LEVELS OF CHLORINE AND PHOSPHOROUS CONTAINING PESTICIDE RESIDUES ON SELECTED VEGETABLES IN ZAMBIA

BY

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DEDICATION

I dedicate this study to my husband, Michael, my children, Akamandisa and Sipho, my mother, Ethel and to the memory of my late father, Edward.

DECLARATION

I, PAULINE MANAYIWA TANAYE INAMBAO, hereby declare that the results in this work are a result of my own work, and that no work has been done on the samples used for the purpose of a degree in this or any other University.

P. M. Tanaye — 15-12-97

Signature — Date

APPROVAL

This dissertation of **Mrs. Pauline M. Tanaye Inambao** is approved as fulfilling the requirements for the award of the degree of Master of Science in Chemistry by the University of Zambia.

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ABSTRACT

Tomatoes and cabbage samples were analysed for chlorpyrifos and monocrotophos residues by Gas Chromatography (GC) with electron capture detector. One field of tomatoes and cabbage was treated for four weeks at the recommended dose of 0.6l/Ha monocrotophos and 2l/Ha chlorpyrifos. A second field treated twice in seven days with chlorpyrifos and monocrotophos. A th ird field was not treated at all. The analysis revealed that tomatoes sprayed twice only showed tolerable levels of chlorpyrifos residues while the monocrotophos residues exceeded these levels. No residues were detected after the twenty first day. The residues detected in the cabbage that was sprayed twice only rose to a maximum on the twenty first day after the initial spraying and then decreased to levels above the FAO/WHO Maximum Residue Limits (MRL's). Both tomatoe and cabbage sprayed weekly retained residues with cabbage showing higher levels than tomatoe. The residues detected in the cabbage, sprayed weekly, were as high as 18.268mg/kg for chlorpyrifos and 29.043mg/kg for monocrotophos i.e. well above the recommended FAO/WHO MRL's.

Twenty percent of the tomatoe and cabbage samples obtained from the markets were found to have residues exceeding the recommended MRL's. This study also showed that despite the same treatment, tomatoe and cabbage responded differently to chlorpyrifos and monocrotophos and as a result retained their residues in unequal quantities.

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LIST OF ABBREVIATIONS

Ach	Acetylcholine
a.i	active ingredient
ACP	Advisory Committee on Pesticide
ADI	Acceptable Daily Intake
BDH	British Drug House
^0C	degree Centigrade
ca	approximately
ChE	Cholinesterases
CH_2CI_2	Dichloromethane
cm	centimetre
DDT	Dichlorodiphenyltrichloroethane
df	film thickness
ECD	Electron Capture Detector
ECZ	Environmental Council of Zambia
EEC	European Economic Community
EPA	U.S. Environmental Proctection Agency
EPPCA	Environmental Protection and Pollution Council Act
equiv	equivalent
ext	extract
EU	European Union
FAO	Food and Agriculture Organisation of the United Nations

FDA	U.S. Food and Drug Administration
FPD	Flame Photometric Detector
GC	gas chromatograph
g	gram
Ha	hectare
HPLC	High Performance Liquid Chromatograph
IAEA	International Atomic Energy Agency
i.d.	internal diameter
i.v.	intravenously
JMPR	Joint Meeting on Pesticide Residues (FAO/WHO)
kg	kilogram
l	Litre
LD_{50}	Lethal dose 50 (the dose at which 50% of the test animals die after exposure)
MAFF	Ministry of Agriculture Food and Fisheries
ml	millilitre
mg	milligram
mg/kg	milligram per kilogram
MRL's	Maximum Residue Limits
NaCl	Sodium Chloride
ng	nano gram
Na_2SO_4	Sodium Sulphate Anhyrdrous

OP's	organophosphosphates
pet ether	Petroleum ether 40-60^0 (boiling point)
TCP	3, 5, 6, - trichloro-2-pyridinol
TLC	Thin Layer Chromatography
µl	micro litre
UK	United Kingdom
UNICEF	United Nation's International Children's Education Fund
USDA	U.S. Department of Agriculture
WHO	World Health Organisation
WWF	World Wildlife Fund International
V	volts

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CHAPTER 1

INTRODUCTION

Chemicals in one form or another have been used since the beginning of life, although their use to assist man to minimise food losses and destroy pests has increased phenomenally with the industrialisation of our society. Major uses of modern chemicals include pesticides, fertilizers, drugs and detergents. The significantly high consumption of chemicals has resulted in a wide range of environmental contamination. In spite of the environmental contamination due to a few persistent ones, chemicals in general have provided immense benefits with regard to the comfort and welfare of human populations. At this stage it appears impossible to abandon the use of chemicals. Table 1 illustrates the extent of chemical use by category in 1993-1994 in Zambia [9].

Table 1: Chemical Use by Category in Zambia

TYPE OF CHEMICAL	NUMBER OF TONS USED PER YEAR IN ZAMBIA(Metric tonnes)
Pesticides - Agriculture	1400
Pesticides - Public Health	600
Pesticides - consumer use	100
Fertilizers	71016
Petroleum Products	1 100,000
Industrial Chemicals (Used in manufacturing/ processing facilities)	125 490.9
Consumer chemicals	7 232.4
TOTAL	1 294 839.6

A pesticide is a substance capable of killing a pest which may be used to protect crops and animals meant for human consumption. This includes substances intended for use as a plant-growth regulator, defoilant. dessicant, fruit-thinning agent or an agent for preventing the premature fall of fruit and substances applied to crops either before or after harvest to prevent deterioration during storage or transportation **[39]**. Pesticides most commonly used are insecticides (to control insects), fungicides (to control fungi), herbicides (to control weeds), acaricides (to control ectoparasites particularly ticks), rodenticides (to control rodents), and nematicides (to control nematodes) **[34]** .They now form an integral part of commercial agricultural production worldwide. Third world pesticide consumption is dominated by insecticides **[6]**.

In developing countries such as Zambia, the pre-harvest and post-harvest crop losses are estimated by FAO to be in the region of 30% or more of the potential production **[15]**. Famine or the threat of famine afflicts many third-world countries. The developed countries enjoy an abundance of relatively cheap food in almost bewildering variety. The success of the more affluent countries in meeting the increasing food demands of their populace has been achieved in a number of ways including : (a) amplification of agricultural production, (b) control of losses during storage, (c) control of losses during distribution and in the home, i.e. increased shell-life. and (d) more efficient use of available resources.

A **pesticide residue** is any substance that remains on any substrate resulting from the use of a pesticide **[3]**.

Though the use of pesticides and other agricultural chemicals results in a substantial increase in yields of food stuffs they can also cause trade and health problems. It is estimated that 5000 deaths and 500,000 pesticide poisonings occur annually in the developing world **[42]**. In order to supply the population with an adequate amount and variety of normal food of high quality at an acceptable price, the use of inseticides, herbicides and fungicides in agriculture and food processing must be tolerated to some extent. Health protection however necessitates the establishment of a legislative framework to keep the use of pesticides within limits **[14]**.

In the United States of America, the US Environmental Ptotection Agency (EPA) is charged with regulating the development, distribution, use and disposal of pesticides. The Agency registers only those pesticides that meet their strict standards for human health, the environment and wildlife. The agency also establishes a tolerance for each pesticide it approves. A tolerance is the maximum residue of a pesticide legally permitted in or on a food. A tolerance ensures that, when pesticides are used according to label directions, the remaining pesticide residues will not pose an unacceptable health risk to anyone who consumes the food.

Tolerances are considered an enforcement tool and are used by the US Food and Drug Administration (FDA) in its monitoring program to ensure a safe food supply.

If any pesticide residue is found to exceed its tolerance on a food, then the food is not permitted to be sold. FDA enforces pesticide tolerances for all foods, except for meat, poultry and some egg products which are monitored by the US Department of Agriculture (USDA).

Most crops grown in the UK receive at least one, and some of them several applications of pesticides a year. Many of these applications include more than one active ingredient (a.i.).

Over the period 1990-1994, average annual pesticide usage on arable crops in Great Britain amounted to 29,200 tonnes of a.i.**[31]**.

The approval and registration procedure for new pesticides is administered by the Pesticides Safety Directorate, an executive agency of MAFF, and the Health and Safety Executive's Pesticides Registration Section (for non-agricultural pesticide usage) under the Control of Pesticides Regulations 1986 and Part III of the Food and Environment Protection Act 1985.

Data on each pesticide are evaluated by the independent Advisory Commitee on Pesticides (ACP) and an expert sub-committee. The data must include information on persistence and fate in soil, potential to leach or move into ground water and effects on target and non-target organisms. The biological and physical aspects of soils to which the pesticide will be applied are also taken into account. Approval is granted (or refused) and the statutory conditions of use specified, on the basis of the evaluation. ACP can review any pesticide already on the UK market and revoke or amend approval should problems become apparent.

The pesticide approval process is being harmonised in Europe by the EC Plant Protection Products Directive (91/414/EEC) which also requires Member states to review existing pesticides.The European Union (EU) is committed to reviewing about 90 active ingredients a year over the next ten years, for which manufaturers may have to provide additional information. The review could lead to the withdrawal of a number of older pesticides that may be toxic or degrade into products of high toxicity.

The Royal Commission on Environmental Pollution in its 19th report , endorsed the government policy of limiting pesticide use 'to the minimum necessary for the effective control of pests compatible with the protection of human health and environment'.

Integrated Pest Management (IPM) defined as 'The application of the best mix of environmentally sound techniques in order to keep pests below the damage threshold' is a practical approach to reducing the use or dependence on synthetic pesticides. IPM is based on the idea of an economic threshold below which pest infestation and damage can be tolerated, together with the use of a variety of pest control techniques (including chemical control) to anticipate and minimise damage **[31]**.

In Zambia the Environmental Protection and Pollution Control Act, EPPCA deals with pesticides and toxic substances. This regulation was passed on the 28th January 1994 i.e. The Pesticide and Toxic Substances Regulations no. 20 under Statutory Instrument 20 of 1994.

It deals with genral registration, labelling and pacgagind, general handling, use and safety of pesticides. It does not specifically deal with pesticide residue monitoring in the country **[42]**. The Food and Drugs Control Laboratory in Lusaka, has some tolerance levels for some of the pesticides and can be used to enforce these.

ORGANOPHOSPHATES

Organophosphorus pesticides are mainly used in agricultural production due to the fact that they are less persistant in the environment and decompose easily compared to the chlorinated pesticides e.g. Dichlorodiphenyltrichloroethane (DDT), that have been in use for many years and have been banned or severely restricted in most countries.

The world wide consumption of organophosphorus compounds from 1974-1983 is shown in table 2 **[40]**. Zambia used approximately 1400 metric tonnes in 1993-1994 **[9]**.

Table 2

Consumption of Organophosphorus Insecticides (in 100kg)[a]

Continent	1974-1976	1981	1982	1983
Africa	89475	14591	479	1121
North/Central America	121216	436913	412352	101714
South America	3249	13530	23285	961
Asia	205678	380788	208760	6524
Europe	251554	322108	275825	77433

[a] From WHO **[40]**

The insecticidal action of these compounds was discovered in Germany during the Second World War in the study of materials related to the nerve gases, sarin, soman and tabun. Initially the discovery was made in search of substitutes for nicotine, which was in critically short supply in Germany.

Some of these pesticides are extremely toxic both to mammals and to the lower animal pests to be controlled, while others are relatively harmless to mammals but very effective against pests **[16]**. They are normally esters, amides or thiol derivatives of phosphoric , phosphonic, phosphorothioic or phosphonothioic acids. Most of them are only slightly soluble in water and have a high oil-to-water partition coefficient and low vapour pressure **[42]**.

The organophosphates (OP's) exert their toxic action by tying up or inhibiting certain important enzymes of the nervous system, namely cholinesterases ChE. At the synapse an impulse is transmitted by acetylcholine (ACh), which is is then destroyed by the ChE enzyme so the synapse will be cleared of transmission. These chemical reactions happen within microseconds and continue constantly, as needed, under normal conditions. However, the OP's attach to the enzyme ChE in a way that prevents it from clearing away the ACh transmitter: in effect the transmission circuits jam because of the accumulation of Ach.

In mammals the accumulation of Ach interferes with the neuromuscular junction, producing rapid twitching of voluntary muscles, tremors, myosis, coma, convulsions, prostration, finally resulting in paralysis and death due to respiratory failure. Symptoms in insects follow the pattern of nerve poisoning: restlessness, hyperexcitability, tremors, convulsions and paralysis **[25, 38]**.

In some cases the pesticide degrades into products that are toxic e.g. the study of the persistence and metabolism of Fenthion in orange fruit. Fenthion degrades into five products/metabolites which are all taken into account when analysing for residues **[28]**.

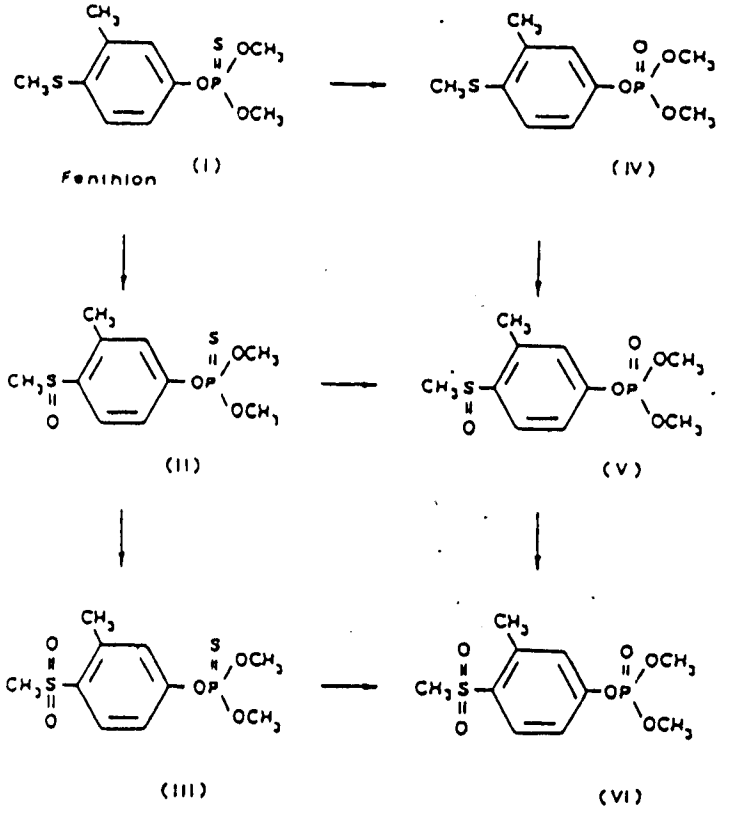
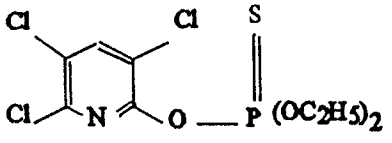


Figure 1. Fenthion (I) and its metabolites: fenthion sulfoxide (II), fenthion sulfone (III), fenoxon (IV), fenoxon sulfoxide (V) and fenoxon sulfone (VI).

Organophosphate insecticides commonly used in Zambia on a wide range of crops are chlorpyrifos and monocrotophos.

CHLORPYRIFOS

Chlorpyrifos whose structure is shown (below) was discovered by Dow Chemical Company in 1965 **[2,5,8, 9, 17]**.



It is a phosphorothioic acid, with the chemical name O, O-diethyl-O (3,5,6-trichloro-2- pyridyl) phosphorothioate and molecular fomula $C_9H_{11}C_{l3}No_3PS$. It has an oral Actute LD_{50} for rats in mg/kg body weight of 135 - 163 defined as very toxic in table 3. This value may be extrapolated to estimate the amount needed to kill a 77 kg human **[38]**. Its acceptable daily intake is 0,5 for tomatoe and 0.05 mg/kg body weight for cabbage **[11]**.

It is usually dispensed as a clear amba liquid of characteristic odour. It is emulsifable with water, has a flash point of 34°C, while its density can be measured in the laboratory. Chlorpyrifos is flammable.

Toxic effects due to cholinesterase inhibition are, headache, nausea, vomiting, incoordination, numbness of the tip of the tongue and face, apprehension, twitching, tremors, confusion and convulsions, laboured breathing and respiratory failure **[44]**.

If poisoning occurs administer atropine sulphate intravenously or intramuscularly if i.v. injection is not possible. Repeat every fifteen minutes until atropinization is achieved and observe patient closely for at least twenty four hours to ensure that symptoms do not reoccur as atropinisation wears off **[44]**.

Chlorpyrifos is a non-systemic insecticide with a wide spectrum of activity by contact, ingestion and vapour action. It is moderately persistent and retains its activity in soil for two to four months and is available against mosquito and fly larvae, cabbage root fly, aphids and codling and winter moths on fruit trees. Chlorpyrifos has become one of the most widely used insecticides **[8]**.

Chlorpyrifos both as a solid and in solution slowly gives rise to the toxin of the 3, 5, 6,-trichloro-2-pyridinol (TCP). This breakdown product can present a toxicological effect on livestock. In soil Chlorpyrifos is initially degraded to TCP and subsequently to organochlorine compounds **[2].**

Table 3 shows the ranges of lethal doses of pesticides.

Table 3

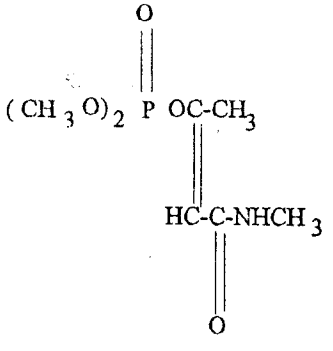
Combined tabulation of Pesticide Toxicity classes.-[b]

Routes of Absorption			
	LD_{90}	LD_{50}	
	Single oral dose for rats (mg/kg)	Single dermal dose for rabbits (mg/kg)	Probable lethal oral dose for humans
Toxicity rating			
6-Supertoxic	< 5	< 20	65 mg
5-Extremely toxic	5-50	20-200	5ml
4-Very toxic	50-500	100-2000	5ml to 30ml
3-Moderately toxic	500-5000	1000-2000	28.4ml to 473 ml
2-Slightly toxic	5000-15000	2000-20000	373.2ml to 1.14l
1-Practically nontoxic	>15000	>20000	>1.14l

[b] From: Ware (1983)**[38]**

MONOCROTOPHOS

Monocrotophos whose structure is shown below, is an aliphatic OP containing Nitrogen. It is a plant systemic insecticide, but has had limited use in agriculture because of its high mamalian toxicity.



This phosphoric acid is a product of the Cyanamid Company with the chemical name, dimethly 1- methyl-3 - (methylamino)-3-oxo-1-propenyl ester. Its molecular formula is C_7H_{14},No_5O. and its structure is given above **[4, 5, 40, 25]**. The oral Acute LD_{50} for rats is given as 14-23 mg/kg body weight **[40]**. Its acceptable daily intake is 1mg/kg for tomato and 0.2 mg/kg body weight for cabbage **[11]**.

Monocrotophos is usually dispensed as a clear reddish brown liquid with a characteristic odour. It has a density at 25^0 C of is $0.982g/cm^3$ and is flammable. Toxic effects due to cholinesterase inhibition are, burning sensation in the mouth, headache, nausea, vomiting, incoordination, numbness of the tip of the tongue and face, apprehension, twitching, tremors, confusion and convulsions, laboured breathing and respiratory failure **[44]**.

If poisoning occurs administer atropine sulphate intravenously or intramuscularly if i.v. injection is not possible. Repeat every fifteen minutes until atropinization is achieved and observe patient closely for at least twenty four hours to ensure that symptoms do not reoccur as atropinisation wears off **[44]**.

There has been very little agrochemical work done in Zambia in the past except that done jointly by FAO and the Ministry of Health on one hand and the World Wide Fund International and the Evironmental Council of Zambia on the other. One such study conducted in 1989 by FAO and the Ministry of Health showed very high residual values of dieldrin in mothers milk; 0.85-1.10mg/l **[30]**. As a result we do not have any readily available information on this subject. Therefore the objectives of the present work are as itemized below:

OBJECTIVES

(1) To provide reliable information on residue levels of monocrotophos and chlorpyrifos in tomatoes and cabbage in Lusaka, Zambia, at the time of sale.

(2) To establish whether residues from the use of insecticides, monocrotophos and chlorpyrifos in tomatoes and cabbage, fall within the maximum residue limits recommended by joint FAO/WHO reports, on pesticide residues in food.

1.3 ANALYTICAL METHODS

Chromatography, a term from the Greek word meaning "colour" and "to write", was first reported by the Russian scientist Tswett in 1906 **[7, 26, 35, 36]**. Tswett studied the pigments from chloroplasts. When filtering their solution in light petroleum through a narrow glass column filled with calcium carbonate he observed that the original mixture began to separate into coloured zones according to the strength of their absorption on the adsorbent. The zones moved through the column at various rates. If instead of the pigment-mixture solution only pure solvent was poured onto the column, the zones moved until their separation was complete. Tswett called the result a **chromatogram** and the method **chromatography** although he was fully aware that it could also be applied to colourless sustances. He demonstrated his findings first in 1903 in Warsaw and then in 1906 before the German Botanical Society. Beginning in 1940 Tiselius and Claesson developed classical procedures with the continuous observation of optical properties of solutions flowing out from chromatographic columns and classified chromatographic processes of all types into three groups differing in the principle of their execution and mechanism of the underlying processes. They are (1) **frontal analysis**, (2) **displacement chromatography**, (3) **elution chromatography**. At the same time Tiselius started the systemic study of column chromatography, Syinge's experiments with the isolation of acetylated amino acids from protein hydrolysates by extraction in funnels from the aqueous into the organic phase. Martin and Syinge constructed an extraction apparatus composed of forty vessels in which acetylated amino acids could be separated on the basis of their distribution constant between a counter current of water and chloroform. Both phases were mixed with a vibrator which pumped them through the apparatus.

However, in the same year (1941) the function of the apparatus was performed by a chromatographic column filled with silica gel particles. A mixture of acetylated amino acids introduced at the top of the column was fractionated according to the distribution constants of the components, similarly to the zones of dyes in Tswett's adsorption chromatography. This is how partition chromatograghy was discovered. For this discovery Martin and Syinge were awarded the Nobel Prize for chemistry in 1952 **[22, 23, 26].**

Chromatography is a separation technique, which encompasses a diverse and important group of separation methods that permit a scientist to separate, isolate and identify related components. Today Chromatography is taken to refer to the separation of components in a sample by distribution of the components between two phases; one that is stationary and one that moves, usually but not necessarily a column **[7]**.

All these methods however make use of a **stationary phase** and **mobile phase**. Components of a mixture are carried through the stationary phase by the flow of the mobile phase; separations are based on the differences in migration rates among the sample components.

CLASSIFICATION OF CHROMATOGRAPHIC METHODS

Chromatographic methods can be categorised in two ways. The first is based upon the physical means by which the stationary and mobile phases are brought into contact. In **column chromatography**, the stationary phase is held in a narrow tube through which the mobile phase is forced under pressure or by gravity.

In **planar chromatography**, the stationary phase is supported on a flat paper or in the interstices of a paper: here the mobile phase moves through the stationary phase by capillary action or under the influence of gravity **[35]**. Thin Layer chromatography (TLC) uses the same principle.

1.3.1 Column Chromatography

A more fundamental classification of Chromatographic methods is one based upon the types of mobile and stationary phases and the kinds of equilibria involved in the transfer of solutes between phases. Two general categories of column chromatography are **gas chromatography** and **liquid chromatography**. In the former, the mobile phase is liquid and can be performed in columns and on plane surfaces: in the latter it is gas and is restricted to column procedures.

The method most commonly used is called **elution chromatography** where elution is defined as a process whereby a solute is washed through a column by additions of fresh solvent. A single portion of sample dissolved in mobile phase, is introduced at the head of the column whereupon the components of the sample distribute themselves between the two phases. Introduction of additional mobile phase (**the eluent**) forces the solvent containing a part of the sample down the column, where further partition between the mobile phase and fresh portions of the stationary phase occurs. The average rate at which a solute migrates **depends upon the fraction of time it spends in that phase**. Ideally the resulting differences in rates cause the components of a mixture to separate into bands located along the length of the column. If a detector that responds

to solute concentration is placed at the end of the column and its signal is plotted as a function of time (or of volume of added mobile phase), a series of symmetric peaks is obtained.

Such a plot called a **chromatogram** is useful for both quantitative and qualitative analysis. The positions of the peaks on the time axis may serve to identify the components of the sample; the areas under the peaks provide a quantitative measure of the amount of each component **[35]**.

Columns

Packed columns are constructed from tubing stainless steel, nickel or glass. Inner diameters may range from 1.6 to 9.5 mm while length is often 3m. These columns are packed with an inert support, usually a diatomaceous earth whose internal pore diameters range from 2 m for material derived from firebrick to 9m for materials derived from filter aids. The internal diameter should be at least eight times the diameter of the support particles. For example, the best particle size is 100/120 mesh (149-125μm) for 2mm-bore column, and 80/100 mesh (177-149 μm) for 4-mm-bore colums **[41]**.

Examples of commonly used mixed phase column materials used in pesticide residue analysis are:

a) 2.5% OV-101 + 3.75% Qf-1 on Chromosorb W (P), (80-100 mesh)

b) 2% OV-101 + 3% OV 210 on Chromosorb 750, (80-100mesh)

c) 1.5% SP2250 + 1.9% SP2401 on Supleçoport, (100-120 mesh) **[13]**.

Currently, the use of capillary columns has gained favour for residue analysis since, much better peak resolution is obtained.

Fused silica is utilized for capillary columns in preference to glass because of better flexibility, strength and inertness.

The high tensile strength of the silica tubing permits the construction of thin-walled flexible columns. To protect the thin wall against scratches, a protective coating of polymide is applied to the outer wall. There are two main types of capillary columns: (1) pakced columns with solid particles over the whole diameter of the column (micropacked) and (2) open tubular columns with an open and unrestricted flow path through the middle of the column. The latter are divided into wall-coated open tubular (WCOT) columns, suppoort-coated open tubular (SCOT) columns and porous-layer open tubular (PLOT) columns **[41]**.

For pesticide residue analysis, typically a 0.3mm i.d. column, 30 meters in length is used, with a film of 0.25 microns DB-5 (polymethyl (5%phenyl) siloxane) **[13]**.

Capillary Injection Systems

The narrow diameter of capillary columns and the low carrier gas flow rate, makes it necessary to use an injection system which will result in the sample being deposited in a band at the start of the column narrow enough to produce the expected degree of resolution.

Three systems have been developed to accomplish this:

a) Inlet Splitters

The sample is vapourised in the injection chamber and a small portion only is deposited upon the column, the remainder being rapidly flushed out of the chamber by carrier gas.

b) Splitless Injection

It is generally applicable to dilute solutions and for small amounts of components **[13]**..

c) 0n column Injection

The sample, of a few microlitres is introduced into the column with a syringe. The needle penetrates the septum during the application until it reaches a small plug of silylated glass wool, in front of the column packing proper **[26]**.

Ovens

Chromatographic columns are coiled and held in basket that is mounted inside an oven. The column oven must be able to be rapidly heated and cooled. This requires a well-designed and adequate system of air flow. In most designs the air is blown past the heating coils, then through baffles that make up the inner wall of the oven, past the column, and back to the bower to be reheated and circulated. Ovens are usually constructed of low-mass stainless steel **[41]**.

Gas chromatography is unquestionally the most widely used determinative procedure for the analysis of Organophosphorous pesticides and many of their metabilites. The procedure is rapid, since a gas can move more rapidly through a packed column than a liquid, provides a good resolution for determining multicomponent residues, and through the use of highly sensitive and specific detectors, trace level residues are quantified with a high degree of precision and accuracy.

This coupled with the very small sample required explains the popularity of the technique **[20]** e.g. the use of GC in the detn of Methyl Bromide and Inorganic Bromide in Fumigated produce i.e. cultivars of sweet celery, apple, rashi, plum and red and green capsicums **[18]**.

The two most widely used detectors for Gas Chromatography analysis of pesticides are the Alkali Flame Ionisation Detector (AFID) and the Flame Photometric Detector (FPD). The AFID is based on the phenomena that the flame ionisation detector (FID) yields enhanced response to heteroatoms in the presence of alkali metal salts. The Electron Capture Detector (ECD) is sometimes used for organophosphorous pesticides, though it is a rather poor choice for the general analysis of organophosphorous pesticides, because of its insensitive response to many of the compounds (especially those with a P=O moiety), the requirement for extensive cleanup and its lack of specificity **[4]**.

Instruments are commercially available in which the gas effluent is automatically fed into a mass spectrometer where they are positively identified according to mass (formula weight and fragmentation pattern).

This important analytical technique is called **gas chromatography-mass spectromtry (GC-MS)** and is capable of identifying and quantifying unbelievably complex mixtures of trace substances **[7 20]** e.g. the determination of Diaminozide and 1,1 - dimethylhydrazine (UDMH by a sensitive gas chromatographic mass spectrometric method in apple juice concentrates) **[32]**. GC is sensitive enough to detect picogram levels.

1.3.2 High-Performance Liquid Chromatography (HPLC)

This includes four basic types of column chromatography in which the mobile phase is a liquid. the four include **partition chromatography**, **adsorption chromatography, ion-exchange chromatography** and **size-exclusion** or **gel chromatography**.

a) Partition chromatography can be sub-divided into **liquid-liquid** and **bond-phased** chromatography. The difference in these techniques lie in the method by which the stationary phase is held on the support particles of the packing. With liquid-liquid, retention is by physical adsorption while with bonded-phase, covalent bonds are involved.

b) Adsorption chromatography or liquid-solid chromatography is based on liquid chromatography and has been adapted to become an important member of HPLC methods. The only stationary phases that are used for liquid-solid HPLC are silica and alumina with silica being preferred for most but not all applications because of its higher sample capacity and its wider range of useful forms.

Adsorption chromatography is best suited for nonpolar compounds having molecular weights less than 5000. A particular strength of adsorption chromatography, which is not shared by other methods, is its ability to differentiate among components of isomeric mixtures.

c) Ion-Exchange chromatography is based upon exchange of equilibria between ions in solution and ions of like sign on the surface of an essentially insoluble, high-molecular-weight solid.

d) Size-Exclusion chromatography is a powerful technique that is particularly applicable to high-molecular weight species. The average residence time in the pores depends upon the effective size of the analyte molecule. Molecules that are larger than the average pore size of the packing material are excluded and thus suffer no retention; such species are eluted. Molecules having diameters that are significantly smaller than the pores can penetrate throughout the pore maze and are thus entrapped for the greatest time and are the last to be eluted. Size-exclusion separations differ from the other procedures in the sense that no chemical or physical interaction between analytes and the stationary phase are involved. One useful application of this procedure is to the separation of high-molecular weight, natural-product molecules from low-molecular weight species and from salts **[35]**.

e) Perfusion Particle Chromatography was introduced recently as a novel development in the field of HPLC for the rapid separation of bimolecules. The advantage this method affords over conventional reverse-phase HPLC is the ability to operate at very high flow rates while maintaining both high sample loading capacity and chromatographic resolution. The principal advantage this technique brings therefore is speed, a critical operating parameter for industries in which major focus is either process development or high throughput applications **[19]**.

1.3.3 Planar Chromatography

Planar Chromatography takes three forms, namely thin-layer (TLC), paper (PC), and electrochromatography. Planar Chromatography differs from its column counterparts in the respect that the stationary phase is a flat, relatively thin layer of material, which may be self-supporting or may be supported on a glass, plastic or metal plate. Currently, most planar chromatography is based upon the thin-layer technique, which is faster, has better resolution and is more sensitive than its paper counterparts.

Typical thin-layer separations are performed on a glass plate that is coated with a thin and adherent layer of finely divided particles, which constitute the stationary phase.

Generally, in thin-layer chromatography samples are introduced onto the coated plates as dilute solutions (0.1 to 1μg/μl) in a volatile solvent. A capillary tube or a micropipet is employed to

deliver a drop of this solution to a spot that is 1 to 2cm from the end of the plate. After evaporation of the solvent the chromatogram is developed by the flow of the mobile phase over the surface. After development is judged to be complete, the flow of the mobile phase is discontinued and the position of the **solvent front** is marked.The position of the separated species is located by a variety of methods, a common method involving spraying the plate with a colorimetric reagent which permits location of the analytes visually. A common example is Palladium Chloride which gives greenish spots for organochlorine and yellowish spots for organophosphorus pesticides.

Enzymes from bees heads or rat livers may be used to study organophosphorous residues. Developed TLC plates are air dried then dipped in an iodine tank, heavily sprayed with the enzyme and incubated at 37 degrees for at least thirty minutes. Where the enzyme is inactivated with the pesticide the plate remains white (unchanged), while visible spots are observed where the enzymes are activated by the pesticide. This method is time consuming, with low sensitivity and non specificity and thus not the most ideal.

The absorbance of the developed chromatogram may be recorded as a function of distance from the original line. The areas and peak heights of the two solute peaks then serve as the basis for quantitative analysis **[35]**.

Separations by paper chromatography are performed in the same way as those on thin-layer plates. Usually, special papers, which are highly purified and reproducible as to porosity and thickness, are employed. Also available commercially are special papers that contain an

adsorbent or an ion-exchange resin, thus permitting adsorption and ion- exchange paper chromatography.

Generally thin-layer chromatography is superior to paper chromatography in resolution, compactness of spots and speed.

Electrochromatography involves electrophoresis which is defined as the migration of particles under the influence of an electrical field i.e. of individual ions and colloidal aggregates. Electrochromatography makes possible the separation of proteins and other large molecules contained in serum, urine, spinal fluid gastric juices and other body fluids.

Other determinative procedures available for organophosphorous pesticides are the following:

1.3.4. SPECTROPHOTOMETRIC METHODS

Infrared Spectrophotometry offers the potential for determining an unusually large number of substances because nearly all molecular species absorb in the infrared region. Moreover, the uniqueness of an infrared spectrum provides a degree of specificity that is matched or exceeded by relatively few other analytical methods **[36]**. Identification of the functional groups in a molecule is seldom sufficient to permit positive identification of the compound and the entire spectrum from 2.5 to 1115 μm must be compared with that of known compounds. Obviously peak overlaps are to be expected with so many compounds absorbing; nevertheless, the method does provide a moderately high degree of selectivity. These are not good for residue analysis

because of the low levels of analytes. e.g the simultaneous detn of carbaryl, chlorpyrifos and its metabolite 3, 5, 6 - Trichloro-2-pyridnol (TCP) by derivative spectrophotometry. This is actually the direct detn of the degradation of a pesticide formulation by measurement of TCP **[2]**.

1.3.5 POLAROGRAPHY

Polarography may be used in pesticide residue analysis especially for organochlorine compounds. Polarography is a class of voltammetry in which the working electrode is a **dropping mercury electrode (DME)** used to investigate solution composition by the reduction or oxidation of ions or molecules at the electrode. This results in current production or comsumption respectively, at the indicator electrode and is displayed as a sigmoidsal curve when current is ploted against applied potential. Polarography is useful for residue analysis of a numbe rof insecticides, both chlorinated (e.g. DDT (p.p-dichlorodiphenylrichloroethane) gives a well developed wave at - 0.9 V in 96% ethano containing lithium and tetraalkylammonium salts) and non chlorinated (e.g. dithiocarbamates, pyrethrins, rotenone) **[7, 21].** This method is usually not used because its selectivity is very poor and many nitrogen compounds are reduced at the same potential thus necessitating a separation technique before analysis **[37]**.

1.3.6. CHEMICAL REACTIONS

The most commonly used ones are colorimetric reactions. In this technique white light is passed through a sample and the percentage of energy absorbed is recorded and related to the sample

properties **[21]**. These employ an instrument for absorption measurements in which the human eye serves as detector. One or more color standards are required. The limits in sensitivity and the quality of the information have discouraged use of this technique **[24].**

CHAPTER 2

EXPERIMENTAL METHODS AND MATERIALS

Apparatus and Reagents

(a) Solvents - Acetone AnalaR from Riedel - de Haen, Aktiengesellschaht Wunstorfer StraBe 40, P.O. Box D-3016 Seelze 1

Hanover Germany

CH_2Cl_2 AnalR from BDH Chemicals Ltd, Broom Road Poole BH 124NN England, pet ether 40-60°C, from Riedel-de-haen, Germany

(b) Sodium Sulphate - anhydrous from BDH

(c) Glass wool from BDH - rinsed with acetone and ethanol several times and dried

(d) An Osterizer High Speed Blender

(e) Kuderna - Danish concentrator - manufactured at National Council for Scientific Research, Lusaka - ca 500 ml with Snyder column and fitted with receiving tube marked at 2cm volume, with hooks held together by rubber bands.

(f) Pyrex Separatory funnels - 250 and 500ml, with Teflon stopcocks.

(g) A Chrompack CP 9002 Series Chromatograph fitted with an Electron Capture Detector, ECD (inlet splitter) with a Hewlett Packard Integrator 1024 Low Emmission. A 50m capillary column of WCOT fused gel with i.d. of 0.25mm and df=0.12μm with the stationary phase CPSil 8CB for presticides.

The working temperatures were: Column 50 - 270° C, Detector 270°C, Injector 200°C, Nitrogen from a LAIND nitrogen generator served as the carrier gas. The flow rate was 40 ml per minute.

(h) Reference Standard Materials - were obtained from the manufacturers ie. Dursban (DowElanco, Belgium) and Azodrin (American Cyanamid Company)

SAMPLING

In the first experiment a field of six week old tomatoes and cabbage was divided into three portions. The control which was not sprayed with any pesticide, a second portion was sprayed twice in the first week only and the last weekly for the remaining four weeks before the end of harvest.

Two tomatoes were randomly picked from given plants and two cabbage leaves were randomly picked from five plants in a specific portion, packed in new polythene bags and frozen in deep freezer.

Both tomatoes and cabbage were sampled weekly for six weeks to the end of the harvest period of the crop.

In the second experiment, tomatoes and cabbage were obtained from markets within Lusaka, namely Soweto Town Centre and Luburma markets. Cabbage was also obtained from Shoprite Checkers shop. A number of these samples were traced back to where they had been grown.

Some tomatoes were traced to Grasmere and Mayflower Farms in Lusaka West, Masebe and Gravistone Farms in Mkushi and unamed farms in Mazabuka and Mumbwa. Some of the cabbage was traced back to J & Y Estates and Natural Resources Development College along Great East Road in Lusaka. Other samples could not be traced back to where they had been grown. All these samples were deep frozen for six to ten months before extraction..

SAMPLE EXTRACTION

Preparation of samples was done by the official methods of AOAC, of 1990 [1]. Each individual frozen sample was finely chopped and 100g weighed into a high-speed blender jar. 200 ml of acetone was added and blended for two minutes at high speed.

This was filtered with suction through a 12cm Buchner funnel fitted with filter paper previosly rinsed with acetone. The filter paper was rinsed before filtration of the sample so as to remove artifacts that could interfere with the analysis. Filtration was complete within a minute and collected in a 500ml suction flask. The extract was then transferred into clean, dry BDH bottles and frozen.

After one to six months later 80 ml of the sample extract was placed in 1l separatory funnel and 100ml petroleum ether (40-60°C) and 100ml CH_2Cl_2 added. The separatory funnel was shaken for one minute upon which the sample separated into two distinct layers. The lower aqueous layer was transferred to a second 500ml separatory funnel. The upper organic layer in the first separatory funnel was dried by passing through ca 200-250 g anhydrous Na_2SO_4 supported on

washed glass wool i.e. glass wool rinsed in acetone and alcohol and then dried, in 10cm funnel, collecting in 500ml Kuderna-Danish concentrator fitted with calibrated receiving tube. To the separatory funnel with aqueous phase 7g of NaCl was added and vigorously shaken for 30 seconds until most of the NaCl had dissolved. 100 ml CH_2Cl_2 was added, shaken for one minute and the lower organic phase dried through the same Na_2SO_4. Extraction of the aqueous phase was done with additional 100ml CH_2Cl_2 and dried as above. The Na_2SO_4 was rinsed with 50ml CH_2Cl_2. The Snyder column was attached onto the Kuderna-Danish concentrator, boiling chips added and slow evaporation started by placing the receiver tube into steam. After ca.one hour 100-150 ml had evaporated the concentrator was exposed to more steam until ca 2ml liquid remained in the hot concentrator tube. Through the Snyder column 100ml of petroleum ether (40-60°C) was added and reconstituted to ca 2ml. Thereafter 50ml of petroleum ether was added and the reconcentration step repeated. 20 ml of acetone was then added and reconcentrated to ca 2ml. The volume of the extract was adjusted to 7ml with acetone and refridgerated in well stoppered pyrex vials.

One hour before injection of samples into the GC the samples were put at room temperature to allow them to come to equilibrium.

The calculation of the equivalent sample weight was as follows :

$$\frac{\text{mg sample equivalent}}{\mu\text{l final extract}} = 100 \times \frac{80}{200 + W - 10} \times \frac{1}{\text{ml final volume}}$$

where 200 = ml acetone blended with 100g sample; W = amount(ml) H_2O present in sample; and 10 = adjustment for water-acetone volume contraction. Thus, when sample contains 85% H_2O (85ml/100g) and the final extract volume is 7ml, each micro litre contains:

$$100 \times \frac{80}{200 + 85 - 10} \times \frac{1}{7} = 4.15\text{mg sample equivalent / } \mu\text{l final extract}$$

i.e. 4.15mg sample equivalent per microlitre of final extract.

For tomatoes the water content value used was 93.4 g while that of cabbage was 89.7 g per 100 g sample **[10].**

These values gave 4.03 mg sample equivalent /µl for tomatoes and 4.09 mg sample equivalent µl for cabbage. One microlitre of sample was injected into the G.C.

Approximate retention times were obtained from repeated runs of the standards as: 12.55 minutes for Monocrotophos and 16.18 minutes for Chlorpyrifos.

Chlorpyrifos and monocrotophos standards were prepared as follows:

100mg of the standard was taken in 100ml of acetone and well shaken in a 100ml volumetric flask to make 1000mg/l of standard. 10ml of this 1000mg/l standard was pipetted into a 100ml volumetric flask and made up to the mark with acetone to prepare 100mg/l of standard. From the 100mg/l standard 5ml was pipetted into a 10ml volumetric flask and made up to the mark with acetone to prepare the 50mg/l standard. Similarly 5ml, 4ml, 3ml, 2ml 1ml and 0.5ml were pipetted into 10ml volumetric flasks and made up to their mark with acetone to prepare 50mg/l, 40mg/l, 30mg/l, 20mg/l, 10mg/l and 5mg/l of standards respectively.

CHAPTER 3

RESULTS

All control samples for both tomatoes and cabbage gave no significant amounts of either chlorpyrifos or monocrotophos residues. They gave peaks of other organochlorines and organophosphates that had earlier been sprayed on the crops.

The retention times, peak areas , relative peak areas of the pesticides were calculated by the programme in the computer . The peak areas corresponding to the retention times of Chlorpyrifos and Monocrotophos for the standard solutions were plotted against their respective concentrations.

The graphs obtained from a plot of areas versus concentrations in mg/l of the standards were not linear due to experimental random errors as shown in Figure 3.1a and 3.1b.

Figure 3.1a

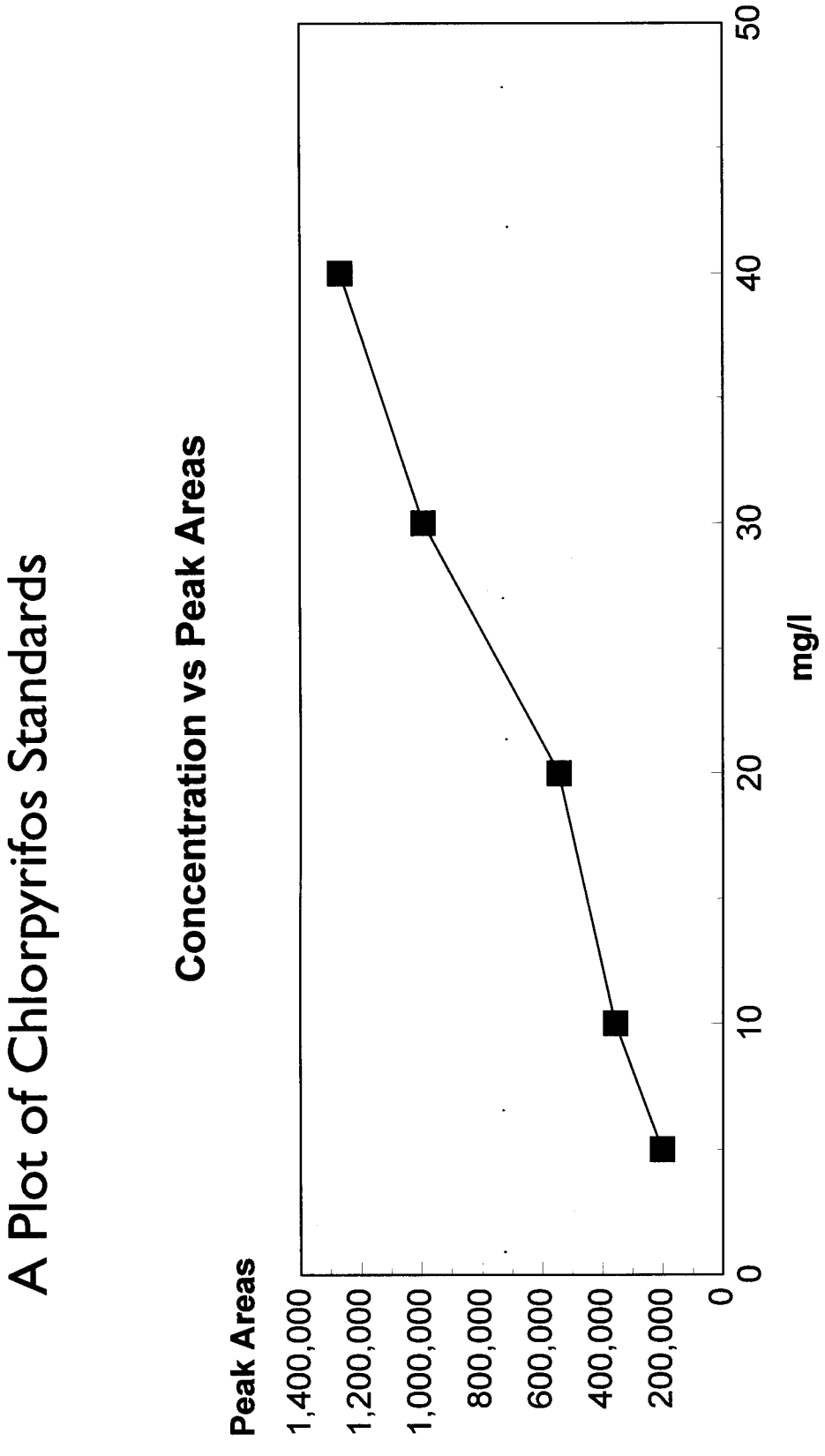
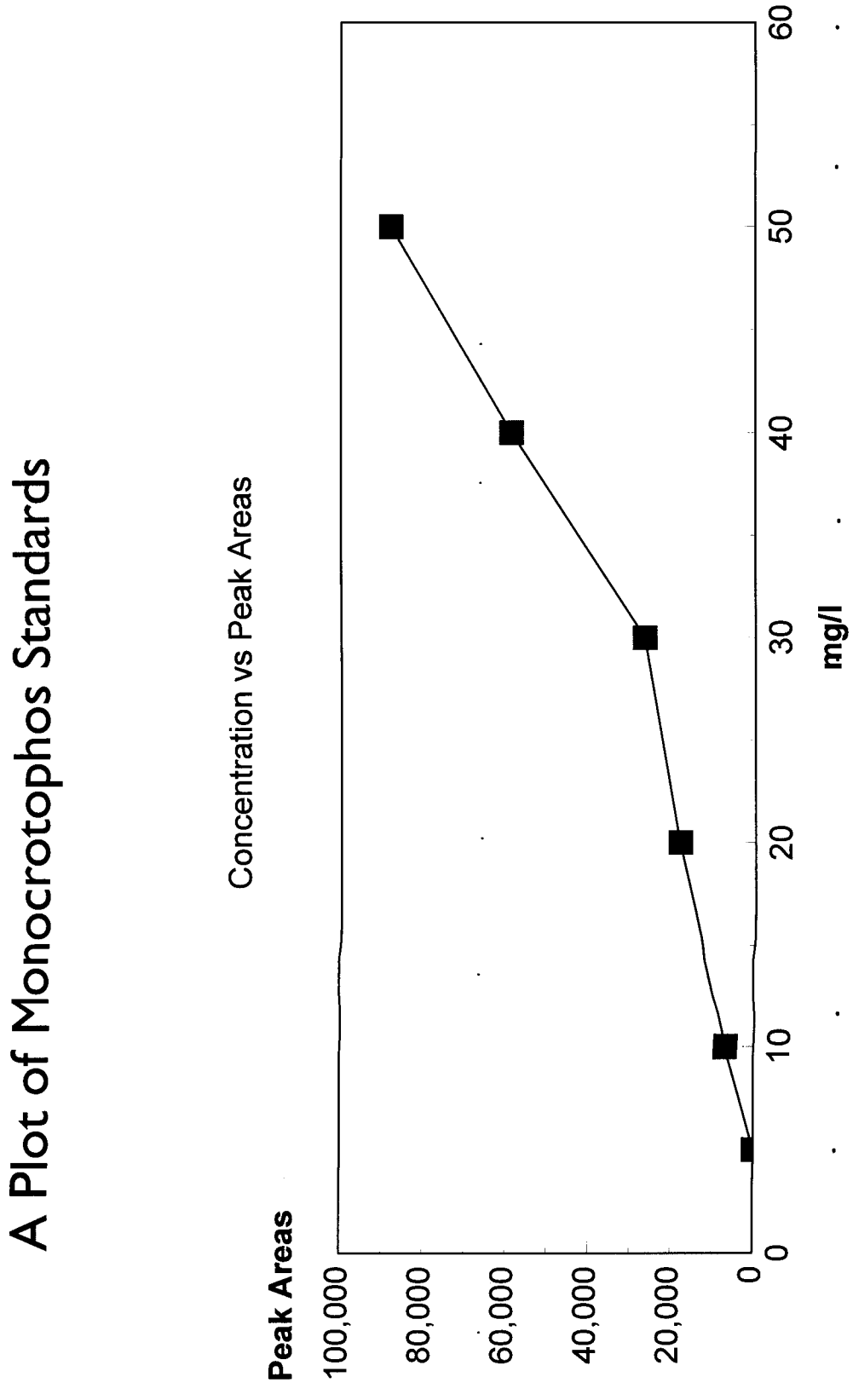

A Plot of Chlorpyrifos Standards
Concentration vs Peak Areas
Peak Areas
1,400,000
1,200,000
1,000,000
800,000
600,000
400,000
200,000
0
0
10
20
30
40
50
mg/l

Figure 3.1b


A Plot of Monocrotophos Standards
Concentration vs Peak Areas
Peak Areas
100,000
80,000
60,000
40,000
20,000
0
0
10
20
30
40
50
60
mg/l

Figures 3.2 show chromatograms of the standards, chlorpyrifos and monocrotophos i.e. 5, 10, 20, 30, 40 and 50 mg/l.

Note that all the chromatograms under 3.2 give a peak at 5 minutes for the solvent, acetone. The other peaks are from organochlorines and organophosphates sprayed on the vegetables other than chlorpyrifos and monocrotopho whose peaks appear at c.a. 16.18 and 12.55 minutes respectively.

3.2a labelled c:\maestro\chrom\97021806 and 3.2b labelled c:\maestro\chrom\97021807 show the 5mg/l and 10mg/l standards respectively . Clearly seen in both chromatograms are the chlorpyrifos peaks at retention time of c.a. 16.18 minutes. No peak is present at the monocrotophos retention time. This indicates that the EC detector is indeed very sensitive to halogen containing substances and as such monocrotophos with no halogen and in such low concentration of 5mg/l cannot be detected and produce a peak.

Figure 3.2a chromatogram of 5mg/l standards

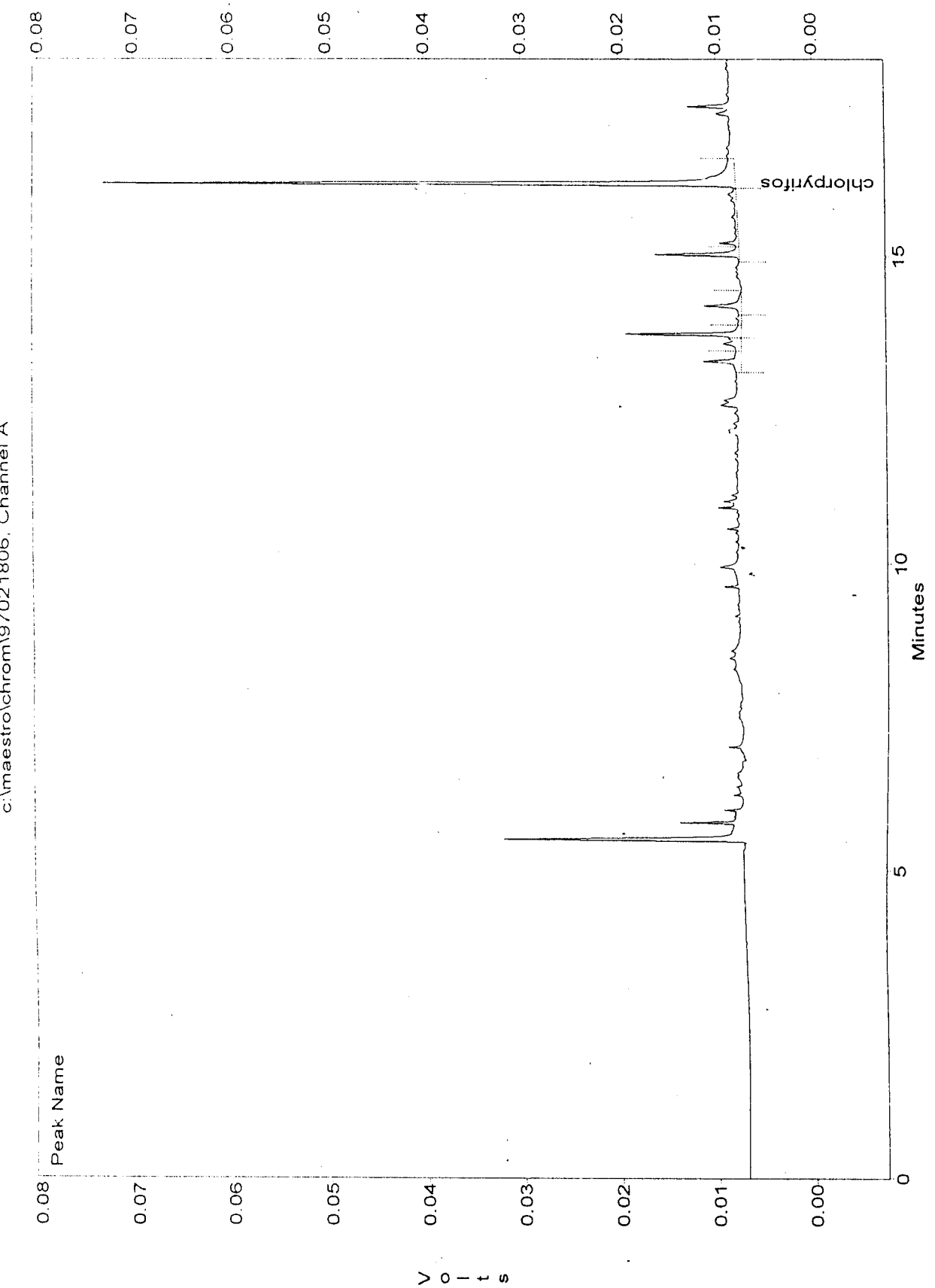
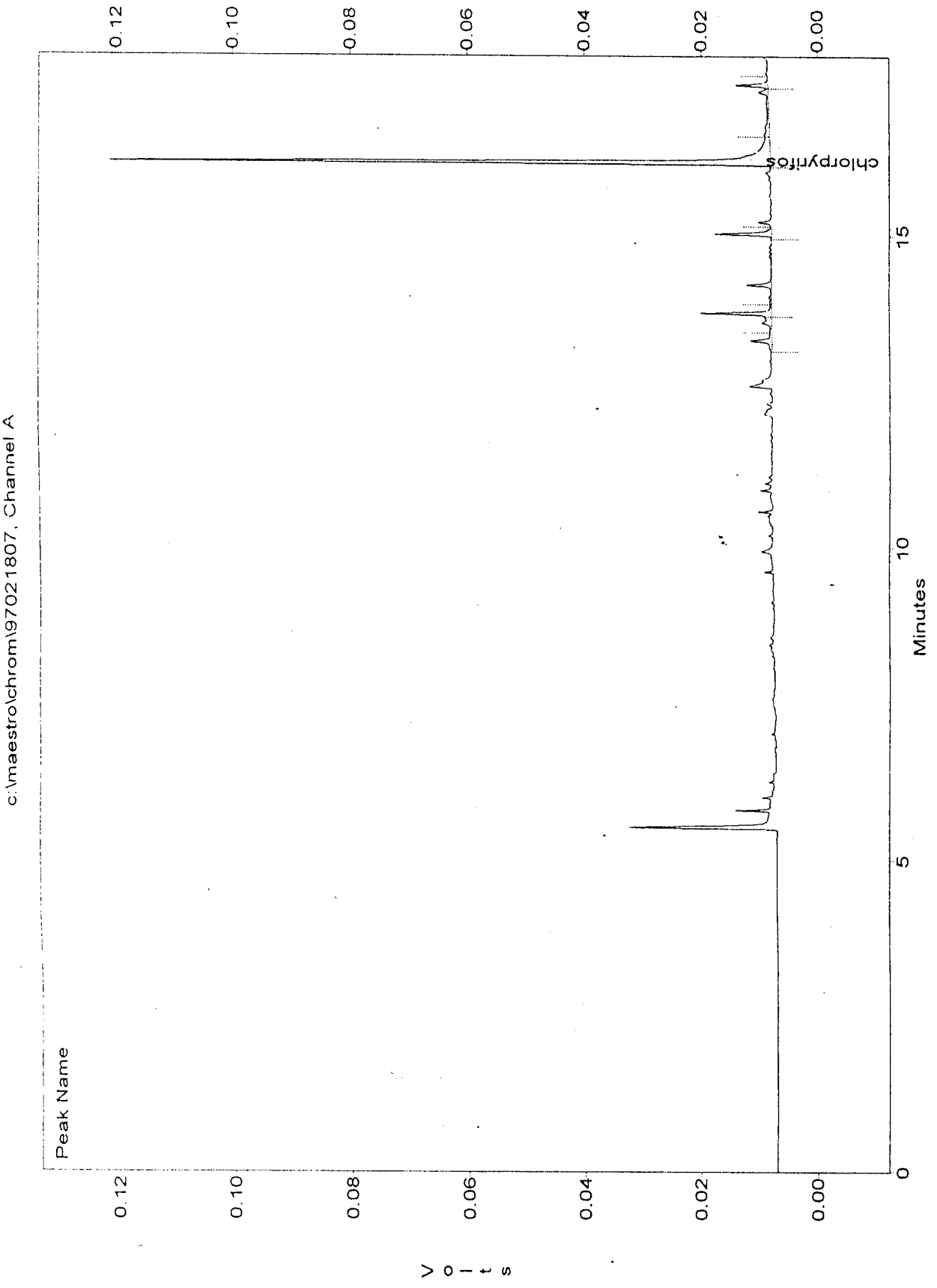


Figure 3.2b chromatogram of 10mg/l standards



Figures 3.2c labelled c:\maestro\chrom\97021808 and Fig 3.2d labelled c:\maestro\chrom\97021809 show the 20mg/l standard and the 30mg/l standard for chlorpyrifos and monocrotophos. Peaks for both pesticides are registered. Though the monocrotophos peak is much lower than that of chlorpyrifos it shows that at a concentration of 20mg/l the Electron Capture Detector is able to detect and register its presence.

Figure 3.2c chromatogram of 20mg/l standards

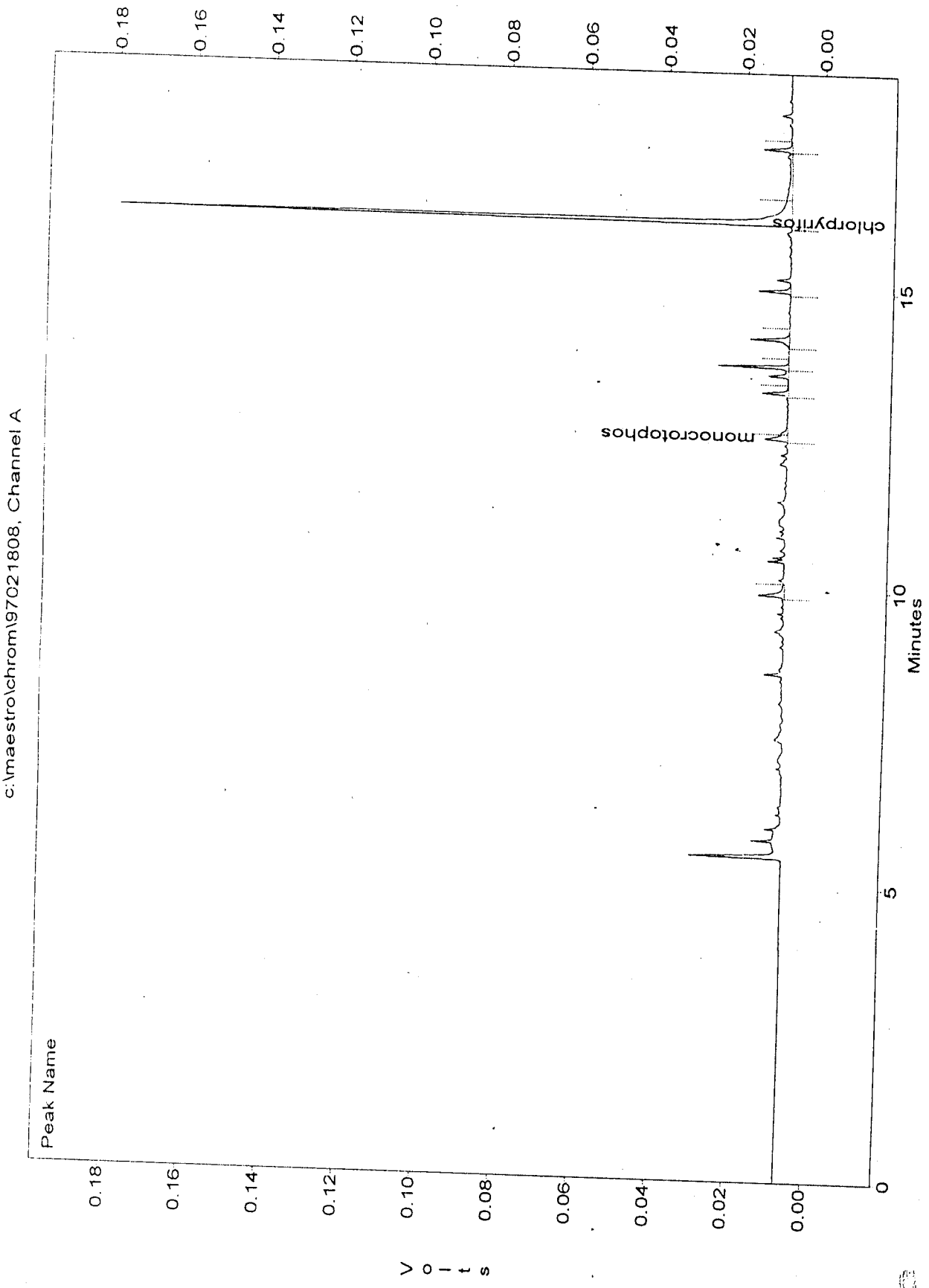
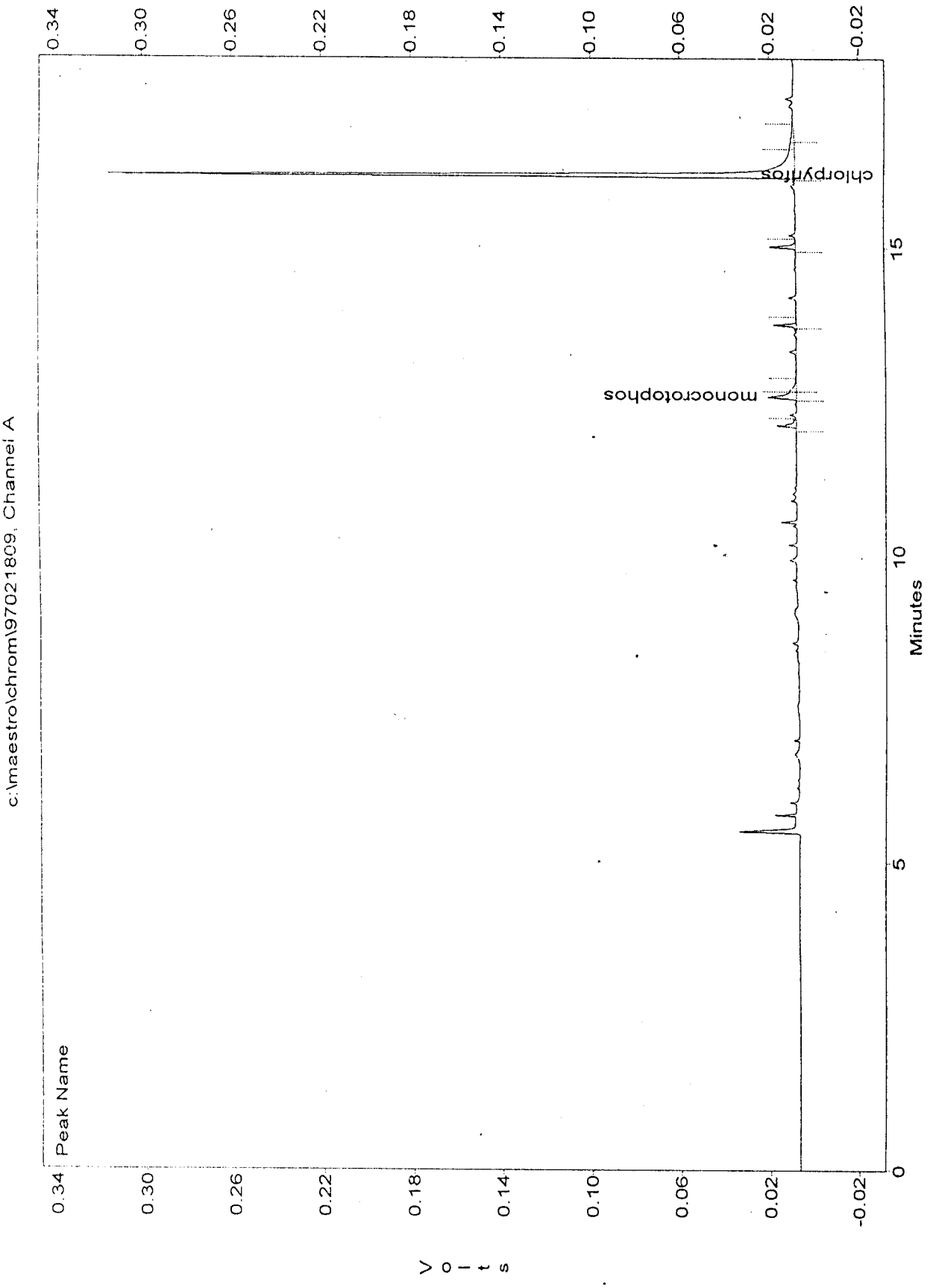


Figure 3.2d chromatogram of 30mg/l standards



Figures 3.2e labelled c:\maestro\chrom\97021810 and 3.2f labelled \maestro\chrom\97021812 show the 40mg/l standard and the 50mg/l standard. These two chromatograms are similar. Both peaks are shown at their respective retention times.

Figure 3.2e chromatogram of 40mg/l standards

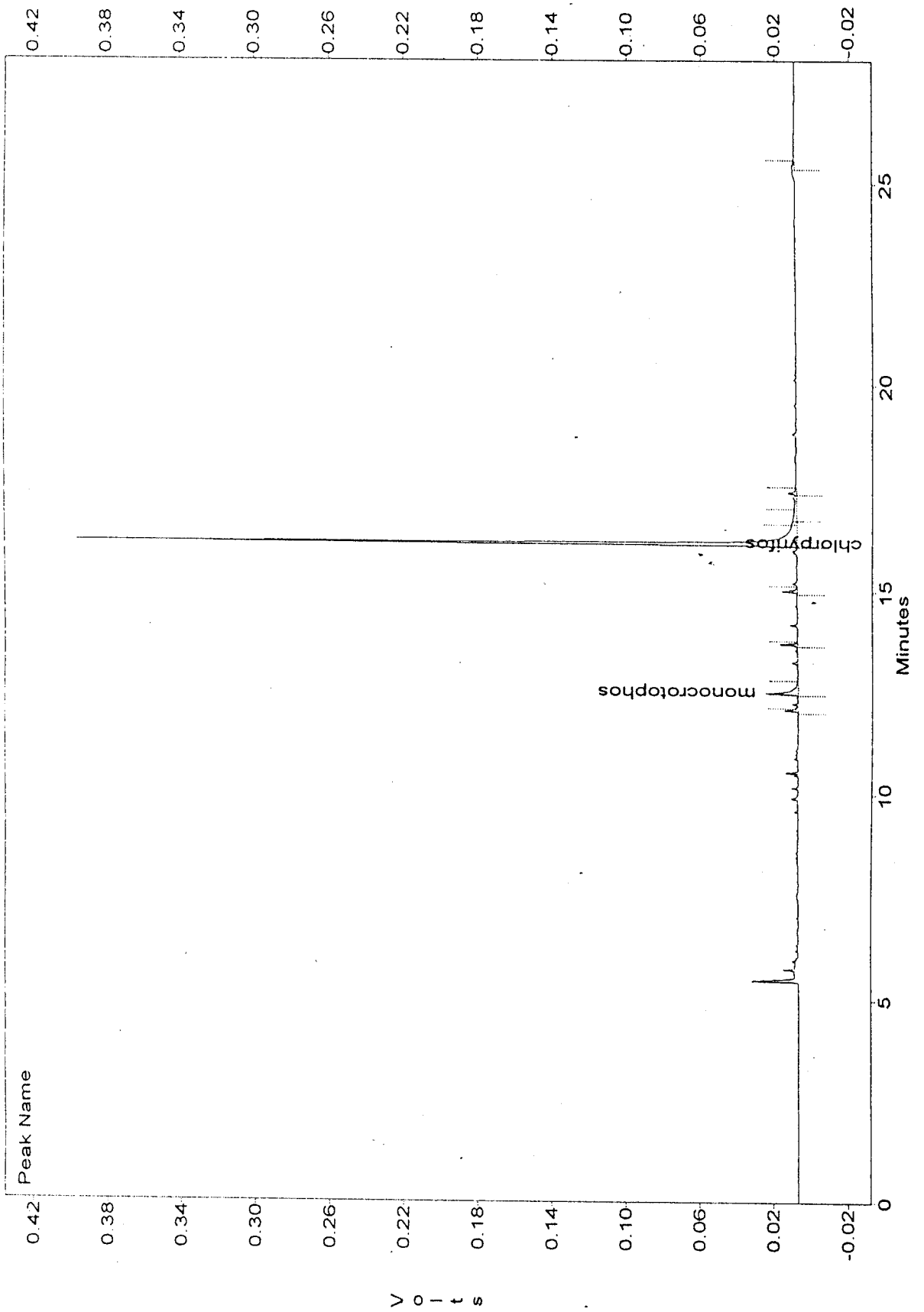
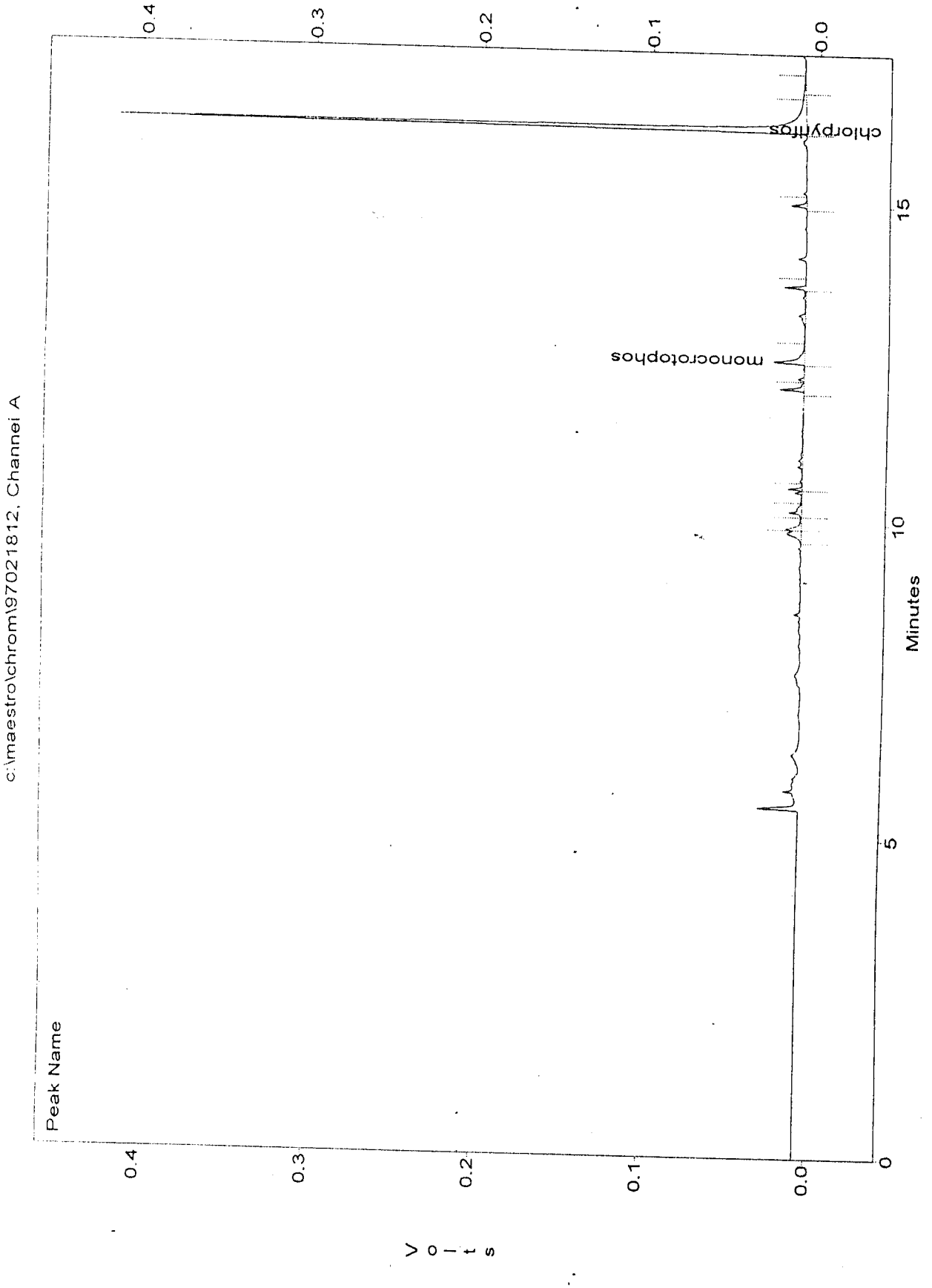


Figure 3.2f chromatogram of 50mg/l standards



Figures 3.3a and Figure 3.3b show the graphs and the equations obtained and used to find the concentrations of the samples of chlorpyrifos and monocrotophos respectively from their peak areas.

The concentrations obtained were then divided by the appropriate value of mg sample equivalent per microlitre of final extract e.g.

The Linear Least Squares Method according to Miller and Miller **[29]** was used to obtain a linear equation for calculating concentrations of the samples as follows:

Chlorpyrifos

x_i	y_i	x_i-x_m	$(x_i-x_m)^2$	y_i-y_m	$(y_i-y_m)^2$	$(x_i-x_m)(y_i-y_m)$
5	203014	-16	256	-470422	2.21×10^{11}	7576752
10	257466	-11	121	-315970	9.98×10^{10}	3475670
20	544390	-1	1	-129046	1.66×10^{10}	129046
30	993374	9	81	319938	1.02×10^{11}	2879442
40	1268936	19	361	595500	3.55×10^{11}	11314500
105	3367180	0	820	0	7.9×10^{11}	25375410

where x_i and y_i are standard concentrations and peak areas respectively and x_m and y_m their means.

The slope b and the intercept a were calculated as 30945.622 and 23577.938 respectively giving the equation $y = 30945.622x + 23577.938$ whose graph is shown in figure 3.3a.

Similarly for monocrotophos the equation was calculated as $y = 2260.43x + 1000$ in the graph shown in Figure 3.3b.

Figure 3.3a

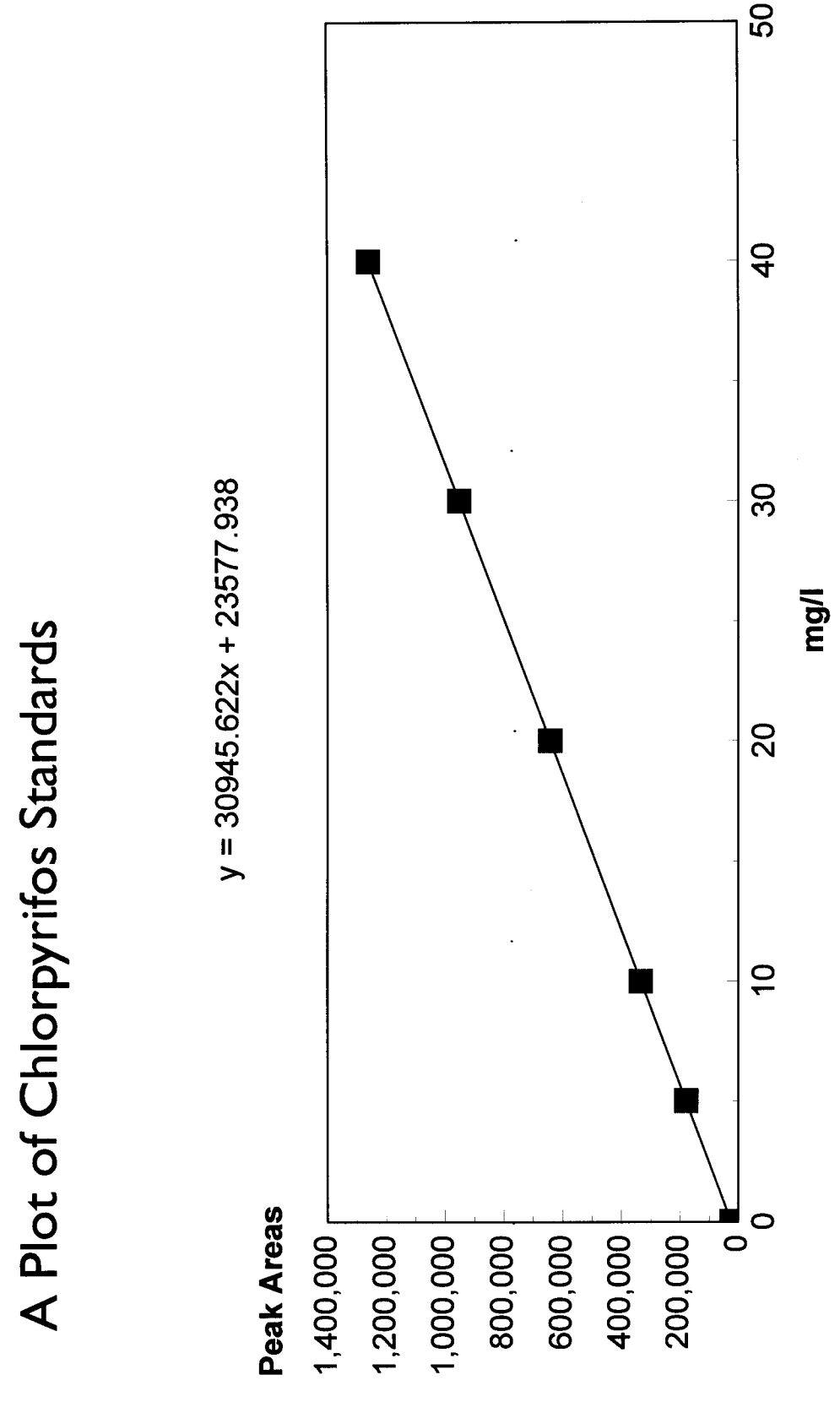
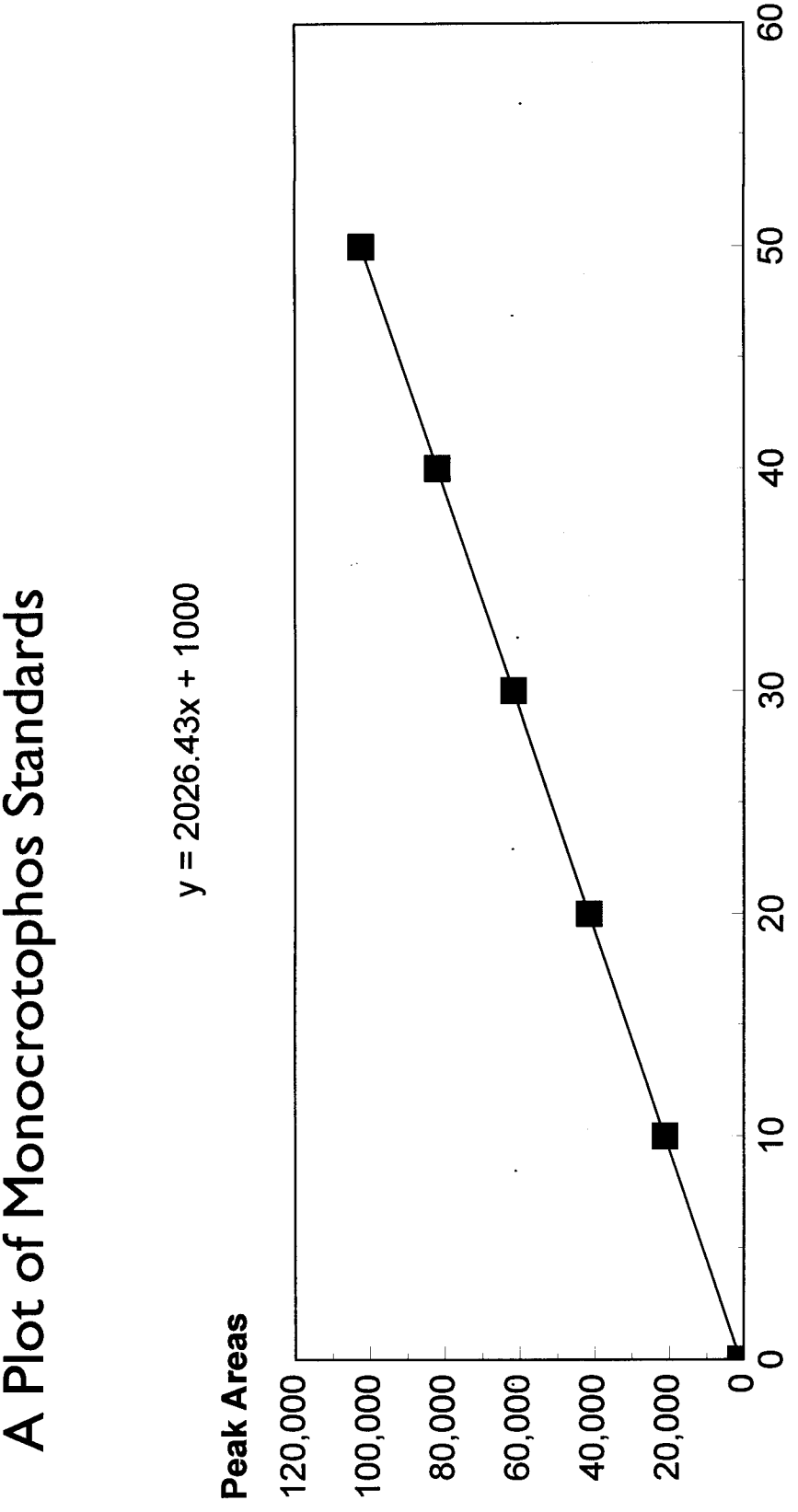

A Plot of Chlorpyrifos Standards
y = 30945.622x + 23577.938
Peak Areas
1,400,000
1,200,000
1,000,000
800,000
600,000
400,000
200,000
0
0
10
20
30
40
50
mg/l

Figure 3.3b


A Plot of Monocrotophos Standards
y = 2026.43x + 1000
Peak Areas
120,000
100,000
80,000
60,000
40,000
20,000
0
0
10
20
30
40
50
60
mg/l

Tomatoe sprayed twice and sampled on the eigth day. the area recorded by the GC was 25811 for chlorpyrifos. Using the equation;

$y = 30945.622x + 23577.938$ from the linear least squares method, where x is the concentration and y the area of the peak on the chromatogram.

The concentration was calculated as 0.072 and divided by 4.03mg/µl to give 0.018mg/kg.

These concentrations are shown in table 4.

Table 4

Approximate concentrations of Chlorpyrifos and Monocrotophos residues in tomatoe and cabbage samples from the experiment in mg/kg.

Number of days after initial spraying	Chlorpyrifos concentrations	Monocrotophos concentrations
Tomatoe sprayed twice		
14	0.02	0.62
1 8	0.04	0.66
21	0.0	0.0
28	0.0	0.0
35	0.0	0.0
Tomatoe sprayed weekly		
14	0.16	3.49
18	1.88	51.26*
21	1.41	2.98
28	0.39	2.45
35	0.01	0.83
Cabbage sprayed twice		
14	1.02	1.73
18	1.36	1.81
21	0.98	3.92
28	0.87	2.40
35	0.364	2.050
Cabbage sprayed weekly		
Day 1	1.29	1.79
Day 8	11.74	4.15
Day 15	18.27	14.26
Day 22	13.18	13.78
Day 29	3.85	29.04*

All control samples gave no significant residues.
These results show a clear pattern apart from two values with * that are completely inconsistent.

Some chromatograms of results shown in table 4 from each experiment are shown in the following order :

Tomato

Figure 3.4a labelled c:\maestro\97012703 was not sprayed with chlorpyrifos and monocrotophos. It shows no peaks at 12.550 nor 16.185 minutes, the retention times for monocrotophos and chlorpyrifos respectively. This was a tomato control sample. The peaks seen are for other organochlorines and organophosphates sprayed on the crop.

Figure 3.4b labelled c:\maestro\chrom\97012406 was sprayed twice and sampled on the twenty second day. It also showed no peaks for both pesticides. The sampling was done when the pesticidee may have already decomposed. In this case the pre-harvest interval had most certainly been observed when sampling was done.

Figure 3.4c labelled c:\maestro\chrom\97012212 was sprayed weekly. It was sampled on the twenty ninth day and shows a peak for chlorpyrifos and no peak for monocrotophos.
In this case monocrotophos may not have been present in quantities large enough to be detected by ECD which is most sensitive to halogenated compounds. The chlorpyrifos peak size indicates that the concentration was very low or the residue was decomposing fast when it was sampled.

Figure 3.4a Tomato control sample

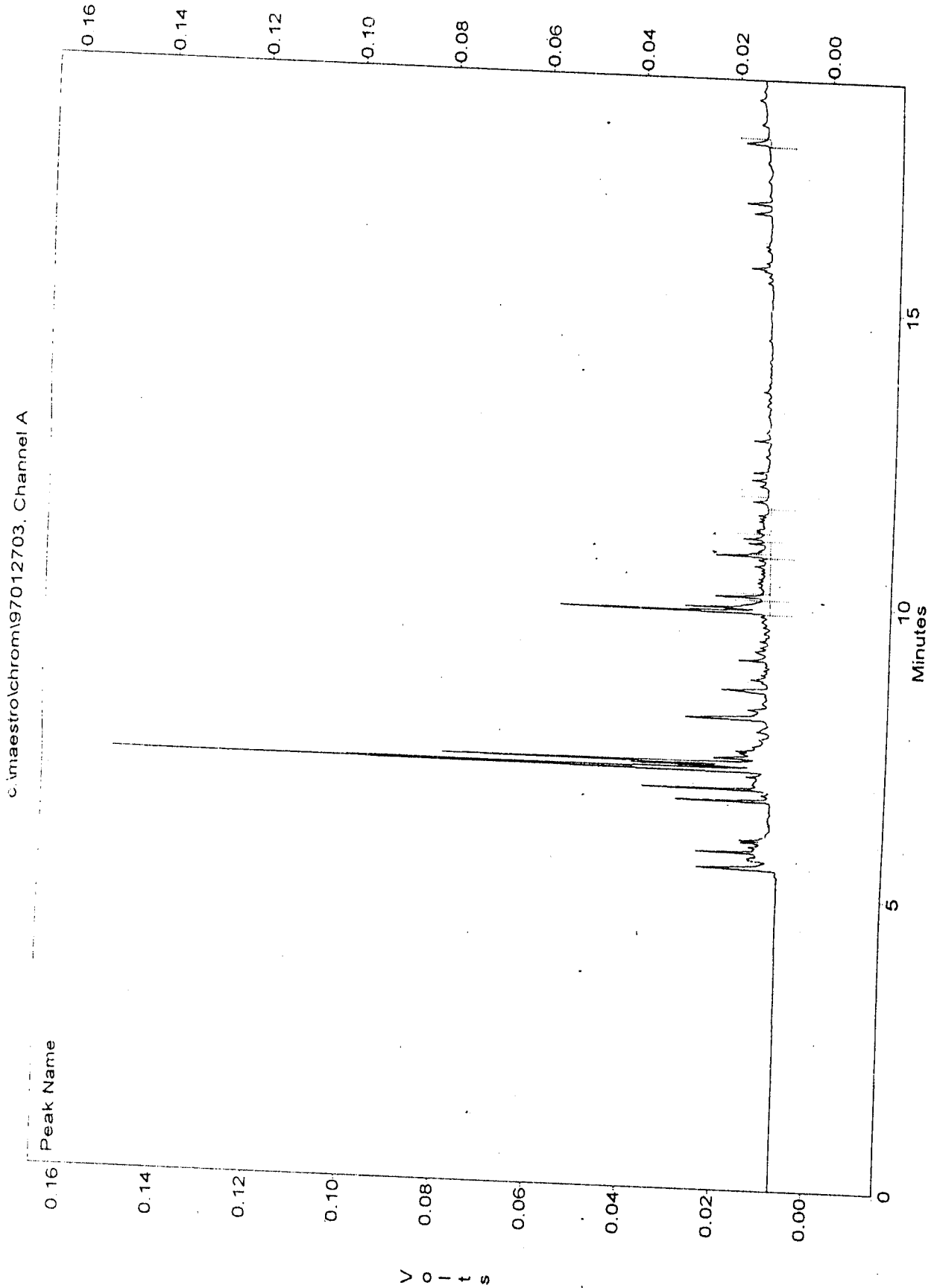


Figure 3.4b Tomato sprayed twice only

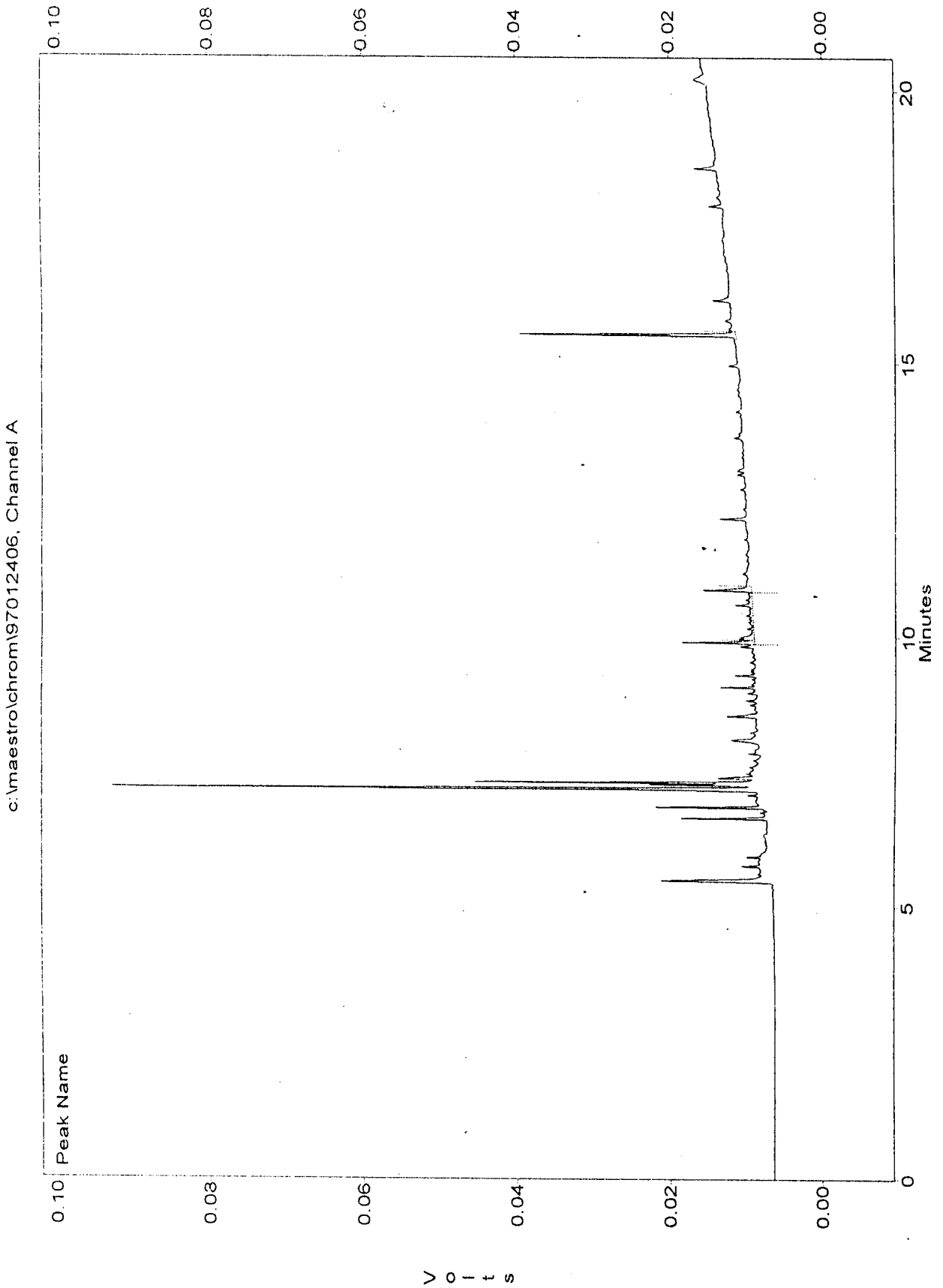
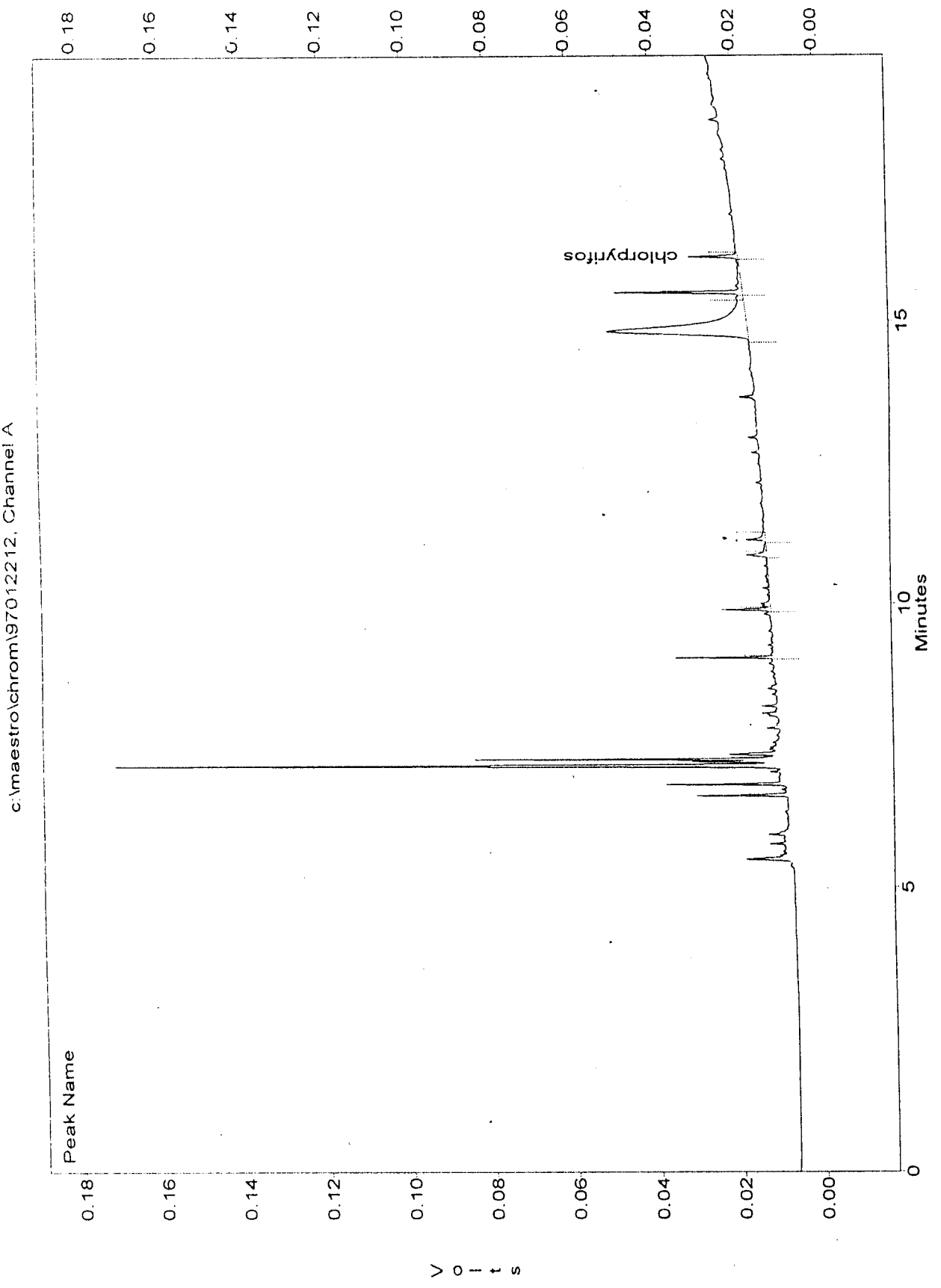


Figure 3.4c Tomato sprayed weekly



Cabbage

Figure 3.4d labelled c:\maestro\chrom\97013008 was a control sample. It recorded no peaks for both chlorpyrifos and monocrotophos since neither pesticide was applied to the crop. The peaks seen at other retention times are from other pesticides applied to the crop.

3.4e labelled c:\maestro\chrom\97020301 was sprayed twice and sampled on the fifteenth day. chlorpyrifos and monocrotophos recorded peaks as shown indicating that residues of both pesticides are present in sufficient quantities to be detected.

Figure 3.4f labelled c:\maestro\chrom\97020404 was sprayed weekly and sampled on the fifteenth day. Peaks for both pesticides were recorded and the peaks were quite high. The chromatograms also tend to suggest that both chlorpyrifos and monocrotophos are heavily adsorbed on cabbage and their residues do not easily decompose in cabbage as compared to the case in tomato.

Figure 3.4d Cabbage control sample

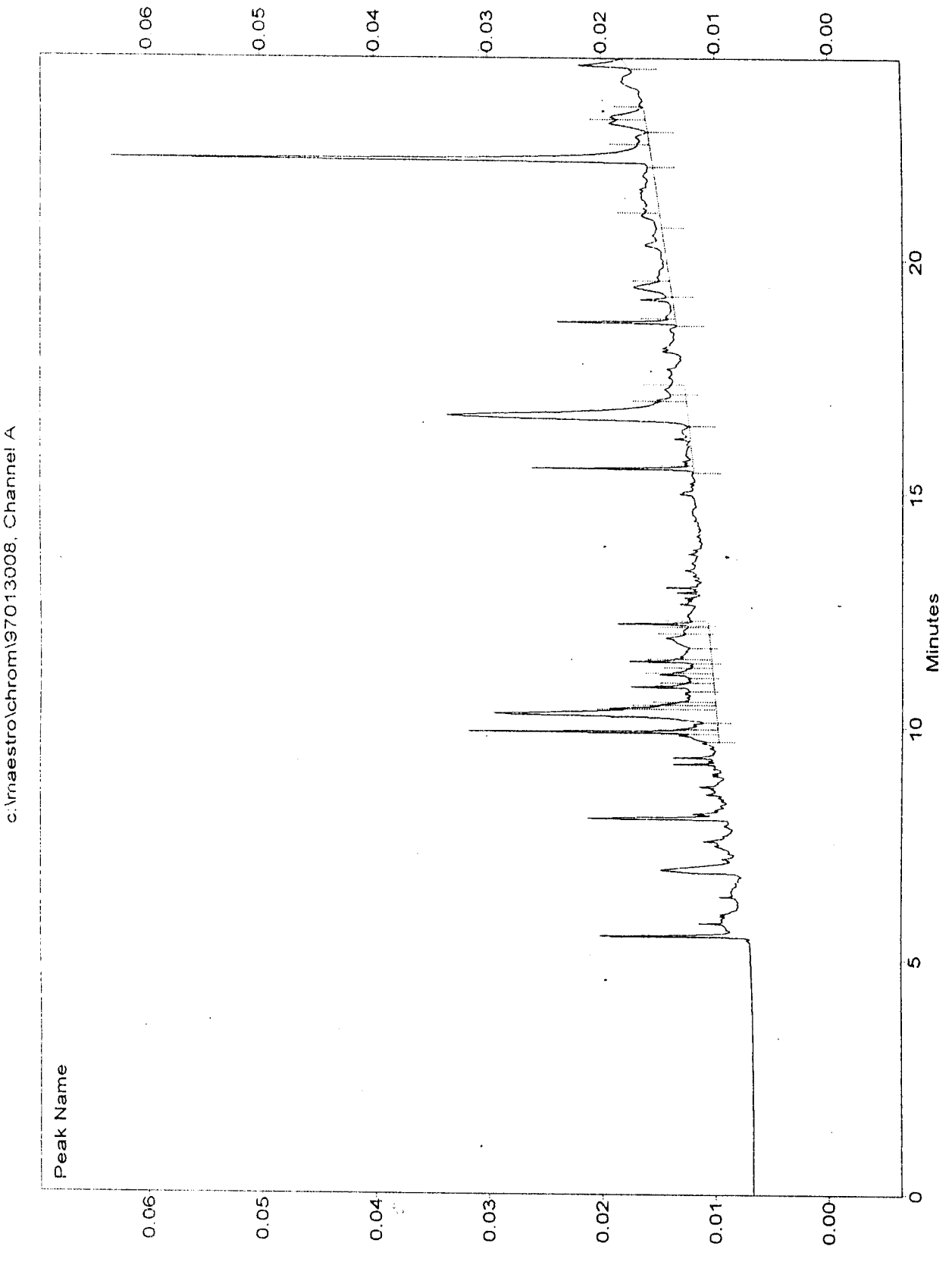


Figure 3.4e Cabbage sprayed twice

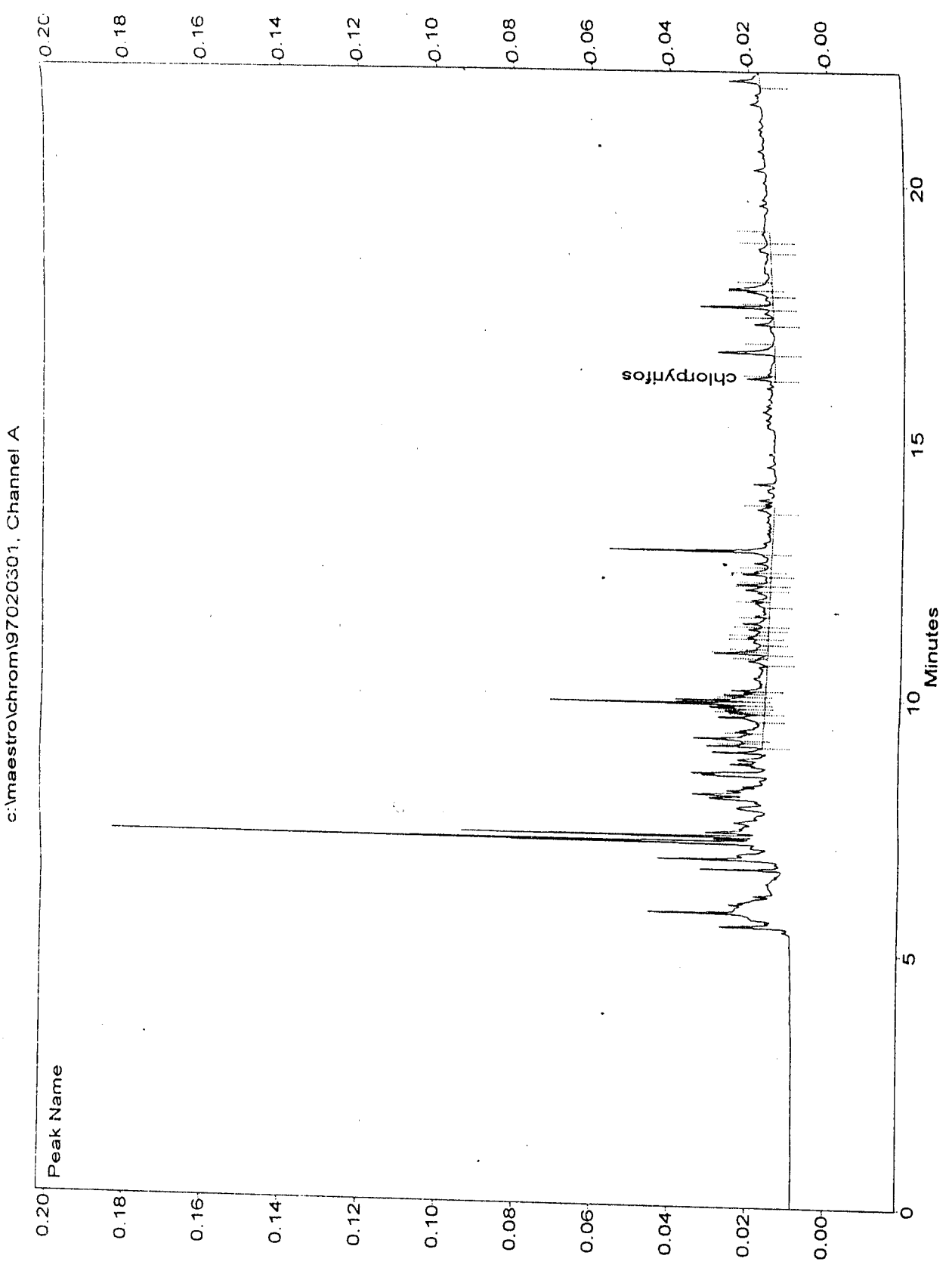
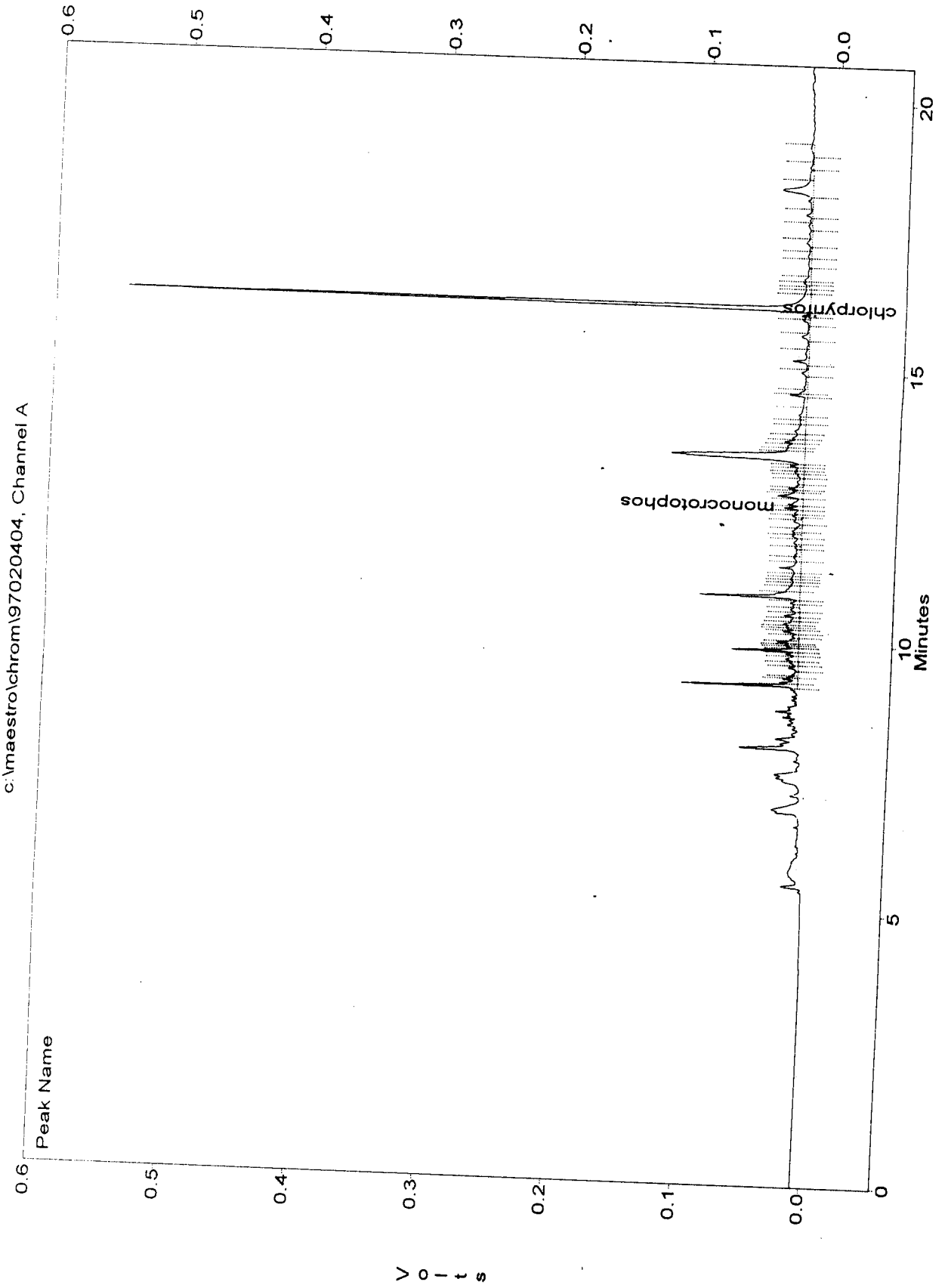


Figure 3.4f Cabbage sprayed weekly



The control experiments were not plotted as they showed no presence of residues. The residues of these samples are shown in table 5. Each experiment was plotted over a thirty five day period i.e. concentration in mg/kg versus days after initial spraying are shown as follows:

Figure 3.5a chlorpyrifos concentrations (residues) mg/kg of tomatoe sprayed twice in seven days. The graph shows the concentration of chlorpyrifos detected in the sample on the fourteenth and eighteenth day after the initial spraying.

Figure 3.5b chlorpyrifos concentrations(residues) mg/kg of cabbage sprayed twice within seven days. The graph shows residues of chlorpyrifos were detected up to the twentyeigth day after the initial spraying indicating that chlorpyrifos is adsorbed more on cabbage than it is on tomato.

Figures 3.5c and 3.5d show chlorpyrifos concentrations (residues) mg/kg of tomato and cabbage sprayed weekly respectively. These residues are given in table 5. Clearly seen is the fact that the cabbage residues are much higher than those of tomato indicating cabbage adsorbs more than tomato and decomposes at a slower

Figure 3.5a

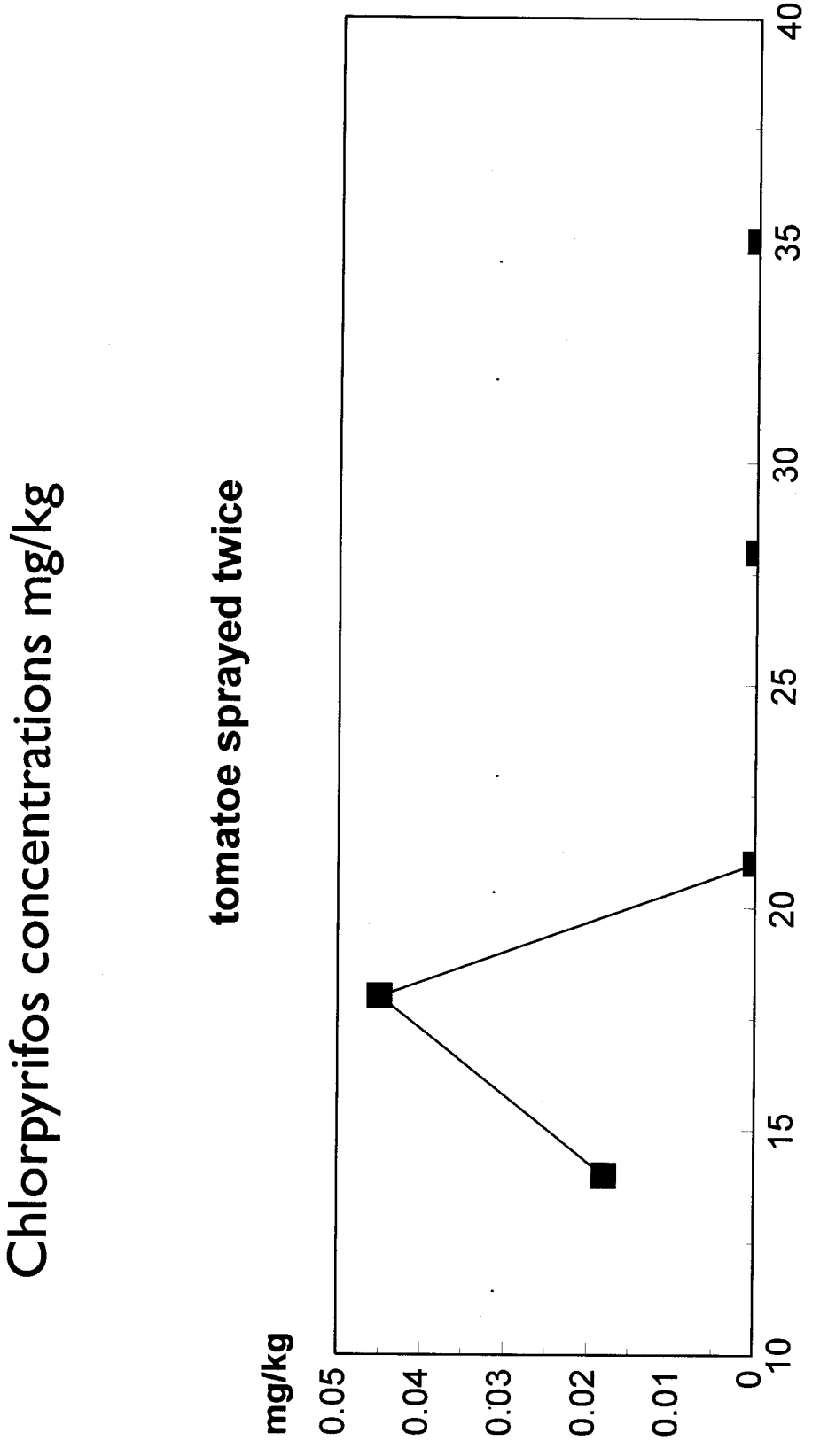

Chlorpyrifos concentrations mg/kg
tomatoe sprayed twice
mg/kg
0.05
0.04
0.03
0.02
0.01
0
10
15
20
25
30
35
40
Days after initial spraying

Figure 3.5b

Chlorpyrifos concentrations mg/kg

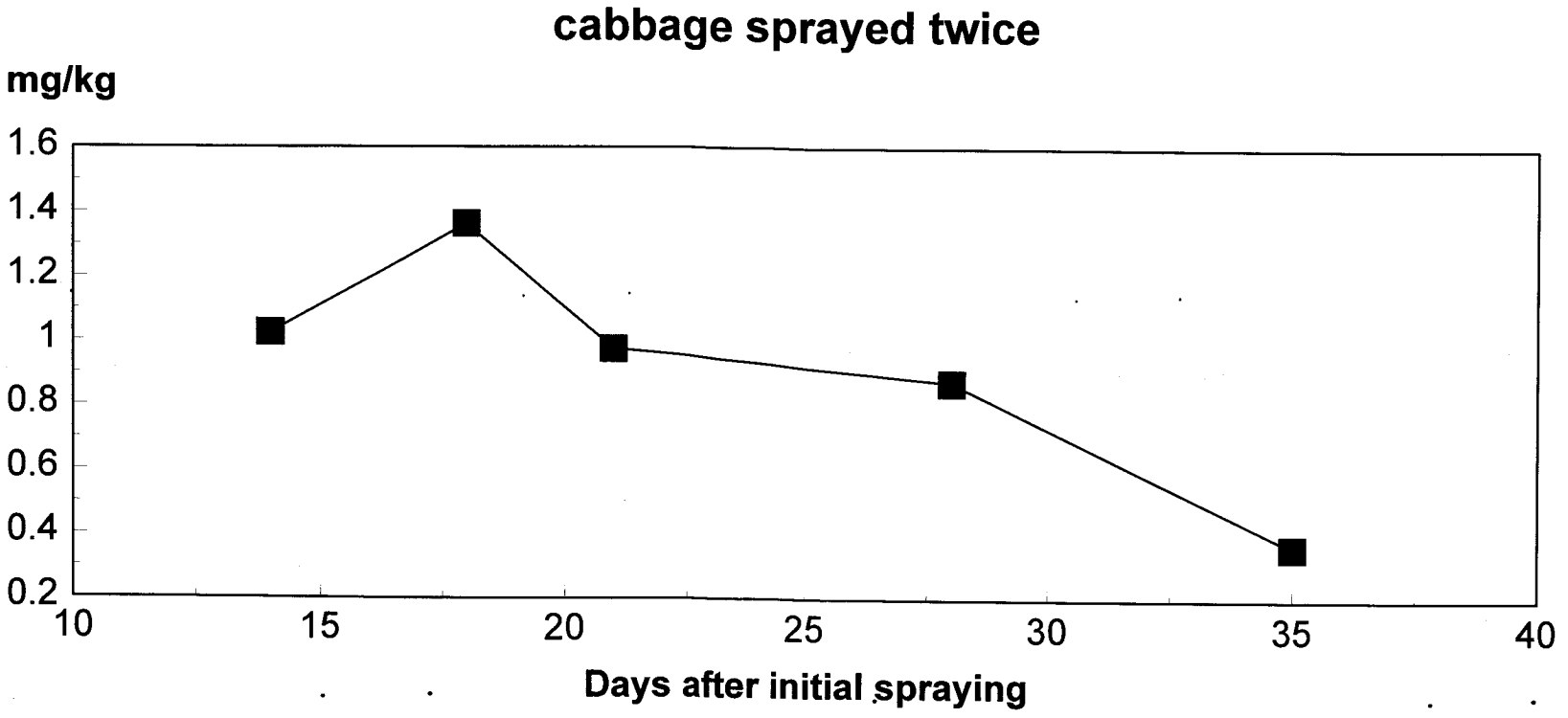


Figure 3.5c

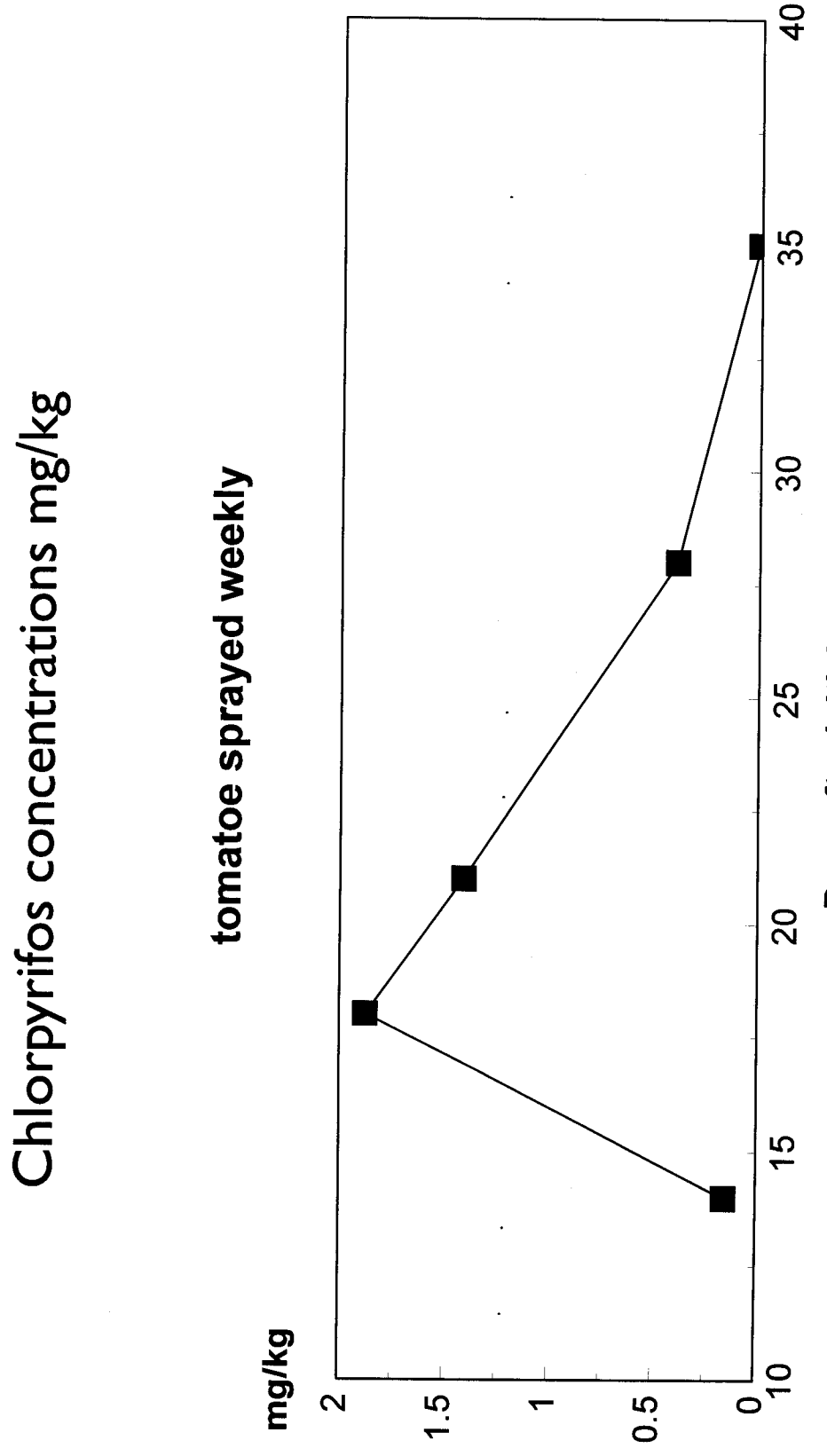
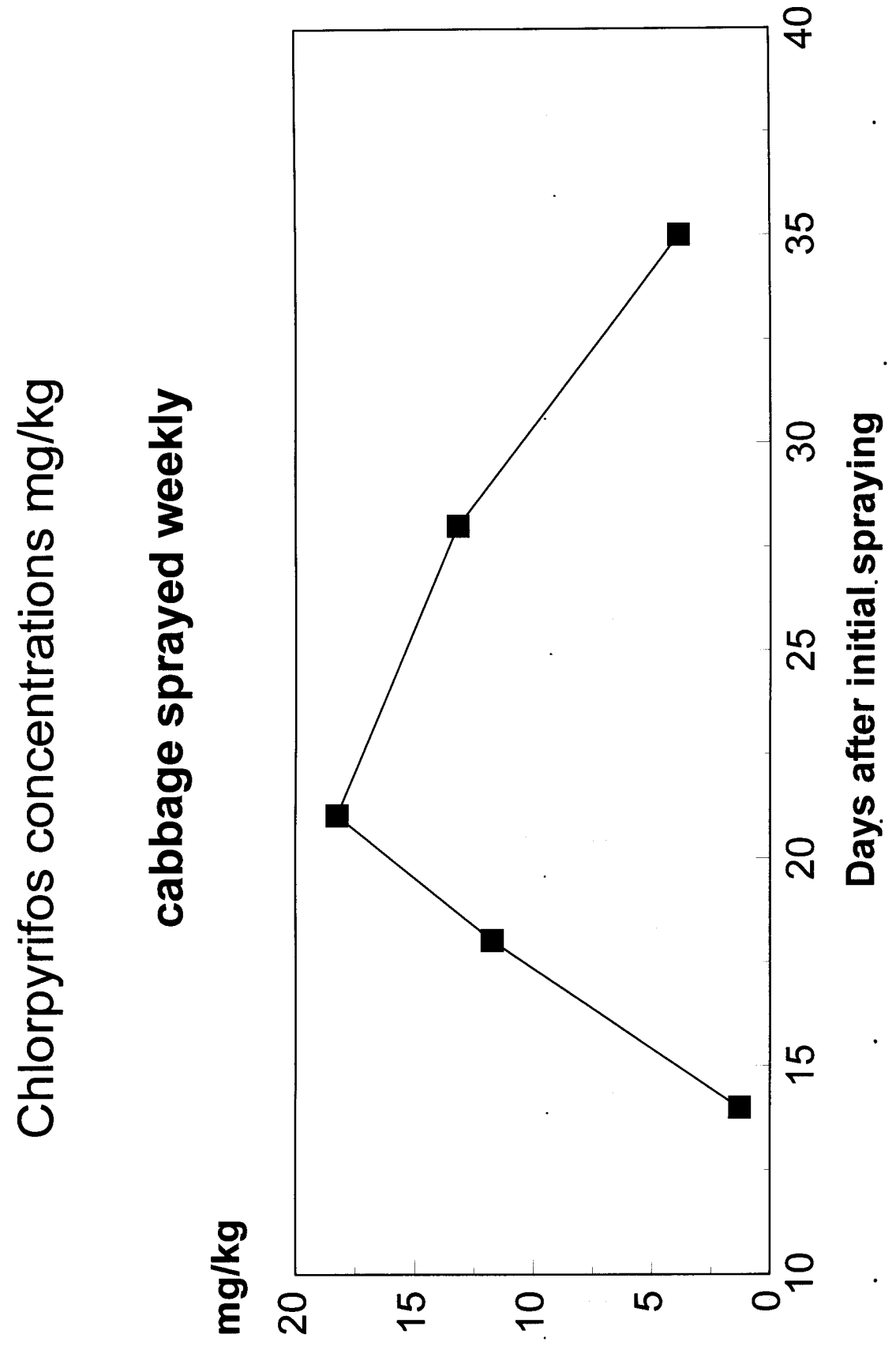

Chlorpyrifos concentrations mg/kg
tomatoe sprayed weekly
mg/kg
2
1.5
1
0.5
0
10
15
20
25
30
35
40
Days after initial spraying

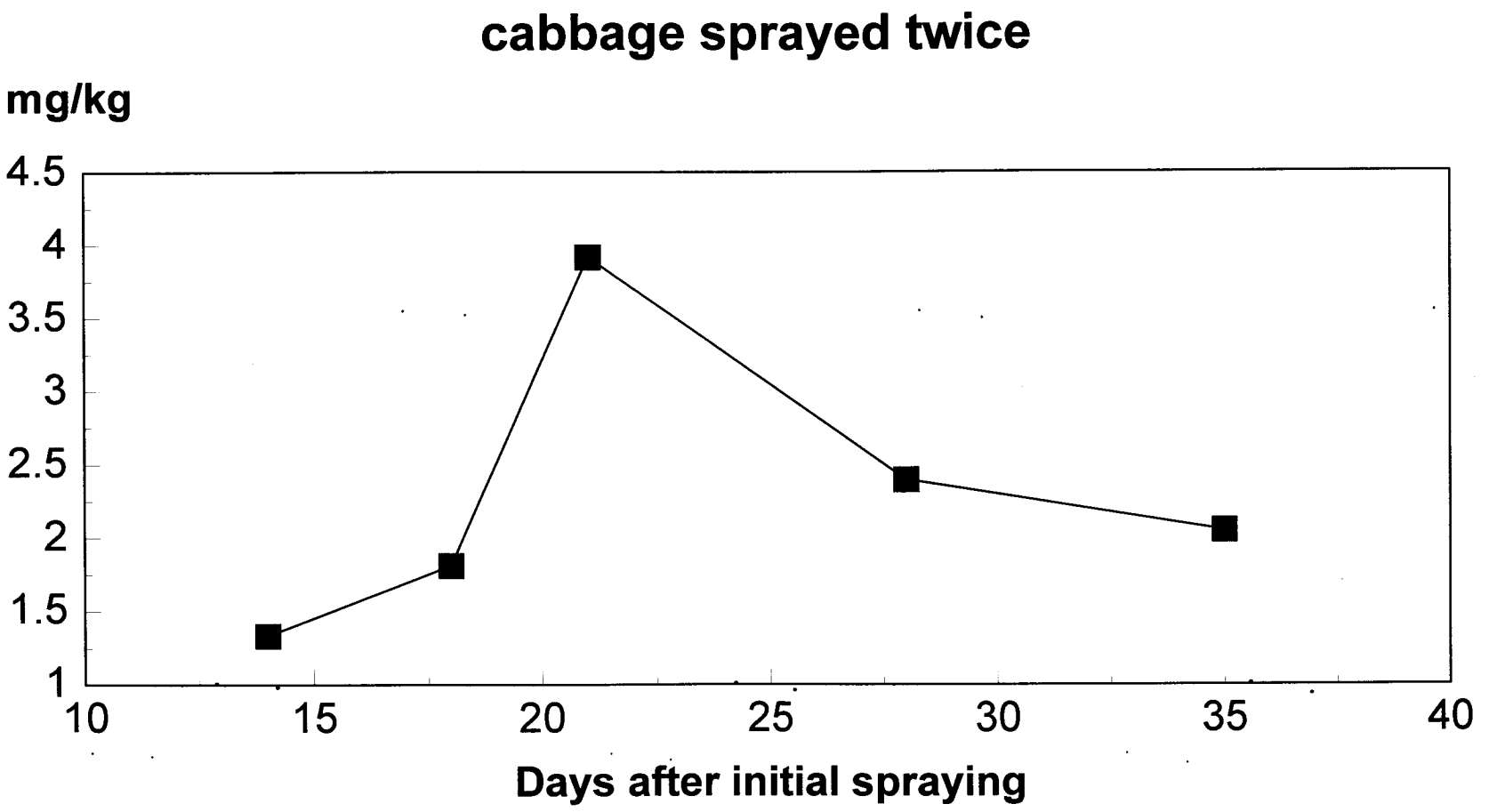
Figure 3.5d


Chlorpyrifos concentrations mg/kg
cabbage sprayed weekly
mg/kg
20
15
10
5
0
10
15
20
25
30
35
40
Days after initial spraying

Figures 3.5e, 3.5f, and 3.5g, show monocrotophos concentrations (residues) mg/kg of cabbage sprayed twice, tomatoe sprayed weekly and cabbage sprayed weekly respectively. These graphs in comparison with those of chlorpyrifos suggest that monocrotophos is adsorbed more than chlorpyrifos. The graph further suggests that cabbage adsorbs more than tomato.

Monocrotophos concentrations

Figure 3.5e



Monocrotophos concentrations mg/kg

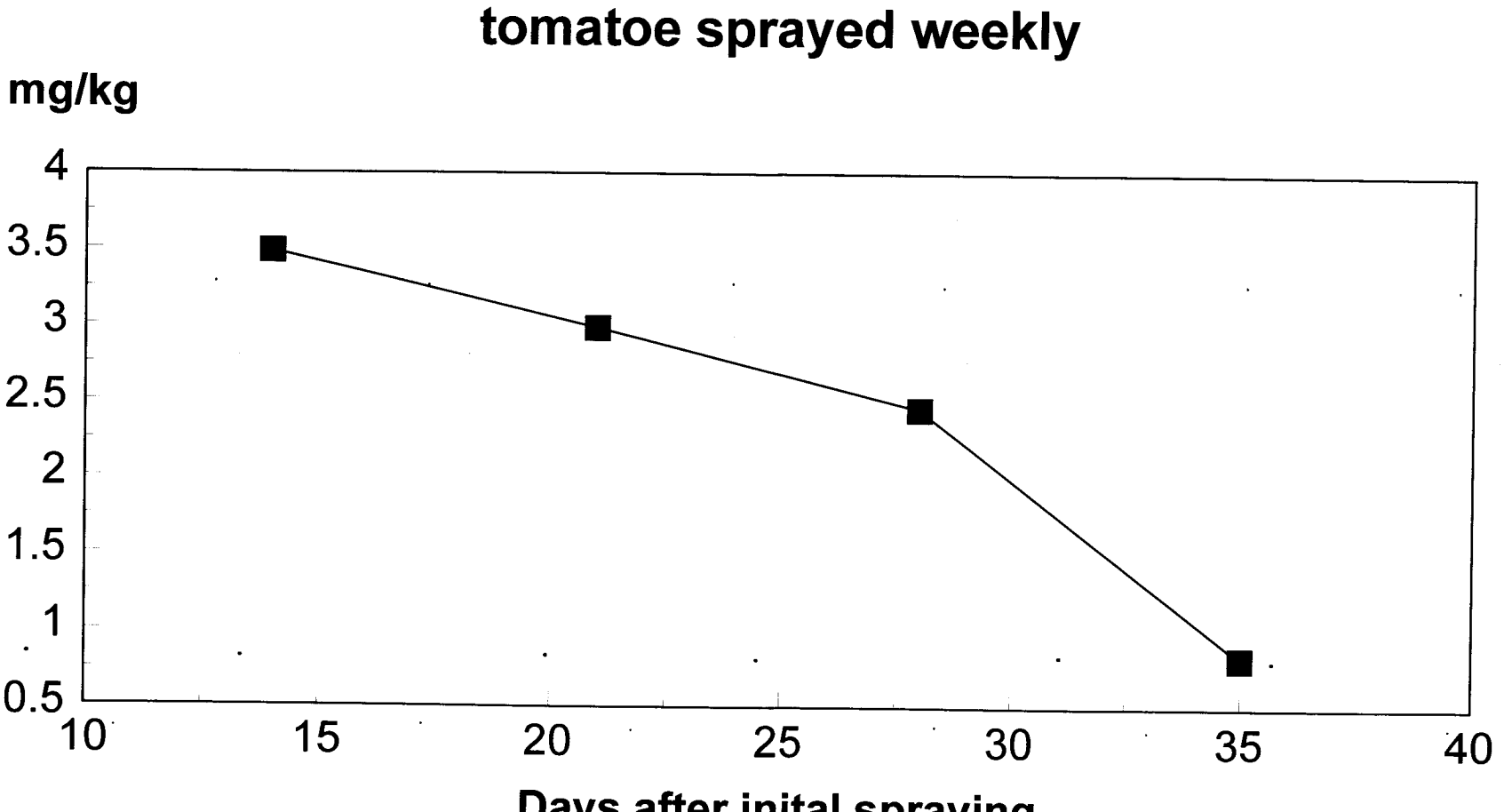
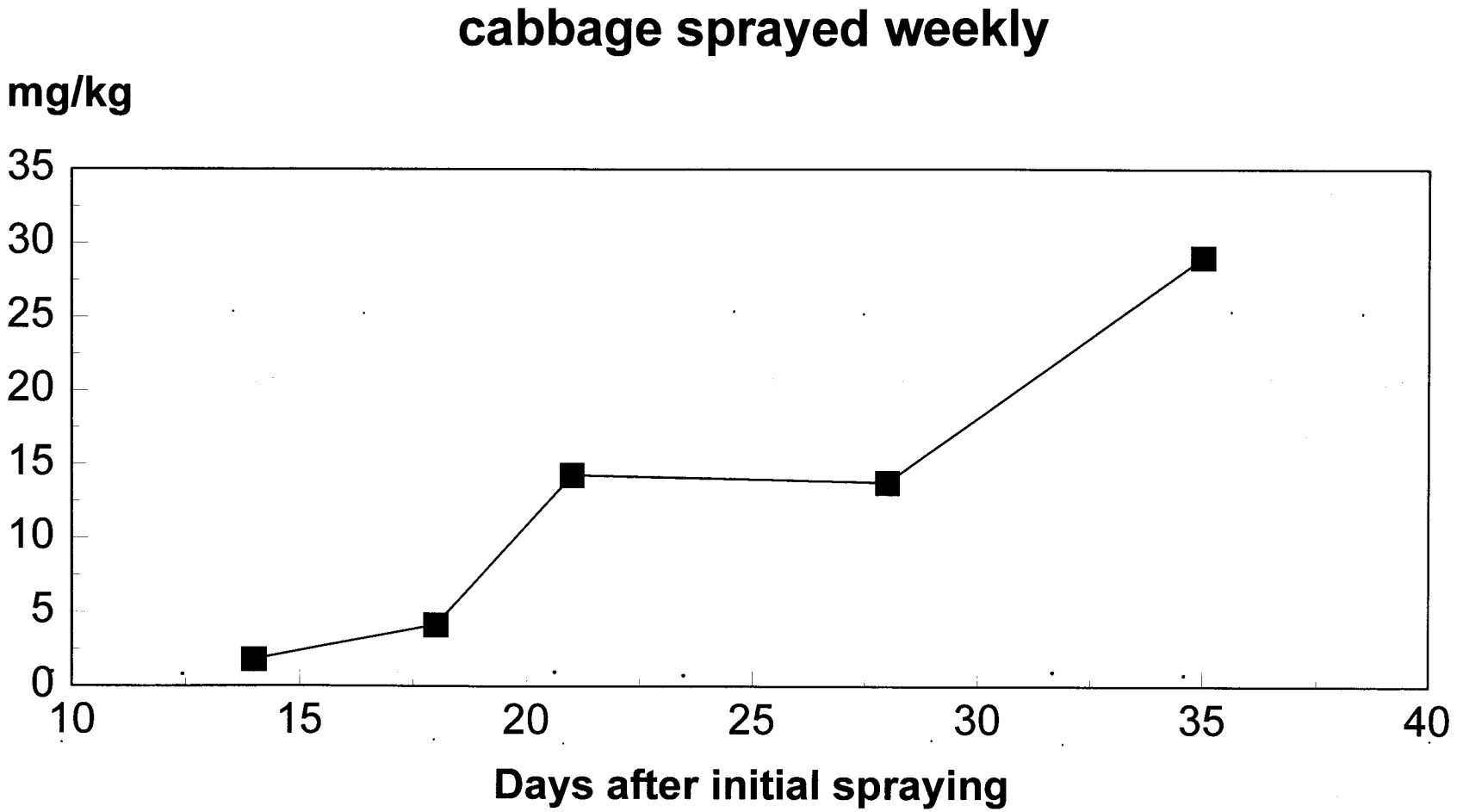


Figure 3.5f

Monocrotophos concentrations mg/kg

Figure 3.5g



The residues found in the samples obtained from the market are given in table 5.

TABLE 5

Approximate concentrations of chlorpyrifos and Monocrotophos in tomatoes and cabbage samples from markets arounmd Lusaka in mg/kg.

Tomatoe	Chlorpyrifos concentration	Monocrotophos concentration
Grasmere farm		
1.	0.0	0.0
2.	0.0	0.0
3.	0.0	0.0
4.	0.0	0.0
Mayflower Farm		
5.	1.46	2.93
6.	0.0	0.0
Unknown source		
7.	0.0	0.0
8.	0.0	0.0
9.	0.06	0.0
10.	0.0	0.0
11.	0.0	0.0
Gravistone Farm		
12.	0.0	0.0
13.	0.0	0.0
14.	0.28	1.92
15.	0.0	0.0
16.	0.0	0.0
17.	0.31	0.0
18.	0.0	0.0
Cabbage		
19.	0.12	0.0
20.	0.16	3.86
21.	10.03	0.0
Shoprite checkers		
22.	0.0	0.0
23.	0.28	0.0
24	0.0	0.0

Two chromatograms are shown below and labelled as ;

Figure 3.6a labelled c:\maestro\ chrom\97020506, shows a broad peak of chlorpyrifos in a cabbage sample obtained from the market. This suggests that the crop was harvested and on the market ready for consumption. The pre-harvest interval may have not been observed.

Figure 3.6b labelled c:\maestro\chrom\97012706, with no peaks for either chlorpyrifos or monocrotophos in a tomatoe sample was either not treated with either chlorpyrifos nor monocrotophos or the pre-harvest interval could have been observed. The latter case is most unlikely since residues of other pesticides are clearly seen on the chromatogram.

Figure 3.6a market cabbage sample

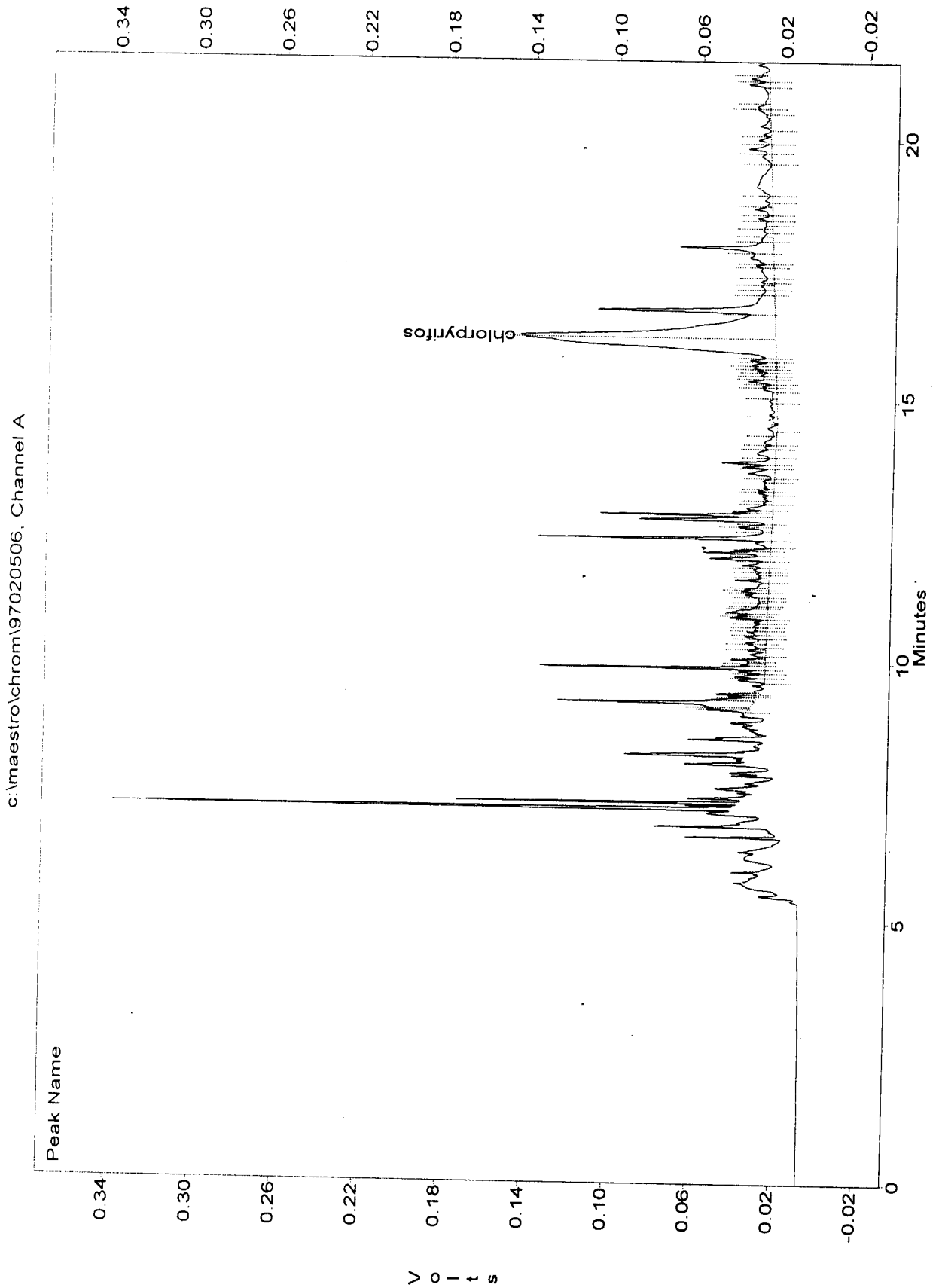
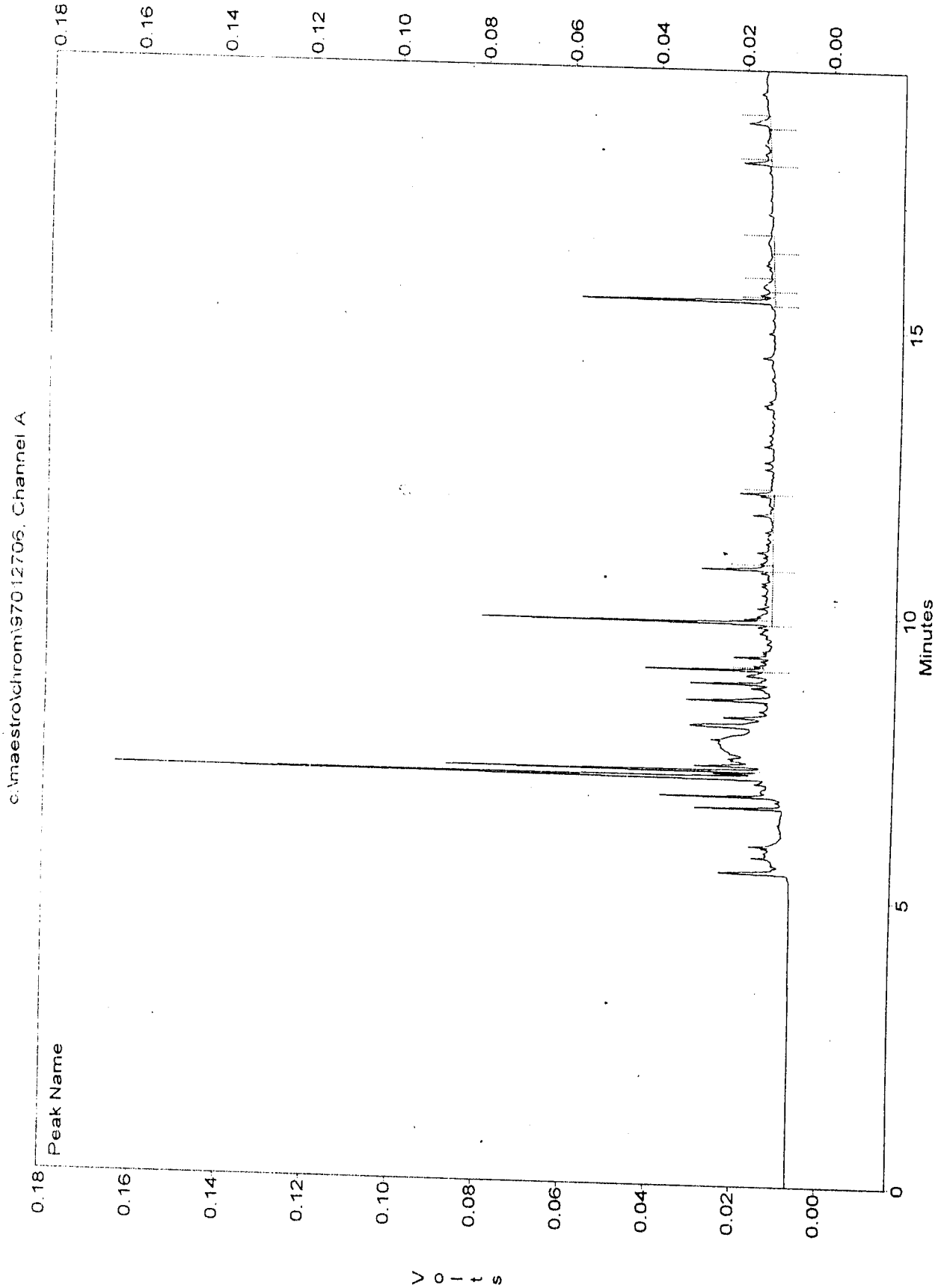


Figure 3.6b market tomato sample



CHAPTER 4

DISCUSSION

4.1 THE GAS CHROMATOGRAPH

The experimental procedure was designed for a GC fitted with a phosphorus sensitive Flame Photometric Detector (FPD). The trail runs were successfully injected in a Pye Unicam 204 Series GC fitted with a FPD. Unfortunately this GC broke down and could not be repaired because the part to be replaced was no longer being made by the manufacturer. Polarography with a Drop Mercury Electrode was also used to analyse the samples. It proved unfavourable because the results were not reproduceable. The alternative GC that was available had only the Flame Ionisation Detector (FID) which was not sensitive enough, and thus Electron Capture Detector (ECD) was used. The use of the ECD necessitated the cleanup of the samples on a Florisil column. The samples that were cleaned all showed no residues even thougresidues even though some of them had residues detected in them before they were cleaned up.

A 10m and 50m column were available and it was found that the 10m column was too short for the analysis and employed the 50m one. The Chrompack chromatograph had a compuerised Hewlett Packard Integrator which gave the retention times and the corresponding peak areas. This made the analysis easier and more accurate.

4.2 SAMPLES

Tomatoe samples sprayed twice with chlorpyrifos rose to 18µg/kg on the fourteenth day. The residue level further increased to 45µg/kg on the eighteenth day. The residues found were below the MRL's recommended by FAO and WHO of 0.5mg/kg. Thereafter there were no chlorpyrifos residues detected. Monocrotophos residues also increased from the fourteenth day to the eighteenth day and then reduced to almost nothing. The residues of monocrotophos were below the MRL's of 1mg/kg. Cabbage samples sprayed twice gave the highest chlorpyrifos residues on the eighteenth day after the initial spraying then steadily decreased. For monocrotophos the highest residue level detected was on the twenty first day and above the MRL of 0.2mg/kg.

The tomato samples sprayed weekly all gave residues of both chlorpyrifos and monocrotophos. In the case of chlorpyrifos the first three samples had these residues; 0.16, 1.88, 1.41mg/kg before decreasing to 0.40 and finally 0.01mg/kg. Of these five values three fall below the recommended MRL's. These high levels of residues could be attributed to drift that may have taken place during spraying of the surrounding field.

For Monocrotophos in these samples, the levels of residues detected all were above the the recommended MRL of lmg/kg with the exception of the sample of the thirtyfifth day.

On the eighteenth day the sample taken gave a high residue suggesting that oversprayiŋg of monocrotophos could have taken place on that particular crop and very little watering may have occurred in that area.

The cabbage that was weekly sprayed with chlorpyrifos increased from 1.29mg/kg on the fourteenth day to 18.27mg/kg, the highest residue on the twenty first day aftyer initial spraying.

The increase was large and suggests accumulative sprays. After the twenty first day when no more spraying was done the levels of the residues decreased to 3.85mg/kg on the thirty fifth day. In all these samples the residues were above the MRL'S recommended by FAO and WHO of 0.05mg/kg. It was observed that successive spraying of cabbage with chlopyrifos made the residue accumulate in large quantities.

For monocrotophos residues the levels increased from the fourteenth day with 1.80 mg/kg to 29.04mg/kg on the thirty fifth day. This suggested that monocrotophos is very easily adsorbed on cabbage and does not easily /rapidly decompose.

All control samples gave no significant residue levels of both chlorpyrifos and monocrotophos.

The market samples of tomato were eighteen from various farms in Lusaka, Mkushi and mazabuka. Four gave residues of chlorpyrifos while two gave residues of monocrophos. For chlorpyrifos one residue was above the recommended MRL of 0.5mg/kg while the other three were below.

Both residues of monocrotophos were above the MRL of 1mg/kg. Of the six cabbage samples from the market four had residues detected in them. All four residues were above the recommended MRL of 0.05mg/kg.

Only one cabbage sample had residues of monocrotophos detected in it, which was above MRL's.

CONCLUSION

The data from this study indicates that 80% of the tomato samples fromthe experiment had chlorpyrifos residues below the MRL's. The remaining 20% wereabove. On the other hand all cabbage samples of the experiment, i.e. 100% , showed residue levels above MRL's.

In 60% of the experimental tomato samples levels of residues of monocrotophos were below the MRL's while 40% were above and all the cabbage samples had unacceptable monocrotophos residue levels.

The data from the market samples gave 5.6% of tomato samples with unacceptable chlorpyrifos residues and 11.1% of unacceptable monocrotophos residues in tomato.

66.7% of cabbage had unacceptable chlopyrifos residue levels while 16.7% had unacceptable monocrotophos levels.

The market study is not a true reflection of the level of residues of chlorpyrifos and monocrotophos in cabbage and tomato at the time of sale. because few samples from very few farms were taken. These cannot be considered a true reflection of the situation.

I wish to recommend that more work be done in this area of pesticide residues on a nation wide level. Also the analysis to be done on a regular basis using both GC-MS and GC fitted with ECD and FPD and the results compared. There is a very big gap in the field of residue analysis while the use of pesticides continues to increase.

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