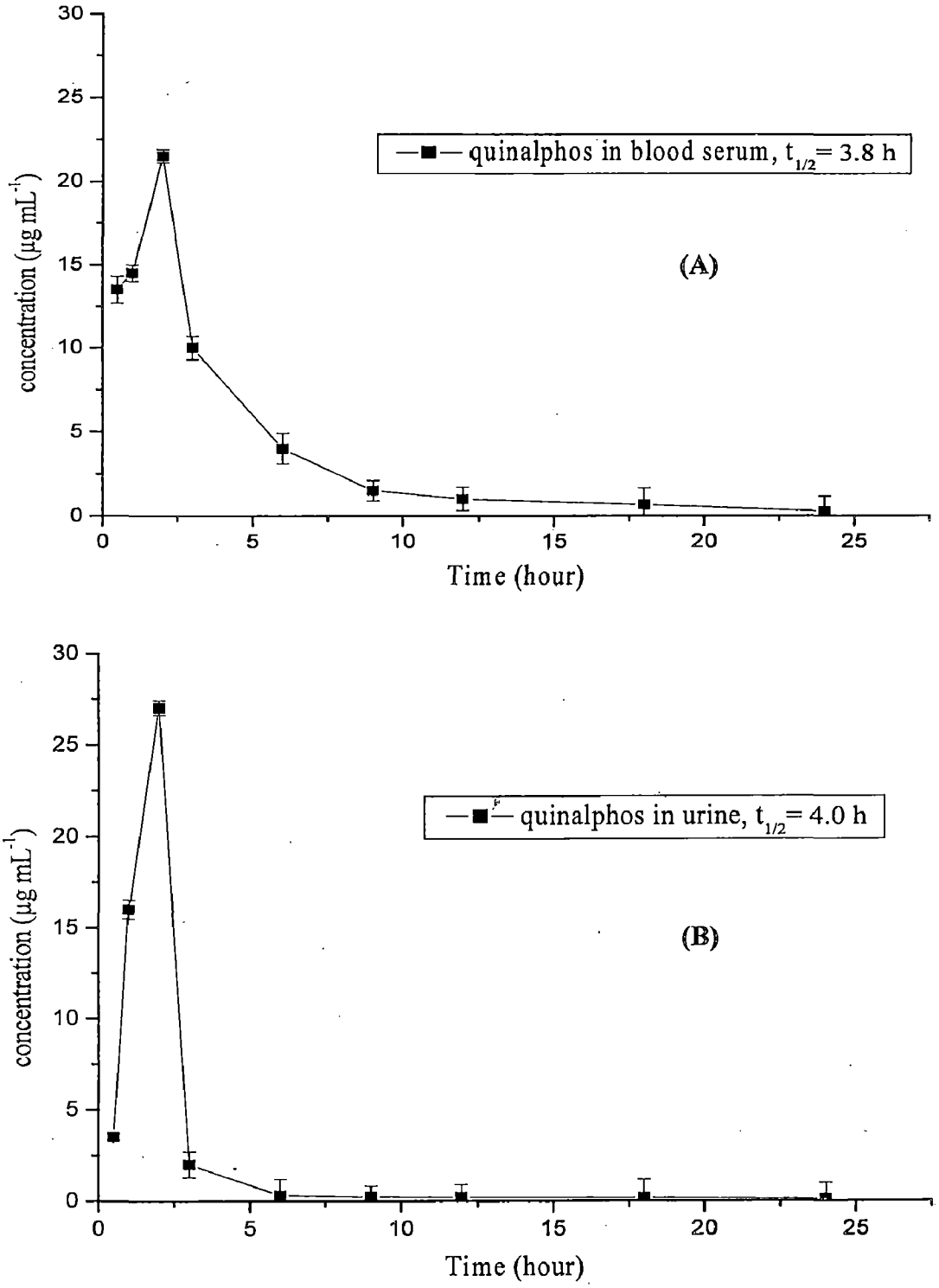


Figure 5.2: Persistence of quinalphos in blood serum (A) and urine (B)

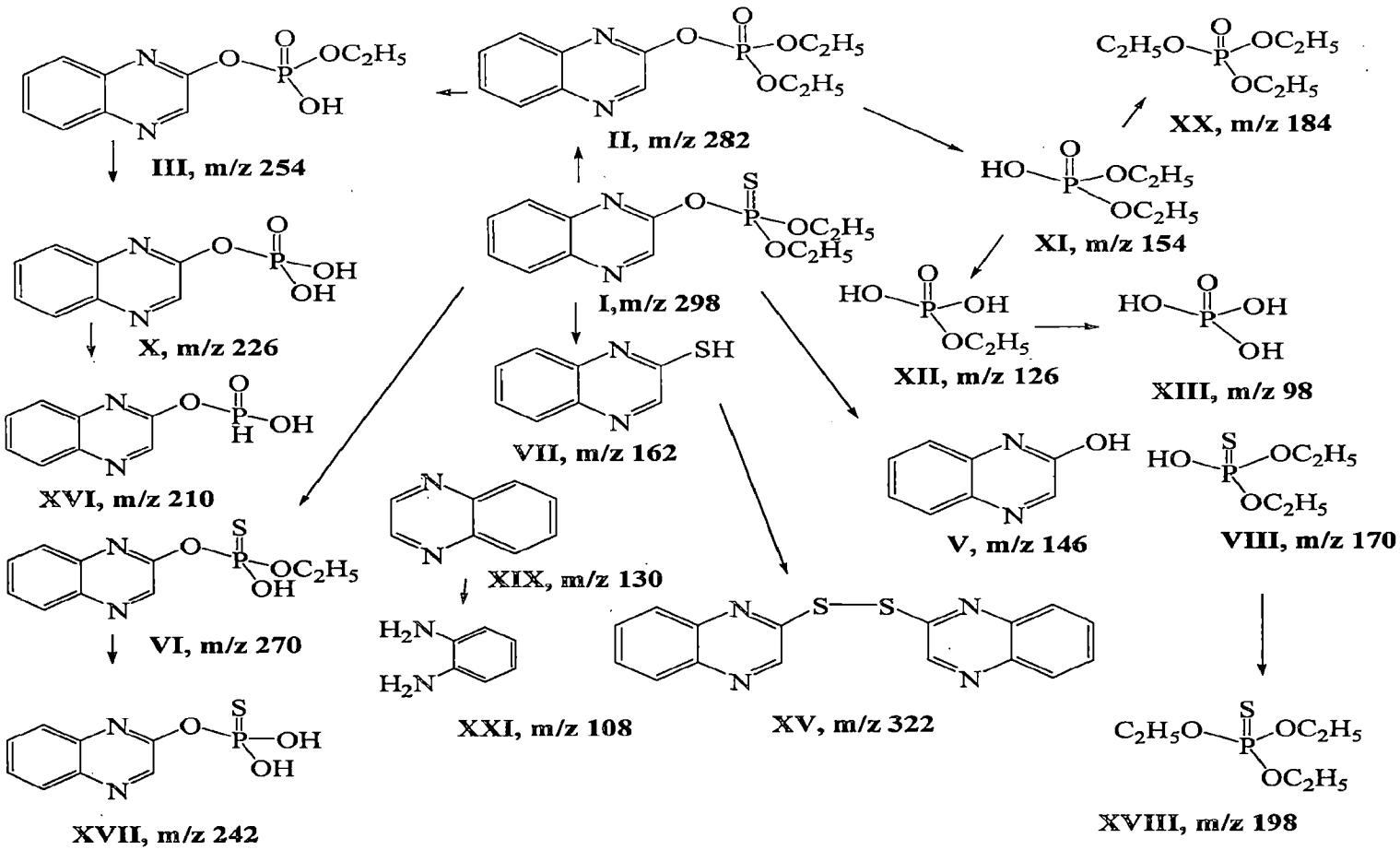


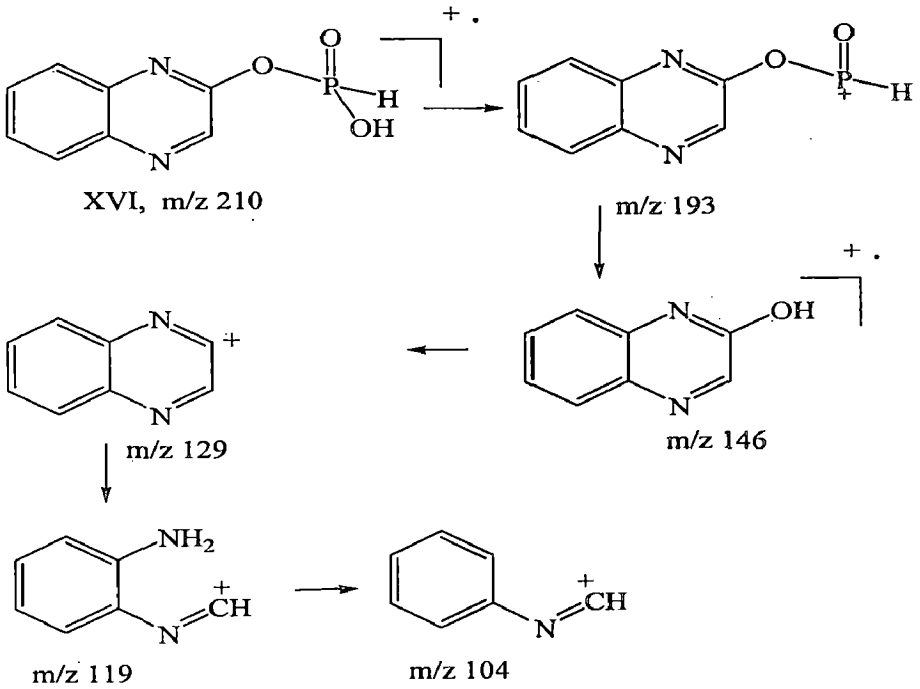
Figure 5.3: Metabolic pathways of quinalphos in *in vitro* (simulated gastric and intestinal phases) and *in vivo* (blood serum and urine) samples

5.3.3.1 *In vitro* studies

5.3.3.1.1 Gastric digestion (pH 2.0)

The GC-MS of the 0.5 hour sample shows the formation of two products along with the parent quinalphos. The first product has a molecular ion peak at m/z 282 and fragment ion peaks at m/z 281, 209, 193, 146 and 129 and is proposed as quinalphos oxon II (Fig. 5.3, Chapter III, Section 3.3.5.1) formed by oxidation of P=S to P=O. The mass spectrum of another product shows a molecular ion peak at m/z 254 and fragment ion peaks at m/z 238, 210, 209, 193, 146, 129, 119, 104 and 91. It is proposed as O-ethyl-O-quinoxalin-2-yl phosphoric acid III (Fig. 5.3, Chapter III, Section 3.3.5.1) formed by dealkylation of quinalphos oxon.

The GC-MS of one hour incubated sample shows the formation of four other metabolites along with (II) and (III). One of the products has a molecular ion peaks at m/z 154 with fragment ion peaks at m/z 153, 137, 126, 98, 80 and 64 and is tentatively identified as diethyl phosphoric acid XI (Fig. 5.3, Chapter III, Section 3.3.5.3). The mass spectrum of the second product has a molecular ion peak at m/z 146 and fragment ion peaks at m/z 129, 119, 104 and 91. This has been proposed as 2-hydroxy quinoxaline V (Fig. 5.3, Chapter III, Section 3.3.5.1) formed by the nucleophilic substitution (SN_2) at the phosphorus atom. The mass spectrum of third product shows a molecular ion peak at m/z 126 along with fragment ion peaks at m/z 98, 82 and 64. It is tentatively identified as monoethyl phosphoric acid XII (Fig. 5.3, Chapter III, Section 3.3.5.3). The mass spectrum of fourth product shows a molecular ion peak at m/z 210 (M+) and fragment ion peaks at m/z 193,146, 129, 119 and 104 (Fig. 5.4). Based on the mass spectrum, it is assigned quinoxalin-2-yl hydrogen phosphonate XVI (Fig. 5.3).



The GC-MS of two hours incubated sample shows the peaks of products (II) and (V) along with a metabolite with a molecular ion peak at m/z 162 and fragment ion peaks at m/z 129, 119, 104 and 91. Based on the molecular ion peak, it is tentatively identified as quinoxaline-2- thiol VII (Fig. 5.3, Chapter III, Section 3.3.5.1) formed by isomerisation of quinalphos to isoquinalphos followed by the hydrolysis.

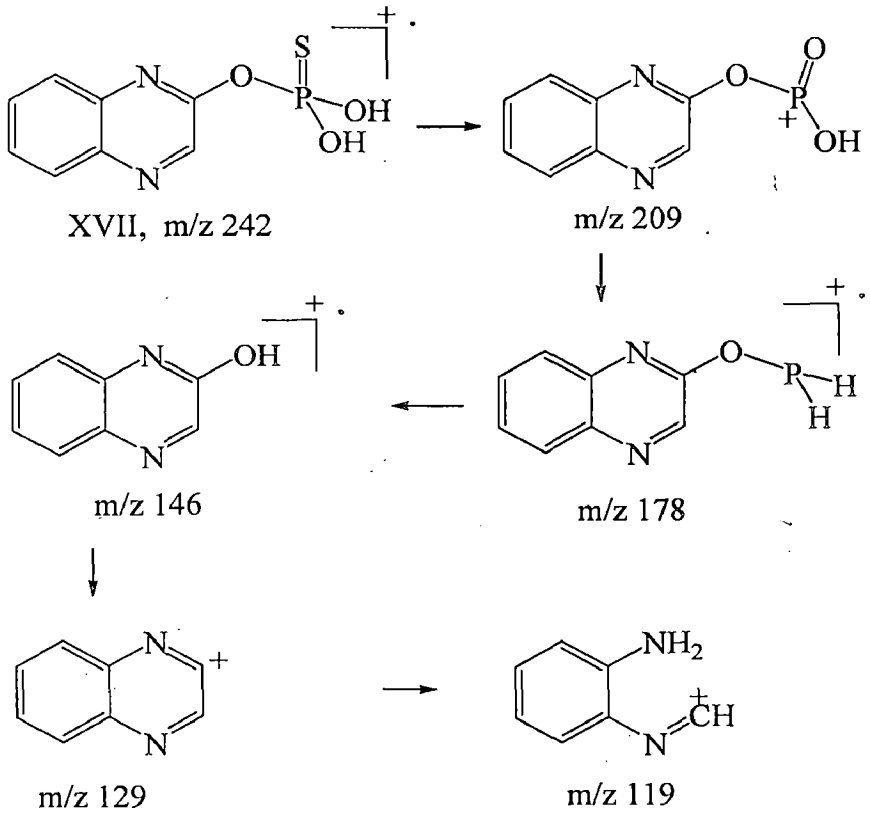
5.3.3.1.2 Intestinal digestion (pH 7.0)

The sample collected after 0.5 hour shows the formation of metabolites (II), (III), (V), (VII) and (XI) along with a molecular ion peak at m/z 270 and fragment ion peaks at m/z 253, 209, 178, 146, 129, 119, 104 and 91. It is tentatively identified as des-ethyl quinalphos VI (Fig. 5.3, Chapter III, Section 3.3.5.1) formed by dealkylation process.

The sample withdrawn after one hour showed the formation of (II), (IV), (V), (VIII) and (IX) along with the two other metabolites having molecular ion peaks at m/z 170 and m/z 242. The mass spectrum of the first product, showing a molecular ion peak at m/z 170

and fragment ion peaks at m/z 169 and 125 has been identified as diethyl thiophosphoric acid VIII (Fig. 5.3, Chapter III, Section 3.3.5.1), a hydrolytic product of quinalphos.

The second product with a molecular ion peak at m/z 242 and fragment ion peaks at m/z 209, 178, 146, 129 and 119 (Fig. 5.5) is tentatively identified as O-quinoxalin-2 yl-O,O- dihydrogen phosphorothioate XVII (Fig. 5.3) formed by dealkylation of desethyl quinalphos.



The two hour sample shows the formation of a dimer, diquinoxalin-2-yl disulfide XV (Fig. 5.3, Chapter III, Section 3.3.5.4) having a molecular ion peak at m/z 322 and fragment ion peaks at m/z 162, 129, 119, 104 and 91. In addition to (XV), the peaks of (II), (III), (V), (VII), (VIII) and (XII) have also been observed.

5.3.3.1.3 *In vitro* blank samples

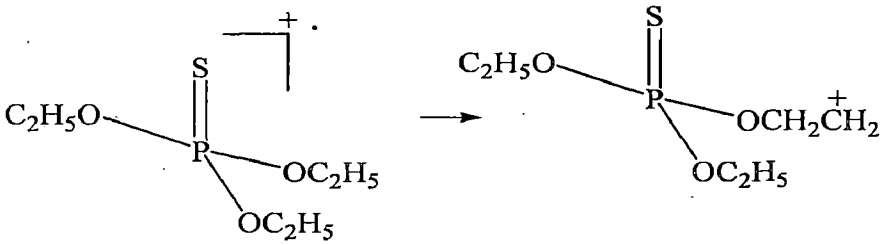
The investigations were also carried out in the absence of enzymes. The blank of gastric phase shows the formation of metabolites (II), (III), (V) and (VIII). A new product having a molecular ion peak at m/z 226 and fragment ion peaks at m/z 209, 179, 146, 129 and 119 is also observed which is identified as dihydroxy quinalphos oxon X (Fig. 5.3, Chapter III, Section 3.3.5.2).

The blank of intestinal phase shows the formation of (II) and (V) along with a peak due to phosphoric acid XIII (Fig. 5.3, Chapter III, Section 3.3.5.3) which has a molecular ion peak at m/z 98 and fragment ion peaks at m/z 81 and 64.

5.3.3.2 *In vivo* studies

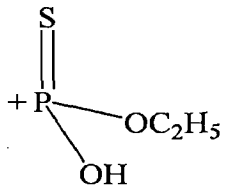
5.3.3.2.1 Identification of metabolites in the blood serum

The blood serum sample collected after 0.5 hour shows the formation of (II) and (V). The one hour sample of blood serum shows the peaks corresponding to structures (II), (V) and (XII), whereas samples collected at 2, 3, 6, 9 and 12 hours intervals show the presence of (II), (III), (V) and (XII). The peaks corresponding to (II), (III), (V), (VIII), (XII) and (XVII) have been identified in the sample collected after 18 hours. The sample collected after 24 hours shows the presence of (VI), (VII), (XV), (XVII) and a new product having a molecular ion peak at m/z 198 and fragment ion peaks at m/z 197 and 125 (Fig. 5.6). It is identified as triethyl thiophosphate XVIII (Fig. 5.3).



XVIII, m/z 198

m/z 197

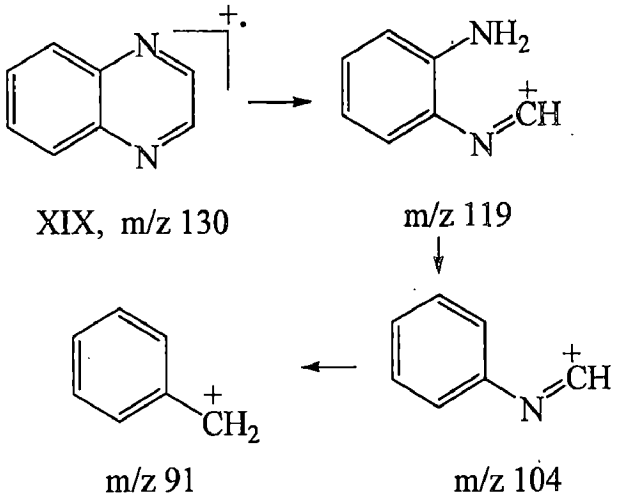


m/z 125

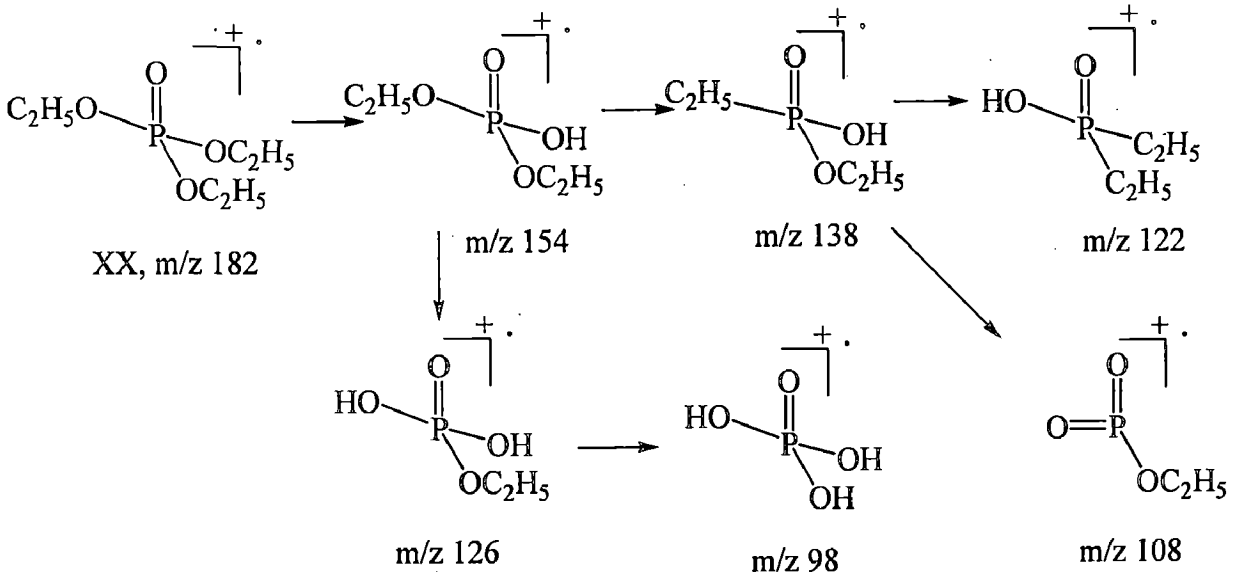
Most of the metabolites identified in blood serum are formed by the oxidation and hydrolysis as the main pathways.

5.3.3.2.2 Identification of metabolites in urine

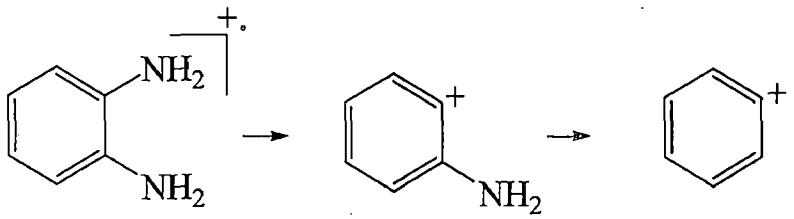
The sample of urine collected after 0.5 hour shows the formation of oxidative products (II) and (III) and a hydrolytic product (V). The urine sample collected after 1 hour, shows the presence of (II), (III), (V) and (XII), whereas samples collected after 2, 3, 6, 9 and 12 hours show the formation of (VI) and (VIII) in addition to (II), (III), (V) and (XII). The sample collected after 18 hours shows the presence of metabolites (VI), (X), (XV) and (XVII) along with a new metabolite with a molecular ion peak at m/z 130 and fragment ion peaks at m/z 119, 104 and 91 (Fig. 5.7). This new product is identified as quinoxaline XIX (Fig. 5.3).



The sample collected after 24 hours shows the presence of two new metabolites alongwith the peaks of (X) and (XIII). The first product has a molecular ion peak at m/z 182 and fragment ion peaks at m/z 154, 138, 126, 122, 108 and 98 (Fig. 5.8). This is identified as triethyl phosphate XX (Fig. 5.3).



The second product has a molecular ion peak at m/z 108 and fragment ion peaks at m/z 92 and 77 (Fig. 5.9). It is tentatively identified as O-phenylene diamine XXI (Fig. 5.3)



XXI, m/z 108 m/z 92 m/z 77

It may be important to mention here that some of the metabolites which appear initially or in earlier samples do not figure in subsequent samples. This may be attributed to the fact that with the time they have decayed to such a low concentration so as to respond to the detector.

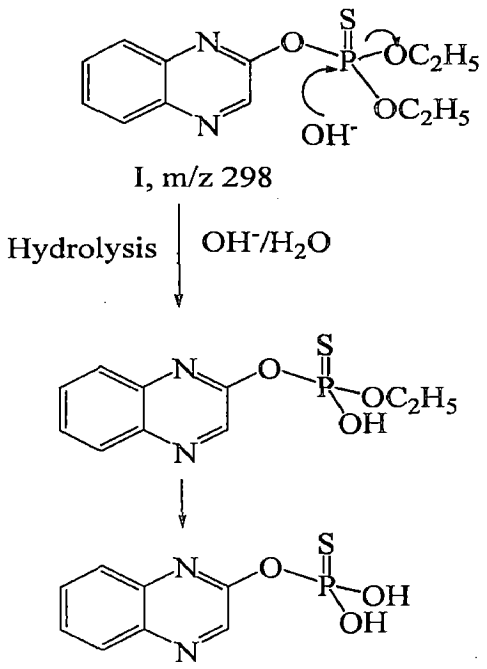
The study carried out on the identification of metabolites of quinalphos in *in vitro* and *in vivo* revealed that the different metabolites are formed as a result of the hydrolysis, oxidation, dealkylation and isomerization. The byproducts formed in the presence and absence of enzymes in *in vitro* studies are almost the same except that a fast reaction rate is observed in the presence of enzymes. The dimer diquinoxalin-2-yl disulfide (XV) is not observed in *in vivo* studies probably it is unstable in the presence of biological juices. The metabolites (XVIII) to (XXI) are observed in *in vivo* studies only. It may be suggested that their formation is favoured by enzymes. The results indicate that the metabolites (II) and (V) which are reported to be highly toxic are reasonably stable in both, *in vitro* and *in vivo* conditions.

A comparison of the metabolites formed in the blood and urine at different time intervals shows that in both the cases more or less the same metabolites with the exception of a few are formed. It is apparent that the time of appearance of a certain metabolite in the two types of biological fluids may not be necessarily the same because of the differences in their composition and the biological processes involved. Such a difference in the mass

spectra may be illustrated by taking a typical case of urine and blood (Fig 5.10 A, B and C). The urine sample collected after 18 hours of dosing shows peaks corresponding to both (VI) and (XVII) besides other peaks (Fig 5.10 A) while the blood sample collected after same time interval shows only the peak of (XVII) along with other peaks (Fig 5.10 B). The blood sample collected after 24 hours has peaks due to both (VI) and (XVII) with other peaks (Fig 5.10 C). These results indicate that the metabolite (VI) in urine appears after 18 hours but in blood after 24 hours which suggests that the direct dealkylation of quinalphos takes place faster in the urine. Similarly the phenomena of ring cleavage is observed in urine only which is supported by the absence of metabolite (XIX) in the blood. The pharmacokinetic studies carried out on the degradation of quinalphos reveal that a number of different metabolites are formed at different time intervals. The pattern of metabolites both in blood and urine samples is more or less same with a few deviations. The presence of these metabolites in the concerned biological fluids can help in the identification of quinalphos as the source of poisoning.

On comparing the data of present study with that obtained for water, soil and plants it can be concluded that more or less the same metabolites are formed. However, a few different metabolites like quinoxalin-2-yl hydrogen phosphonate, O-quinoxalin-2 yl- O,O-dihydrogen phosphorothioate, triethyl thiophosphate, triethyl phosphate, quinoxaline and O-phenylene diamine appear in the biological studies. On the basis of the above results it can be concluded that the metabolite formation both at the acidic and neutral pH (2.0 and 7.0) is initiated by hydrolysis. Results further suggest that in alkaline/neutral condition the cleavage of P-O bond results in the formation of (XVII) (base catalyzed ester hydrolysis) while in acidic condition the cleavage of P-O bond results in the formation of (XVI) i.e. P-

O bond is more prone to cleavage in the presence of biological enzymes of animals. The probable mechanism for the formation of (XVII) can be proposed as given below.

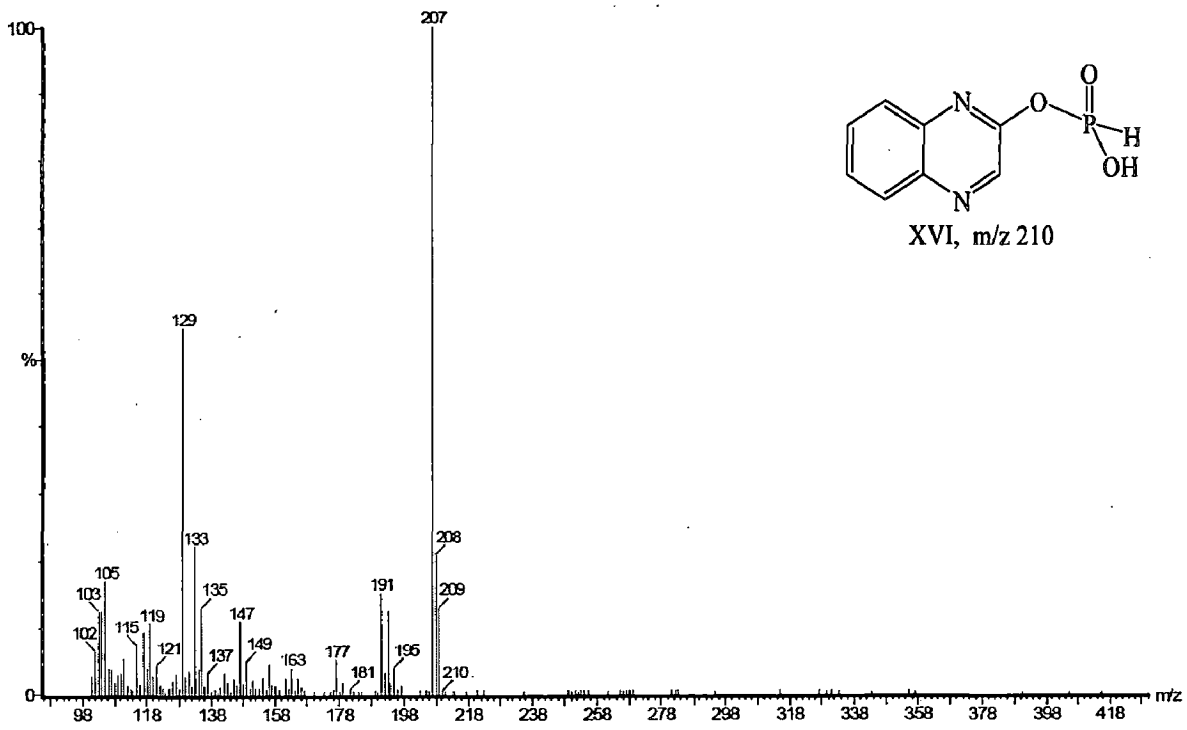


XVII, m/z 242

However, with the available data a suitable mechanism for the formation of (XVI) could not be proposed.

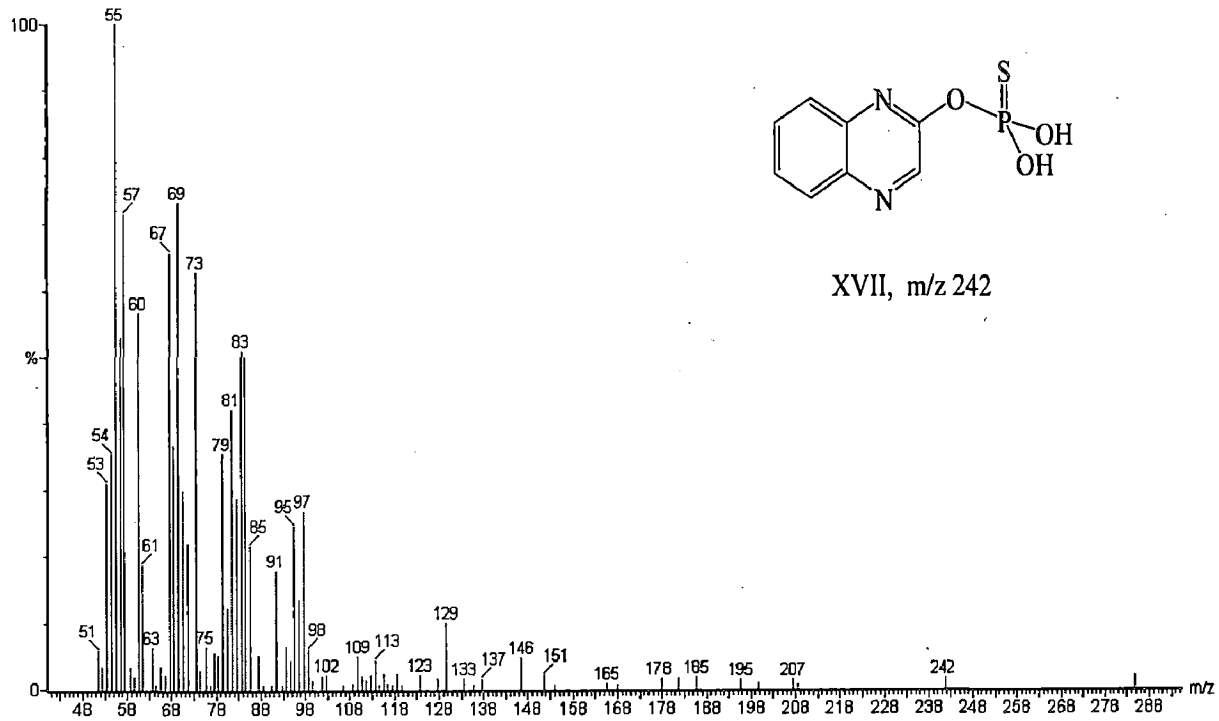
A perusal of data indicates that metabolites quinalphos oxon (II) and 2-hydroxyquinoxaline (V) are formed in all the matrices investigated and are reported to be more toxic than quinalphos. They may independently or synergistically enhance the toxicity of the pesticide. Besides these two compounds many other metabolites are formed for which the information on toxicity is not available. The possibility of their contribution towards the toxicity cannot be ruled out. These pharmacokinetic studies will help in refining a biologically based risk assessment for the exposure to quinalphos under a variety of scenarios.

The contents of this chapter have been accepted in "**Journal of Hazardous Materials**".



XVI, m/z 210

Figure 5.4: Mass spectrum of quinoxalin-2-yl hydrogen phosphonate (XVI)



XVII, m/z 242

Figure 5.5: Mass spectrum of O-quinoxalin-2 yl- O,O- dihydrogen phosphorothioate (XVII)

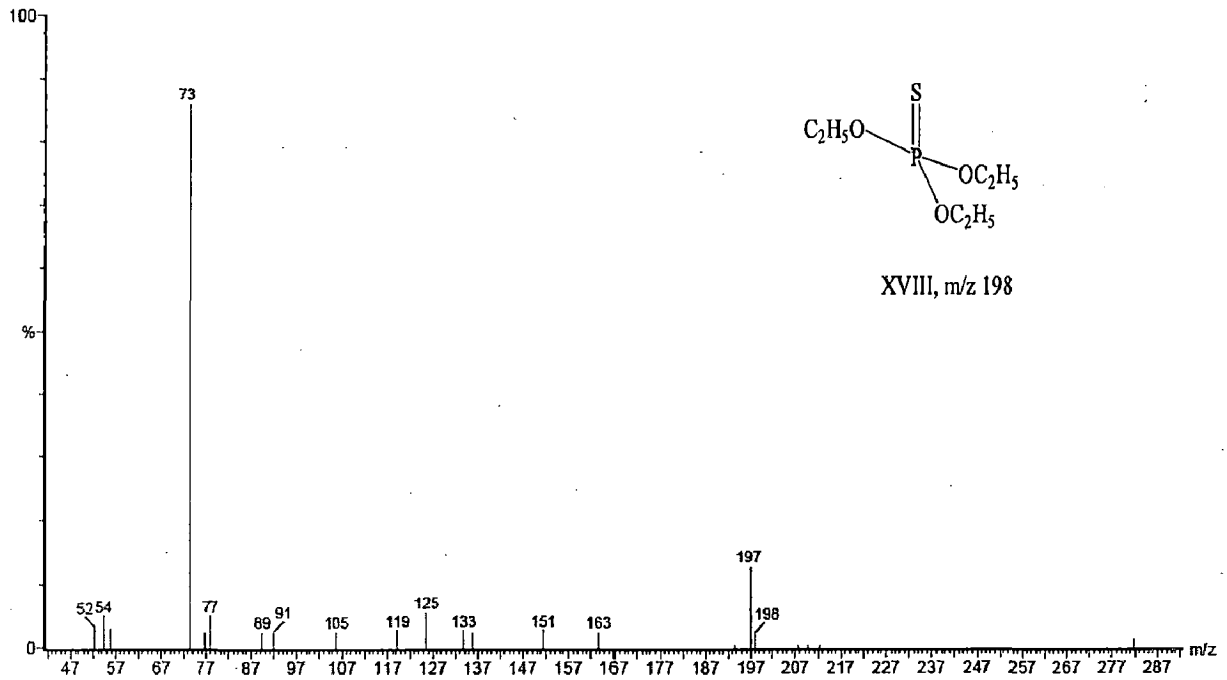


Figure 5.6: Mass spectrum of triethyl thiophosphate (XVIII)

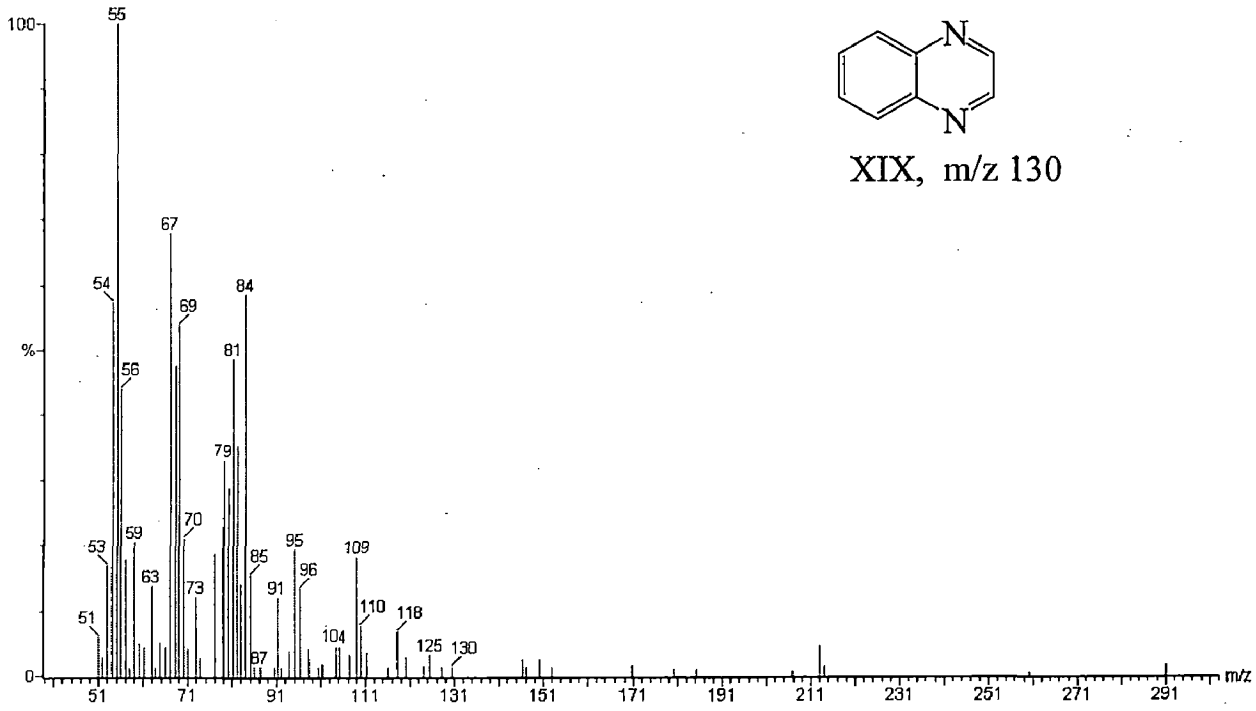


Figure 5.7: Mass spectrum of quinoxaline (XIX)

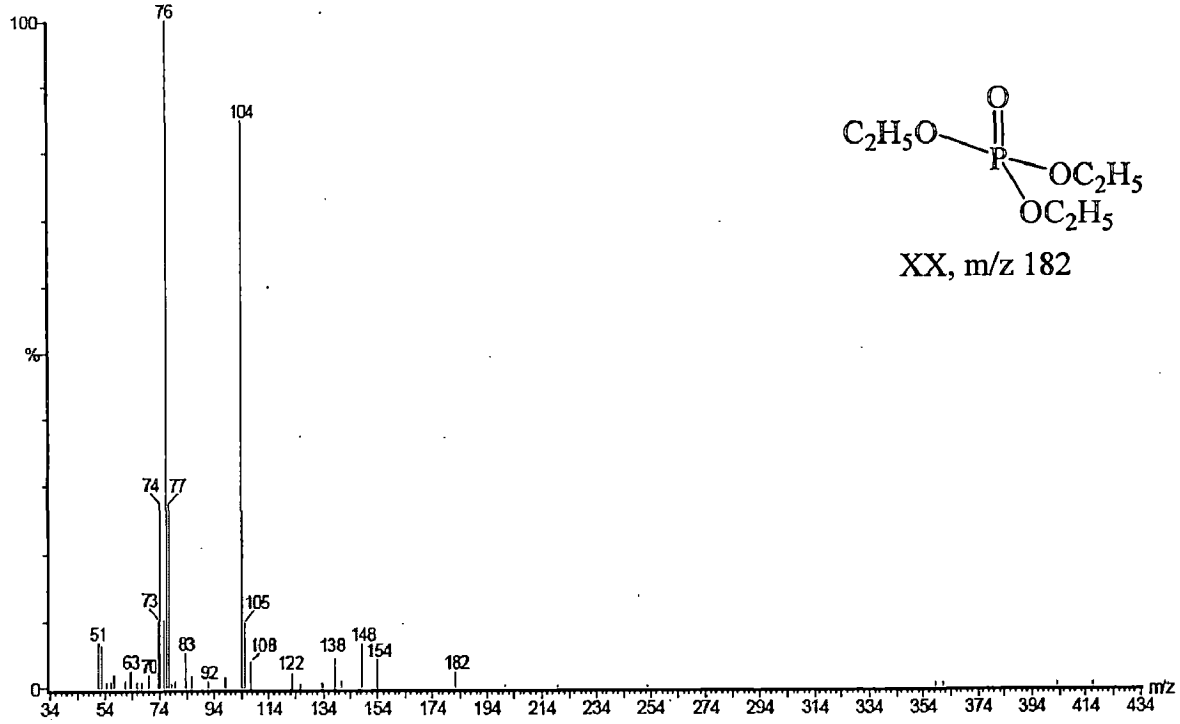


Figure 5.8: Mass spectrum of triethyl phosphate (XX)

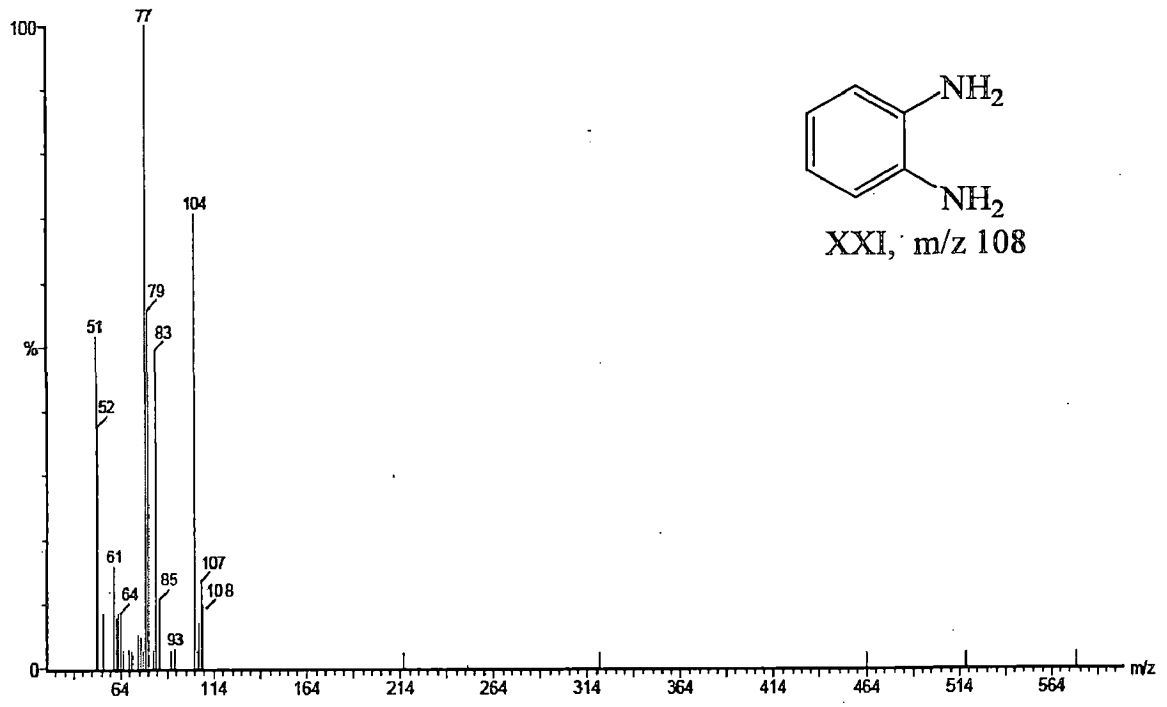


Figure 5.9: Mass spectrum of O-phenylene diamine (XXI)

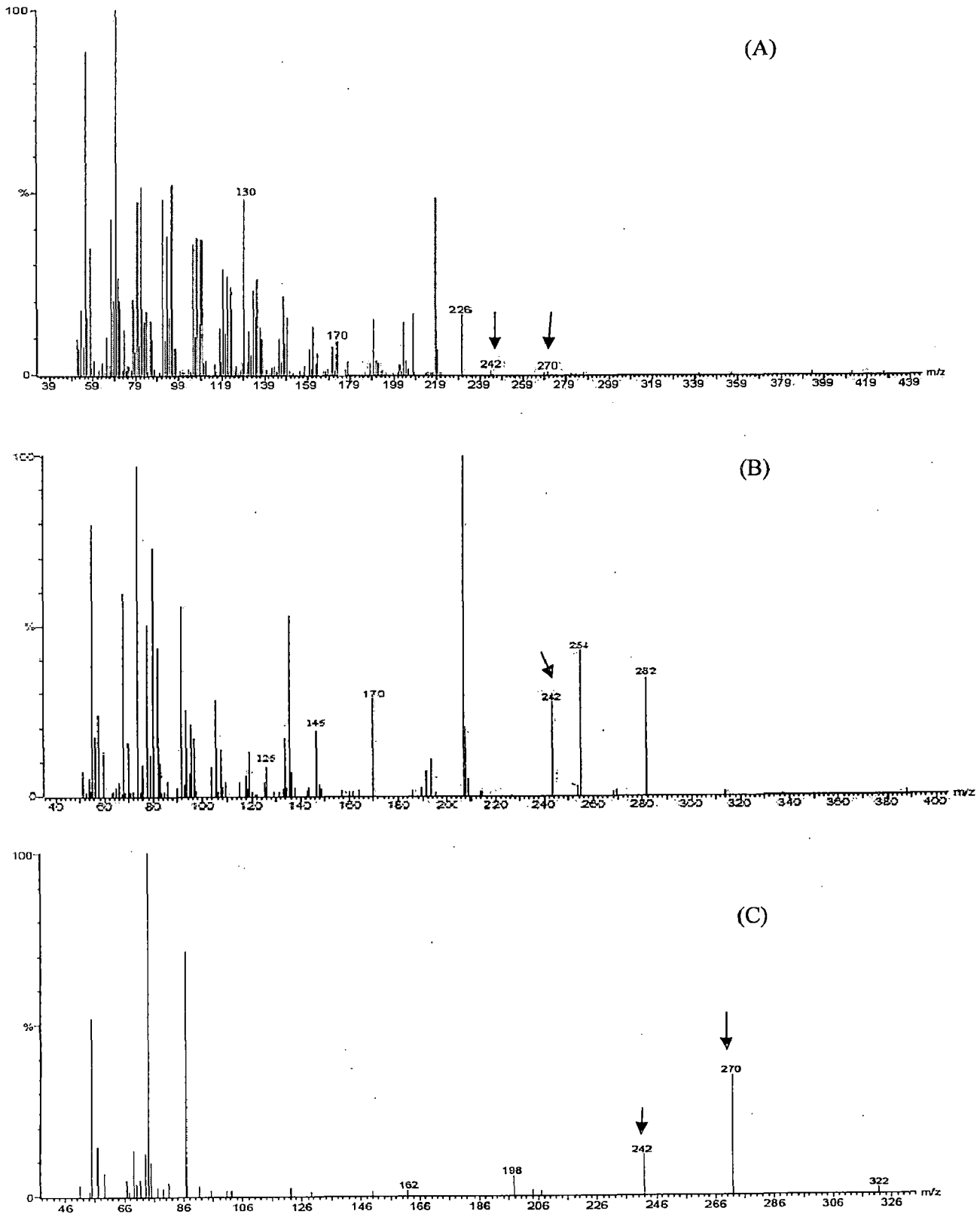


Figure 5.10: Mass Spectra for the degradation of quinalphos (A) urine after18 h dosing (B) blood after18 h dosing (C) blood after 24 h dosing (arrows indicate the peaks due to dealkylated products)

5.4 References

[1]. World Health Organization, "Public health impact of pesticides used in agriculture", 0 edn. WHO, Geneva, (1990).

[2]. Gunnell, D., Eddleston, M., Phillips, M. R. and Konradsen, F., "The global distribution of fatal pesticide self-poisoning: systematic review", BMC Public Health **7**, 357 (2007).

[3]. Agarwal, S. B., "A clinical, biochemical, neurobehavioural and socio psychological study of 190 patients admitted to hospital as a result of acute organophosphorus poisoning", Environ. Res. **62**, 63 (1993).

[4]. Goel, A., Joseph, S. and Dutta, T. K., "Organophosphate poisoning: Predicting the need for ventillatory support", J. Assoc. Physcians. Ind. **46**, 786 (1998).

[5]. Das, R. K., "Epidemiology of insecticide poisoining at A.I.I.M.S emergency services and role of its detection by gas liquid chromatography in diagnosis", Medico. Update **7**, 49 (2007).

[6]. Gannur, D. G., Maka, P. and Reddy, K. S. N., "Organophosphorus compound poisoning in Gulbarga region - a five year study", Indian J. Forensic Med. Toxicol. **2**, 3 (2008).

[7]. Mehrpour, O., Dolati, M., Soltaninejad, K., Shadnia, S. and Nazparvar, B., "Evaluation of histopathological changes in fatal aluminum phosphide poisoning", Indian J. Forensic Med. Toxicol. **2**, 34 (2008).

[8]. Kumar, R., Singh B., Singh, N. and Singh J., "Review of management of common poisoning in India", Medico. Update **5**, 31 (2005).

[9]. Palimar, V., Arun, M., Saralaya, K. M. and Singh, B., "Spectrum of organophoshorous poisoning in manipal", Medico. Update **5**, 55 (2005).

[10]. Esquivel-Sentíes, M. S., Barrera, I., Ortega, A. and Vega, L., "Organophosphorous pesticide metabolite (DEDTP) induces changes in the activation status of human lymphocytes by modulating the interleukin 2 receptor signal transduction pathway", Toxicol. Appl. Pharmacol. **248**, 122 (2010).

[11]. Salazar-Arredondo, E., Solís-Heredia, M. J., Rojas-García, E., Hernández-Ochoa, I., Quintanilla-Vega, B., "Sperm chromatin alteration and DNA damage by methyl-parathion, chlorpyrifos and diazinon and their oxon metabolites in human spermatozoa", Reprod. Toxicol. **25**, 455 (2008).

[12]. Eyer, F., Roberts, D. M., Buckleyc, N. A., Eddleston, M., Thiermann, H., Worek, F. and Eyer, P., "Extreme variability in the formation of chlorpyrifos oxon (CPO) in patients poisoned by chlorpyrifos (CPF)", Biochem. Pharmacol. **78**, 531 (2009).

[13]. Bicker, W., Lammerhofer, M., Genser, D., Kiss, H. and Lindner, W., "A case study of acute human chlorpyrifos poisoning: novel aspects on metabolism and toxicokinetics derived from liquid chromatography-tandem mass spectrometry analysis of urine samples", Toxicol. Lett. **159**, 235 (2005).

[14]. Gälli, R., Rich, H. W. and Scholtz, R., "Toxicity of organophosphate insecticides and their metabolites to the water flea *Daphnia magna*, the microtox test and an acetylcholinesterase inhibition test", Aquat. Toxicol. **30**, 259 (1994).

[15]. Drevenkar, V., Vasilić, Z., Stengl, B., Fröbe, Z. and Rumenjak, V., "Chlorpyrifos metabolites in serum and urine of poisoned persons", Chem. Biol. Interact. **87**, 315 (1993).

[16]. Drevenkar, V., Stengl, B. and Fröbe, Z., "Microanalysis of dialkylphosphorus metabolites of organophosphorus pesticides in human blood by capillary gas

chromatography and by phosphorus-selective and ion trap detection", Anal. Chim. Acta **290**, 277 (1994).

[17]. Tsatsakis, A. M., Barbounisa, M. G., Kavalakisa, M., Kokkinakisa, M., Terzia, I. and Tzatzarakisa, M. N., "Determination of dialkyl phosphates in human hair for the biomonitoring of exposure to organophosphate pesticides", J. Chromatogr. B **878**, 1246 (2010).

[18]. Timchalk, C., Busby, A., Campbell, J. A., Needhamb, L. L., and Barr. D. B., "Comparative pharmacokinetics of the organophosphorus insecticide chlorpyrifos and its major metabolites diethylphosphate, diethylthiophosphate and 3, 5, 6-trichloro-2-pyridinol in the rat", Toxicology **237**, 145 (2007).

[19]. Cáceres, T., Megharaj, M. and Naidu, R., "Toxicity of fenamiphos and its metabolites to the Cladoceran *Daphnia carinata*: The influence of microbial degradation in natural waters", Chemosphere **66**, 1264 (2007).

[20]. Cáceres, T., Megharaj, M. and Naidu, R., "Toxicity and transformation of fenamiphos and its metabolites by two micro algae *Pseudokirchneriella subcapitata* and *Chlorococcum sp.*", Sci. Total Environ. **398**, 53 (2008).

[21]. Vega, L., Valverde, M., Elizondo, G., Leyva, J.F. and Rojas, E., "Diethylthiophosphate and diethyldithiophosphate induce genotoxicity in hepatic cell lines when activated by further biotransformation via Cytochrome P450", Mutat. Res. **679**, 39 (2009).

[22]. Vasilic, Z., Drevenkar, V., Stengl, B., Frobe, Z. and Rumenjak, V., "Diethylphosphorous metabolites in serum and urine of persons poisoned by phosalone", Chem. Biol. Interact. **87**, 305 (1993).

[23]. Vasilic, Z., Stengl, B. and Drevenkar, V., "Dimethylphosphorus metabolites in serum and urine of persons poisoned by malathion or thiometon", Chem. Biol. Interact. **119**, 479 (1999).

[24]. Anam, K. K. and Maitra, S. K., "Impact of quinaiphos on blood glucose and acetylcholinesterase (AChE) activity in brain and pancreas in a rose ringed parakeet (Psittacula krameri borealis: Newmann)", Arch. Environ. Contam. Toxicol. **29**, 20 (1995).

[25]. Das, B. K. and Mukherjee, S. C., "Sublethal effect of quinalphos on selected blood parameters of *Labeo rohita* (Ham.) fingerlings", Asian Fish. Sci. **13**, 225 (2000).

[26]. Debnath, D. and Mandal, T. K., "Study of quinalphos (an environmentaloestrogenic insecticide) formulation (Ekalux 25 E.C.)-induced damage of testicular tissues and antioxidant defence systems in Sprague–Dawley albino rats", J. Appl. Toxicol. **20**, 197 (2000).

[27]. Dikshith, T. S. S., Datta, K. K. and Raizada, R. B., "Effect of repeated oral administration of quinalphos to male goat (*Capra hircus*)", J. Biosci. **4**, 405 (1982).

[28]. Dwivedi, P. D., Das, M. and Khann, S. K., "Role of cytochrome P-450 in quinalphos toxicity: effect on hepatic and brain antioxidant enzymes in rats", Food Chem. Toxicol. **36**, 437 (1998).

[29]. Gupta, A., Agarwal, A. K. and Shukla, G. S., "Effect of quinalphos and cypermethrin exposure on developing blood-brain barrier: role of nitric oxide", Environ. Toxicol. Pharmacol. **8**, 73 (2000).

[30]. Vig, K., Singh, D. K. and Sharma, P. K., "Endosulfan and quinalphos residues and toxicity to soil microarthropods after repeated applications in a field investigation", J. Environ. Sci. Health B **41**, 681 (2006).

[31]. Ray, A., Chatterjee, S., Ghosh, S., Kabir, S. N., Pakrashi, A. and Deb, C., "Suppressive effect of quinalphos on the activity of accessory sex glands and plasma concentrations", Arch. Environ. Contam. Toxicol. **21**, 383 (1991).

[32]. Riediger, S., Behrends, A., Croll, B., Vega-Naredo, I., Hänig, N., Poeggeler, B., Böker, J., Grube, S., Gipp, J., Coto-Montes, A., Haldar, C. and Hardeland, R., "Toxicity of the quinalphos metabolite 2-hydroxyquinoxaline: growth inhibition, induction of oxidative stress, and genotoxicity in test organisms", Environ Toxicol. **22**, 33 (2007).

[33]. Chebbi, S. G. and David, M., "Respiratory responses and behavioural anomalies of the carp *Cyprinus carpio* under quinalphos intoxication in sublethal doses", ScienceAsia **36**, 12 (2010).

[34]. Sarkar, R., Mohanakumar, K. P. and Chowdhury, M., "Effects of an organophosphate pesticide, quinalphos, on the hypothalamo–pituitary–gonadal axis in adult male rat", J. Reprod. Fertil. **118**, 29 (2000).

[35]. Shukla, Y., Singh, A. and Mehrotra, N. K., "Evaluation of carcinogenic and co-carcinogenic potential quinalphos in mouse skin", Cancer Lett. **148**, 1 (2000).

[36]. Srivastava, L. P., Singh, R. P. and Raizada, R. B., "Phototoxicity of quinalphos under sunlight *in vitro* and *in vivo*", Food Chem. Toxicol. **37**, 177 (1999).

[37]. Srivastava, M. K. and Raizada, R. B., "Assessment of the no-observed-effect level (NOEL) of quinalphos in pregnant rats", Food Chem. Toxicol. **37**, 649 (1999).

[38]. Vairamuthu, S. and Thanikachalam, M., "The effect of quinalphos on blood and brain esterase activity in chicken", Indian Vet. J. **80**, 1160 (2003).

[39]. Praveen, U. S., Yogaraje, G. C. V., Gowtham, M. D., Nayak, V. G. and Mohan, B. M., "Quantitative densitometric determination of quinalphos in postmortem blood by HPTLC", Int. J. Med. Toxicol. Legal Med. **9**, 30 (2007).

[40]. Vasilic, Z., Drevenkar, V., Rumenjak V., Stengl, B. and Frobe, Z., "Urinary excretion of diethylphosphorus metabolites in persons poisoned by quinalphos or chlorpyrifos", Arch. Environ. Contam. Toxicol. **22**, 351 (1992).

[41]. Erah, P. O., Goddard, A. F., Barrett, D. A., Shaw, P. N. and Spiller, R. C., "The stability of amoxycillin, clarithromycin and metronidazole in gastric juice: relevance to the treatment of *Helicobacter pylori* infection", J. Antimicrob. Chemother. **39**, 5 (1997).

[42]. Stoeckel, K., Hofheinz, W., Laneury, J. P., Duchene, P., Shedlofsky, S. and Blouin, R. A., "Stability of cephalosporin prodrug esters in human intestinal juice: implications for oral bioavailability", Antimicrob. Agents Chemother. **42**, 2602 (1998).

[43]. Timchalk, C., Nolan, R. J., Mendrala, A. L., Dittenber, D. A., Brazak, K. A. and Mattsson, J. L., "A physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for the organophosphate insecticide chlorpyriphos in rats and humans", Toxicol. Sci. **66**, 34 (2002).

CONCLUSIONS

Agricultural development continues to remain the most important objective of planning and policy of a country. In the process of development of agriculture, pesticides have become an important tool as a plant protecting agent for boosting food production. No doubt, several countries are reaching to a state of self-sufficiency in food grains because of widespread applications of these agrochemicals but the darker side of their use envelops the total human environment. There is an alarming rise in the number of deaths due to pesticide poisoning and it is feared that the future generation will also be affected. This is mainly attributed to the flooding of the market with an increasing number of these toxic chemicals without properly testing their persistence and toxicity. In the developing countries the scenario in this regard is depressing. Despite restrictions and regulations on their use India accounts for one third of pesticide poisoning cases in the third world. With the growing awareness about the pesticide pollution the studies on decay profiles and formation of metabolites in water, soil, plants and animals have assumed a paramount significance. The present thesis is an endeavour in this direction and includes investigations on the parameters affecting the decay of quinalphos and thiram pesticides. Efforts have also been made to trace the metabolic pathways of the two pesticides. Based on the studies it is possible to draw some significant conclusions.

Under controlled laboratory conditions the decay of both the pesticides in water increases with the increase in temperature, pH and humic acid concentration. In the soil a change in pH from acidic to alkaline range enhances the rate of decay of thiram but in the case of quinalphos the behaviour of soil of neutral pH does not fall on the expected lines. This can be attributed to the fact that decay of pesticide in soil is very complex and may be governed by the different factors namely moisture, organic content and microbial activity other than the simple hydrolysis. This is aptly reflected by the fact that in acidic medium, the decay of quinalphos in soil is slower than in water but in alkaline medium, the

reverse is true. On comparing the $t_{1/2}$ of thiram in deionised water and soil at pH 5 and 8 it is observed that at the acidic pH the persistence of thiram in water is almost four times than that in soil of similar pH. But at alkaline pH the persistence of thiram in water is half as compared to the soil of similar pH. The persistence of quinalphos is exceptionally higher in acidic water and soil.

On the plants quinalphos and thiram pesticides follow a first order kinetics. The degradation is faster in the radish root than that on the radish leaf and tomato fruit. The faster decay rate in the root can be due to its moisture content and the microbial activity of the soil with which it is in contact. The slower decay on the tomato fruit and the radish leaf may be attributed to the fact that after the pesticide is applied on them, solvent gets evaporated and the pesticide remains reasonably in a dry state.

The pharmacokinetics of quinalphos in *in vitro* (simulated gastric and intestinal phase) shows that the degradation of the pesticide follows a first order kinetics. The degradation of quinalphos is faster in the gastric conditions. A faster degradation in simulated phases than that in the blank solutions indicates that the presence of pepsin and pancreatin enzymes is leading to a faster decay. The concentration profiles observed in the biological fluids of Wistar albino rats (*in vivo*) suggest that quinalphos after dosing is rapidly absorbed and reaches a peak concentration and thereafter it is metabolized.

The studies on the identification of metabolites of these pesticides reveal that they are formed as a result of a variety of reactions namely hydrolysis, oxidation, dealkylation, isomerization or thiono thiolo rearrangement, dimerization, methylation sulfuration, desulfuration, cyclization and photodegradation. The metabolic pathways seem to be complex and the different metabolites are observed with the change in the matrix. On the basis of metabolites obtained it appears that the metabolite formation in water, soil, plants, *in vitro* and *in vivo* is invariably initiated by hydrolysis.

In the case of quinalphos, hydrolysis is favoured both in acidic and alkaline water but based on the appearance of the byproducts the oxidation seems to be slower in acidic water. The formation of isoquinalphos is observed only in water (pH 8.0), soil (pH 5.1) and radish leaf and root. The absence of isoquinalphos in acidic water, alkaline soil, tomato fruit, *in vitro* and *in vivo* probably indicates towards its rapid hydrolysis to quinoxaline-2-thiol (VII) in the said conditions of the matrices.

The study on the identification of metabolites of quinalphos indicates that of all the investigated conditions dimerized products are observed only in acidic soil and intestinal digestion (*in vitro*). This suggests that the dimerized metabolites are unstable in most of the investigated matrices and are either not formed or are converted to other stable products. The compounds such as triethyl thiophosphate and triethyl phosphate are observed in *in vivo* studies only. It may be suggested that their formation is favoured by the animal enzymes. The metabolites quinoxaline and O-phenylene diamine are observed only in *in vivo* investigations. Probably ring cleavage is facilitated in the animal system. On comparing the results of studies on simulated and biological fluids with those obtained for water, soil and plants it can be concluded that the two metabolites 2-hydroxy quinoxaline and oxon which are known to be poisonous, persist for a longer period in all the investigated matrices.

The appearance of the mass peaks for the different metabolites of thiram suggests that N-dealkylation is not as prominent in the water as in the soil. A perusal of data further indicates that oxidation products are observed mainly in the water and soil and not in the plants. This suggests that in water and soil hydrolysis is followed by oxidation and dealkylation but in plants oxons are unstable. The pattern of metabolite formation in water and soil is more or less similar but cyclized products appear only in the plants. Of the different metabolites formed dimethyl dithiocarbamate and oxons are reported to be highly

toxic. Apparently they seem to persist for a longer period. The metabolites 2(N,N-dimethyl amino)thiazoline carboxylic acid and 2-thioxo-4-thiazolidine identified in the case of plants are also reported to be toxic.

It may be interesting to point out here that in some cases the half life of a pesticide in different media is nearly the same but the metabolites are not necessarily the same. This amounts to the fact that the half life of a pesticide cannot be the sole criteria to evaluate the environmental hazard. The results presented in the thesis also reflect that the pesticides quinalphos and thiram indirectly continue to be hazardous even after their substantial decay because of the formation of some metabolites which are known to be more toxic than the parent compound. The byproducts identified would be highly useful for providing a data bank of metabolites for forensic and epidemiological investigations. A number of metabolites identified during the decay cycle of both the pesticides have been reported by some earlier workers but some of the metabolites have been observed for the first time such as quinoxaline and O-phenylene diamine. The claim to have reported some new metabolites is purely based on the information available with the author. The toxicity of many of the metabolites could not be traced from the literature and it is difficult to comment upon their toxicological aspects.

This study cannot be rated as complete in itself but is definitely a pointer to predict the fate of quinalphos and thiram under the laboratory and field conditions. The author is conscious of the fact that in order to correlate residual and metabolic findings some more data should have been collected. But there are some constraints which cannot be always overcome.

APPENDIX

METABOLITES OF QUINALPHOS AND THIRAM

APPENDIX

SOME IMPORTANT METABOLITES OF QUINALPHOS

*S.NO.	METABOLITES
(II)	Quinalphos oxon (m/z 282)
(III)	O-ethyl-O-quinoxalin-2-yl phosphoric acid (m/z 254)
(IV)	Isoquinalphos (m/z 298)
(V)	2-hydroxy quinoxaline (m/z 146)
(VI)	Desethyl quinalphos (m/z 270)
(VII)	Quinoxaline-2-thiol (m/z 162)
(VIII)	Diethyl thiophosphoric acid (m/z 170)
(IX)	Trihydroxythiophosphoric acid (m/z 114)
(X)	Dihydroxy quinalphos oxon (m/z 226)
(XI)	Diethyl phosphoric acid (m/z 154)
(XII)	Monoethyl phosphoric acid (m/z 126)
(XIII)	Phosphoric acid (m/z 98)
(XIV)	Monosulphide dimer (m/z 290)
(XV)	Disulphide dimer (m/z 322)
(XVI)	Quinoxalin-2-yl hydrogen phosphonate (m/z 210)
(XVII)	O-quinoxalin-2 yl- O,O- dihydrogen phosphorothioate (m/z 242)
(XVIII)	Triethyl thiophosphate (m/z 198)
(XIX)	Quinoxaline (m/z 130)
(XX)	Triethyl phosphate (m/z 184)
(XXI)	O-phenylene diamine (m/z 108)

* The number of metabolites is on the basis of metabolic pathways (Figs. 3.7 and 5.3).

SOME IMPORTANT METABOLITES OF THIRAM

*S.NO.	METABOLITES
(II)	Dimethyl dithiocarbamate (m/z 121)
(III)	Dimethyl dithiocarbamoylsulfenic acid (MH^+ 138)
(IV)	Bis (dimethyl dithiocarbamoyl) trisulphide (m/z 272)
(V)	Methyl dimethyldithiocarbamate (m/z 135)
(VI)	Bis (methyl dithiocarbamoyl) disulphide (m/z 212)
(VII)	N,N-dimethyl carbamoyl-N,N-dimethyl thiocarbamoyl disulphide (m/z 224)
(VIII)	1, 1, 3, 3-tetramethylurea (m/z 116)
(IX)	Tetramethylthiuram monosulfide (m/z 208)
(X)	Dimethylcarbamo (thioperoxic) acid (m/z 105)
(XI)	1,1,3,3-tetramethyl-2-thiourea (m/z 132)
(XII)	Bis(Dimethyl carbamoyl) disulphide (m/z 208)
(XIII)	N-methyl-N,N-dimethyl dithiocarbamoyl-disulphide (m/z 226)
(XIV)	N-methyl-amino-dithiocarbamoyl sulphide (m/z 166)
(XV)	Bis (thiocarbamoyl) sulphide (m/z 152)
(XVI)	Bis (dithiocarbamoyl) disulphide (m/z 184)
(XVII)	2(N,N-dimethyl amino)thiazoline carboxylic acid (m/z 174)
(XVIII)	2-thioxo-4-thiazolidine (m/z 120)

* The number of metabolites is on the basis of metabolic pathways (Fig. 4.6).