DEVELOPMENT OF METHODS FOR SCREENING PESTICIDE RESIDUES IN PLANT MATRICES IN GHANA

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DECLARATION

It is hereby declared that this thesis is the outcome of research work undertaken by the author. Any help obtained has been duly acknowledged.

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DEDICATION

To my mum, Rose, Yvonne and all my brothers and sisters.

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In a project of this nature it is impossible to accomplish the work without the support of many people all of whom one may not be able to acknowledge by name. I would therefore wish to express my sincere gratitude to all the people who assisted me either physically or spiritually.

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ABSTRACT

TLC has been used in combination with micro-extraction and clean-up methods to provide an alternative cost effective analytical procedure for screening pesticide residues in plant matrices. Thirty-five (35) agrochemicals, which are used in priority crops in Ghana, were used in this study.

Ethylacetate extraction in the presence of anhydrous sodium sulphate, followed by gel permeation chromatographic clean up and additional purification on silica gel cartridges provided clean extracts enabling the application of 300mg sample equivalent on the TLC plates.

Detection method involving the use of O-tolidine was found to be suitable for general screening of residues, having medium sensitivity for several compounds. The method involving the use of silver nitrate was the only one found to be most suitable for detecting the organochlorine pesticides. Lindane was the most sensitive to this reagent and had a Minimum Detectable Quantity (MDQ) value of 5ng/5μL. This method was suitable for use on only alumina plates and detection was also possible even under sunlight.

The enzyme inhibition methods were very sensitive to the carbamate and phosphoric acid type insecticides with MDQ values between 0.2 and 2000ng.

Other detection methods involving p-nitrobenzene, p-dimethylaminobenzaldehyde and photosynthesis inhibition were also tried and discussed.

The database developed has been successfully used for screening and semi-quantitative determination of some ranges of pesticide residue in soil and plant matrices.

CHAPTER ONE

OVERVIEW OF PESTICIDE RESIDUE ANALYSIS

1.1 USES OF PESTICIDES

Environmental pollution by pesticides is becoming a global problem. It is evident that our environment has been flooded with pesticides, some of which have had effects other than those intended. There is no doubt that indiscriminate use of pesticides in this part of our world has the potential to upset temporarily and in the long term permanently, many ecological patterns.

Pesticides are chemicals designed to combat the attacks of various pests on agricultural and horticultural crops in order to increase crop yield, protect stored products to meet the proportionate increase in the demand for food. Pesticides are also used in the eradication of some vector borne diseases like malaria etc. Pesticides are therefore indispensable tools for increasing agricultural production per unit area, as the area available for cultivation is limited and in the area of human health.) Pesticides therefore, are an integral part of world agriculture/health and under present conditions may be considered indispensable.

In general, substances used as plant growth regulators and defoliants are also included in the term Pesticides [1]. In 1980, 530,000 tons of pesticides were used in the production of food as well as clothing and other industrial goods in the United states for more than 270 million inhabitants [1]. That means, an annual input of 2Kg of pesticides per person into their environment. Plants that supply our main source of food are susceptible to between 80,000 to

100,000 diseases caused by fungi, viruses, bacteria and other types of micro-organisms [1]. They compete further with 30,000 species of weed, of which about 1800-cause economic losses worldwide [1]. About 30% of crop losses due to pest have been recorded in developed countries despite the use of pesticides and other sophisticated control methods [2]. Estimations by various people also agree that at least 10% of the harvested crops worldwide are lost during storage [3]. Losses occur mainly due to insects, fungi, mites and rodents, which are all susceptible to pesticide control. In third world countries it is reckoned that at least a third of potential crops are lost to pests [4].

1.1.1 PATTERN OF USE

In 1980, the world-wide output of pesticides was valued at $11.5 billion with 34% for insecticides, 19% for fungicides and 42% for herbicides [5]. The relative amounts of insecticides and herbicides used in the developed countries have dramatically changed in the past two decades. Whiles in the U.S.A. (1964), only 34,500 tons (3.1×10^4t) of herbicides were used together with 64,900 tons of insecticides, the use of herbicides has been increased to 196,400 tons (1.8×10^6t) and that of insecticides decreased to 26,800 tons (2.4×10^4t)[5] after 18 years. Today in the developed countries, herbicides are the main pesticides used, while in Africa, Latin America and Asia insecticides are the most important products.

In Ghana and other parts of Africa, some organochlorine pesticides are those extensively used. These organochlorines are known to have high fat solubility, accumulate in fatty tissues of animals (including human beings),

and hence tend to accumulate in food chains. They degrade possibly into other toxic residues, which can have long term adverse effects on the environment [6,7]. Lindane and endosulfan are organochlorine pesticides which are still being used in Ghana. All of these chemicals have either been banned or their use restricted in the developed and temperate countries [8].

Pesticides use has assumed an increasingly significant role in the production of food and the prevention of vector borne disease in Ghana. They have saved millions of human lives in Ghana. Pesticides are one of the most important weapons in the fight against hunger. Before 1980, pesticides were mainly used for pest and disease control in the Cocoa Industry, notably the Cocoa mirids [9]. Annual imports during that period for the cocoa industry alone increased from 270,000 gallons in 1970 to 500,000 gallons in 1981/82 (official release from the Cocoa Services Division of the Ministry of Agriculture) [10]. These chemicals were mostly organochlorines such as DDT, lindane and dieldrin.

DDT was first introduced in the country as an emulsion spray for the Cocoa Industry in 1948 [11]. Lindane, later in 1950 was found to be more effective [12]. Their persistence, that is, their high stability, is regarded as problematic. But this - and their low prices - is precisely the reason why they have been and are still very useful pesticides in the last 35 years and the mainstay of agricultural and vector - control programs in Ghana. Resistance of Cocoa mirids to lindane after 1961 led to the proliferation of other pesticides in the Ghanaian Cocoa System. Trials with organophosphates and Carbamates then began in 1963. Currently lindane is alternated with unden (a carbamate) every two

years for control of mirids in the cocoa industry.

After 1980 and with the recent launching of the economic recovery Programme (ERP), the use of other plant protection chemicals including Herbicides, Fungicides and growth regulators increased drastically. By the mid 80's,[10] large quantities of hazardous pesticides were being used for agricultural pest control. There was virtually no control in the importation, distribution, marketing, and use of pesticides in the country [10] Compounding the problem was the careless abuse of pesticides by consumers. Data however on health of workers occupationally exposed to pesticides was lacking, though it was apparent that large numbers of consumers were exposed on a regular basis. Their use at the time was so widespread that fishermen sometimes used them in harvesting fish from rivers and lakes. There were calls at the time by government and individuals for measures to streamline and promote safe and efficient use of pesticides in agriculture and public health. Against this background, the Environmental Protection Council (EPC at the time) set up the Toxic Chemicals sub-committee on pesticides to advise on measures to ensure safe and efficient use of pesticides in Ghana. The culmination of their deliberation were (i) the banning of five pesticides. (ii) The drafting of a Legislation which was later passed into law by the Parliament of the 4th Republic as the Pesticide Control and Management Act (Act 528, 1996).

The consumption pattern however indicates that the bulk of pesticides used in agriculture are insecticides, followed by herbicides and fungicides [10] The insecticides are used mainly to combat cocoa capsids,

grasshoppers, army worms and other insect pests on food crops.

Pesticides are also used in public health programmes for control of mosquitoes, black flies and other disease vectors. The high use of pesticides initially was partly attributed to the low cost of the chemicals due to government subsidy on the items.[10] In spite of the increasing trends in the retail prices of pesticides, partly due to the removal of the subsidy, consumption of pesticides in Ghana has increased drastically from year to year due to numerous pest problems facing the Ghanaian farmer [10].

Some of the other agrosystems apart from the cocoa and coffee which use agrochemicals include the palm tree ecosystem. Here monocrotophos, an organophosphate is used to control leaf miners. Also Endosulfan an organochlorine is used in cowpea and tomato cultivation. Whereas the cocoa ecosystem uses glyphosate, simazine etc, the maize ecosystem uses Atrazine, Diuron etc as herbicides. All ecosystems virtually now use chloropyrifos (Dursban) as control for termites. A wide range of fungicides including Kocide, Cocobre, Ridomil Plus and karate are used on pepper and cocoa ecosystems.

1.1.2 DISSIPATION OF PESTICIDES

Pesticide residue is defined as the amount of pesticide remaining in the environment after application or spill. It can be either the parent pesticide or its metabolites or both. These arise in a commodity directly or indirectly from application of the pesticide. Pesticides breakdown in the environment after

application. The rate of breakdown or dissipation varies between less than a day to several years. Depending on the ecosystem under consideration, residues are left in food. Residues left in food must conform to the Maximum Residue Limits (MRL) [13]. MRL is the maximum concentration for a pesticide residue resulting from the use of a pesticide according to good agricultural practice that is recommended by the Codex Alimentarius commission to be legally permitted or recognised as acceptable in an agricultural commodity or animal feed.[13, 14] The dissipation, persistence and fate of pesticidal chemicals is dependent on the nature of the chemical itself and a multitude of environmental factors, such as soil types, temperature, light, moisture, micro-organisms, pH etc.[15] For example, the movement of pesticidal chemicals in and through soils is primarily a function of the water solubility of the chemical and of the adsorptive capacities of the soil type. Some researchers[16] have shown that increased volatility of organochlorine pesticides in wet soils is due to displacement of the pesticide from the soil surfaces, resulting in an increased vapour density or partial pressure of the pesticide.

As a means of minimizing the use and hence the undesirable effects of pesticides, Integrated pest management (IPM) methods are being used. IPM is a method of pest control which combines different pest control techniques and integrates them into the overall farming systems[17] which may reduce the dependence of chemical pesticides. According to Smith and Reynolds [18] definition, which has been embraced by the Food and Agricultural Organisation (FAO) of the United nations, "IPM is a pest management system which in the context of the associated environments and the population

dynamics of the pest species, utilises all suitable techniques and methods in as compatible a manner as possible, and maintains the pest population levels below those causing economic injury". Notwithstanding IPM, there will be residue problems, because of (i) world-wide demand for adequate supplies of pest-free, good quality commodities and foodstuffs that will necessitate the continued use of pesticides and hence the continued associated problems of pesticide residues (ii) the perception that pesticides use are a relatively easy and quick method of regulating an upsurge in the pest population and more especially in cases where disease resistant cultivars of a plant may be available but the farmer will continue to grow varieties which are susceptible to diseases because of other qualities, such as taste and yield.

Pesticide residue monitoring is therefore important for the following reasons:

(i) Assessing the extent of any pesticide use or misuse.

(ii) Reassuring consumer about the quality or safety of basic foodstuffs.

(iii) For research purposes.

The residues are monitored using analytical methods.

1.2 ANALYTICAL PROCEDURE

1.2.1 SELECTION OF METHOD

Directly pertinent to pesticide residue analysis is the analytical methodology used. The method selected will reflect the nature of the samples under analysis and the range of pesticides involved. Further factors to be considered

include resources available to the laboratory, reasons for performing the analyses and the required lower limits of analytical detection.

1.2.1.1 SAMPLE TYPES

Generally samples for residue analysis and identification are of solid or/liquid origins. Complexity of samples therefore range from relatively simple matrices such as drinking water, wine, beverages to complex ones of plant, animal and soil origin. For convenience, some researchers[19] have grouped samples, each group presenting different analytical problems as follows:

1. Root and bulb vegetables such as carrots and yams
2. Fruits and vegetables of low chlorophyll and oil content. Eg. Bananas and citrus.
3. Plants and crops of high chlorophyll content such as legume vegetables.
4. Dried fruits of high sugar content such as figs.
5. Dry crops of low oil content such as cereals, grains and tea.
6. Foods of high oil such as oil seeds and cocoa beans.
7. Foods of animal origin such as meats and fish.
8. Dairy products such as milk and eggs.

1.2.1.2 NATURE OF RESIDUES TO BE ANALYSED

To the chemist in the pesticide residue laboratory, classification of pesticides according to chemical structure has helped group them to enable choice of analytical method to be used:

1. Chlorinated pesticides such DDT, endosulphan.
2. Organophosphates such as pirimiphos-methyl.
3. Fungicides such as benomyl.
4. Pyrethroids such as cyhalothrin and deltamethrin.
5. Carbamates such as carbaryl, propoxur and carbofuran.
6. Dithiocarbamates such as thiram and mancozeb.
7. Fumigants such as methyl bromide and phosphine.

A multiresidue method, a more general method of analysis is used when the identity of the residue to be analysed is not known. A single sample is used to provide a reliable identification and quantification of a large number of compounds at very low concentrations.[20, 21, 22, 23, 24]

1.2.1.3 RESOURCES AVAILABLE TO THE LABORATORY

Pesticide analysis is one of the most demanding forms of chemical analysis in terms of both labour and materials. Recurrent costs for analytical consumable materials and instrument maintenance, particularly for developing countries, and large quantities of consumables required for a particular method may prevent its choice. Staff time and availability can make a method too costly to use.

1.2.1.4 PURPOSE OF ANALYSIS AND REQUIRED LOWER LIMITS OF ANALYTICAL DETECTION

The last two decades has seen a remarkable increase in sensitivity of chemical analysis, especially in pesticide residue analysis. It is from this field, more than any other that high sensitivity figures such as ppm, ppb, and ppt have

been brought to the public by the news media. These high levels of sensitivity are normally achieved when representative samples are taken and properly cleaned before analysis. For example, routine screening will often involve the use of well-evaluated, locally developed or simplified procedures, whereas samples analysed for legislative or other legal purposes, or those showing residues on or about national or international tolerance levels, may need to be analysed or re-analysed using established or standard procedures. On the other hand there is little merit in using a detailed referee method for the analysis of a group of samples from, for example, a field study where a number of compounds are being screened for biological activity and only an indication of the likely pesticide residue levels is required. A simplified method, with labour and material savings, will then be a wise choice.

1.2.2 SAMPLE PREPARATION

The main aim of sample preparation is to get a sub-sample from the 'analytical basket' in a form that would enable efficient extraction of the residues present and representative of the original material. Preceding the subsampling involves chopping, (sieving), grinding/homogenising/macerating sample.

1.2.3 EXTRACTION PROCEDURE

This normally involves the extraction of pesticide residues into a suitable solvent with minimum of contamination from sample. Sample is divided into very small particles (homogenising) to create a large surface area with which the solvent reacts. Solvents that dissolve the pesticides but cause minimal solubilization of the other sample material are then applied. The extract is then

separated from the solid material by vacuum filtration and collected. It is then ready to undergo cleanup. Solvents commonly used include hexane, acetonitrile, ethylacetate and mixtures of these. Soils and high fat samples are normally extracted through classical procedures like soxhlet extraction whiles ordinary samples employ shaking with solvent, maceration with solvent. The most important basis for the choice of a solvent is that the residue of interest is extracted by the chosen solvent. For example, improved methods for organophosphorus and organochlorine residues use acetronitrile and elaborated in the Mills procedure (MOG method).[25] Other researchers developed similar extraction procedures using acetone[26] and methanol.[27] Their reason is that water-miscible solvents will break up the sample matrix and better dissolve the more polar pesticide residues. To ensure quantitative recovery of pesticides in this modified extraction set-up, the ratio of water to acetone is kept constant.[28, 29]

Solid Phase Extraction (SPE), Microwave Extraction (ME) and Supercritical fluid Extraction (SPE) are some of the newer techniques of extraction.[19]

1.2.4 EXTRACT CLEAN UP AND REMOVAL OF CO-EXTRACTIVES.

The presence of co-extractives from the sample can alter not only the extraction efficiency but seriously interfere with subsequent analysis. This is a major separation step that eliminates the plant extractables and many polar pesticide components. The clean up procedure used is dictated by both the sample type and the nature of the pesticide residues to be determined as well as the sensitivity of results needed. Ideally, these co-extracts should be completely removed or their concentration reduced to a level that does not

cause problems in succeeding steps of the analysis. Clean up and removal of co-extractives usually protects and lengthens life span of analytical columns and sensitive instrument detectors used for residue analysis. It also minimises risk of false positive results and masking of true results as well as giving more consistent detector response and better baselines from instruments. Liquid-liquid partition, adsorption column chromatography, gel permeation chromatography and sweep co-distillation are some of the commonly used clean-up techniques.[1]

1.2.5 DETECTION OF RESIDUES

A variety of techniques involving GLC, HPLC, TLC, IR etc. are used to determine residues of compounds of interest after the steps described previously.

1.3 RATIONALE AND OBJECTIVE OF PROJECT

At present, little work has been done on pesticide residue pollution of the environment from the application of pesticides by vegetable and cash crop farmers. The Cocoa Research Institute of Ghana (CRIG) which tests and recommends most of these chemicals for use by cocoa farmers and eventually vegetable farmers, have samples like the beans sent to NRI, Britain (in rare cases) after application of a particular chemical for residue studies and taste analysis to be done. Thus monitoring of pesticide has been done to satisfy export trade regulations and to protect foreign consumers. Unfortunately nothing is being done to protect the Ghanaian rural population from the hazards posed by the use of pesticides and the levels of these environmental pollutants have not been properly determined and documented.

Contamination of soils, water bodies, food crops in mixed farms as well as accumulation in crabs, snails, birds and mushroom can precipitate unknown environmental hazards. Many scientists interested in the field are deterred by the fact that the Gas Chromatography (GC) and HPLC, GC-Mass Spectrometer (GC-MS) systems which are mostly needed for such analysis are normally not available because of their prohibitive capital cost and maintenance, lack of servicing facilities/or break down within a short time after installation, high cost of pure reagents/solvents (consumables) needed for this kind of equipment. It is therefore the aim of this study to investigate the possibility of applying TLC detection in combination with micro extraction and clean-up methods for providing an alternative cost effective analytical procedure for screening pesticide residues in plant and soil.

1.4. SPECIFIC OBJECTIVES

In this proposed study TLC was used to undertake qualitative and quantitative analysis of some selected pesticides commonly used in priority crops in Ghana. The work carried out include:

(a) Identification of pesticide residues in samples of unknown origin by:

  (i) Building up Rf and RRF data base for these selected pesticides that are used extensively in Ghana's agriculture.

  (ii) Run samples with marker compounds simultaneously with all nine detection methods listed in the methodology.

(b) Quantitative determination of pesticide residues by:

  (i) Determining the average diameter of spots.

(ii) Applying calibration curve based on standard spots or embrace sample spot with two standards and estimating concentration.

(iii) Comparing some of the results with those run on a GLC to calculate reliability of the results.

(c) Application of the method to analysis of samples collected from a cocoa ecosystem (CRIG experimental plots) for analysis of propoxur, atrazine and selected vegetables spiked with some of the pesticides.

CHAPTER TWO

TRENDS AND ADVANCES IN PESTICIDE RESIDUE ANALYTICAL METHODOLOGY

2.1 CLEANUP OF SAMPLE EXTRACTS

2.1.1 LIQUID – LIQUID PARTITIONING METHOD

Liquid–liquid partitioning is applied in clean up of sample extracts. The process allows residues from the initial extraction solution into a non-aqueous solvent. The compound's distribution coefficient (p-value) between two solvents will indicate if the compound will pass through the liquid-liquid partitioning. The underlying principle allows the separation of extraneous co-extractives or their transfer into a suitable solvent from residues of interest by shaking the medium with two immiscible solvents or combinations of immiscible solvents. The process generally can be repeated two or more times depending on the p-values. The most popular solvent used here against aqueous acetone extracts is dichloromethane. To increase the transfer of pesticides to the dichloromethane phase, by a salting-out effect, sodium sulphate or chloride is added.[1]

Lipid samples, however, are dissolved in petroleum ether or hexane and partitioned with acetonitrile.[1] The lipid remains in the ether whilst the pesticides go into the acetonitrile phase. Pesticides are later partitioned again into hexane by addition of excess water and sodium chloride to dissolve acetonitrile in the aqueous phase. Other procedures involve partitioning pesticides from hexane solutions of extracted fats into dimethyl-formamide

and then extracting pesticides back into hexane after the addition of aqueous sodium sulphate solution.[30]

2.1.2 ADSORBTION COLUMN CHROMATOGRAPHY METHOD

Many adsorbent materials such as alumina, florisil, activated charcoal, celite, magnesium oxide etc. have been used to clean up extracts before analysis. Adsorbent column chromatography is a clean-up step designed to remove non-volatile high molecular weight coextractants. It is usually used as a cleanup on its own or to complement other techniques. The pesticides are either preferentially adsorbed onto the adsorbent in the column whiles the coextractives are eluted or the coextractives adsorbed while the pesticides are selectively eluted. The order depends on the adsorbent, the pesticides of interest and the solvent used in the elution.

Good cleanups are achieved when columns are eluted with solvent mixtures of low polarity, eluting less polar residues and leaving more polar co-extractives behind on the column. In the presence of very polar residues, the adsorbent may have to be changed to accommodate adequate elution of the required residues. Solvents of high polarity sometimes deactivate some adsorbents and change the pesticide elution profile. The grain (quality, quantity and particulate size) of the adsorbent affect the flow rate and homogeneity of the chromatographic column and the sharpness of separation of the zones of adsorbed material. Grain sizes used vary considerably[31]. Very fine particles fill the column unevenly and cause the formation of channels, and also reduce the flow-rate. Unsatisfactory separation occurs due to uneven flow of liquid and disturbance of the planar front of the adsorption zones. In the case of too

coarse particles, again uneven and diffuse adsorption zones result. Because the thickness of the film of liquid between the grains become greater and vary over a wide range, the average time taken for solute to diffuse to the interface becomes longer, consequently, equilibrium is established more slowly and less uniformly. The average size of adsorbent grains according to Zechmeister and Cholnoky[32] is 1.5 to 10.5μ. The activity of sorbent, activation and deactivation of the sorbent by the solvents, nature of residues and the co-extractives as well as temperature and loading of the column are some of the other factors which affect the separation. The adsorption activity can be changed within limits by the process of activation. This consists of the removal of substances such as water, which block the active centres of the sorbent or of improvement in the surface properties by a change in the functional groups or the size of the pores[33]. If the most active adsorbent is used, all substances are very strongly bound on to it and the differences in their adsorptivity disappear; if partial deactivation is carried out, the resolving power of the adsorbent is increased, and the possible decomposition of the less stable substances is prevented.

2.1.2.1 ALUMINA (ALUMINIUM OXIDE)

Alumina is one of the most widely used adsorbents, because of its high adsorption capacity. It is easily activated to different but reproducible degrees, has many applications. Moreover it is easily obtainable and can be regenerated.

Alumina in the activated form is very retentive, allowing slow passage of pesticides through when used in a cleanup. Addition of 3 – 6% water

deactivates the alumina to a less adsorptive state.[35] The main disadvantage is the possible occurrence of unwanted side-reactions with the substances adsorbed. It tends to decompose some organophosphates, with some of the more polar pesticides only partially recovered.[35]

Three forms of alumina are commercially available (Acidic, base, neutral). The most commonly used in the field of pesticide analysis is the neutral. This lends itself particularly in cleaning extracts of some pyrethroid and organochlorine pesticide residues.

2.1.2.2 FLORISIL

Florisil is a synthetic selective adsorbent and a magnesia – silica gel catalyst suitable for use with either water or organic solvents. It is made by mixing magnesium sulfate with sodium silicate, filtering, drying, and calcining at 650 700°C. The active form can be obtained by heating at 130°C for 168h (1 week)[34] or 130°C overnight[35].

The deactivated form (addition of 2 – 10% water) lends itself for clean – up of fatty materials since it retains some lipids preferentially.

Florisil clean up has formed part of several multiresidue procedures for the analysis of fruits and vegetables and particularly for fatty foods for the analysis of organochlorine pesticides.

Elution of florisil column with solvents of low polarity results in quantitative recovery of non-polar residues, which are sufficiently clean for GLC with electron capture detector (ECD) as well as for TLC.[35] As with Alumina, even well deactivated Florisil decomposes some pesticides[34, 35] (eg. the phthalimide fungicides, will oxidise organophosphates with thio-ether groups). Because of

the varying amounts of sodium sulphate contamination, activity varies considerably from lot to lot and therefore each batch needs to be carefully standardised before use to prevent variable recovery and clean-up . These form the main disadvantages.

2.1.2.3 OTHER ADSORBENTS

Apart from alumina and florisil, there are other adsorbents such as magnesium oxide, deactivated silica gel, silica gel, Carbon and Celite. These other adsorbents have limited uses but useful in certain situations. For example, carbon, because of its lipophilic nature, absorbs preferentially non-polar, lipophilic and high molecular weight substances. It is therefore very useful in cleaning-up extracts with high chlorophyll content (vegetables).

Combinations of these adsorbents have been used in an attempt to produce more efficient multiresidue cleanup methods. A charcoal-silinized celite column is used to clean up polar, medium polar, and non-polar pesticides. It is the column of choice for carbamates.

Columns composed of layers of different adsorbents have found occasional use. The most active adsorbents are packed in the bottom of the column, the least active near the top. Because it is impossible to foretell at what position substances will be completely separated and because substances already separated upon one layer may form a single band when washed onto a more active region, columns of mixed adsorbents have not found extensive use in research.

2.1.3 GEL PERMEATION CHAROMATOGRAPHY METHOD (GPC))[36]

GPC is mainly based on size exclusion chromatography. This mode relies on the accessibility of the pores in the stationary phase to molecules of differing molecular sizes. Here the stationary phase is a polymeric porous bead suspended in a suitable solvent (eg. ethylacetate: cyclohexane 1:1). As sample passes through column, smaller components are able to penetrate further into the pores and are therefore retained longer on the column than larger molecules, which are excluded from the pores. Molecules larger than the pore size of the gel tend to pass straight through. Both plant and animal samples can excellently be cleaned up using this method.[28] The method can also be applied to all kinds of environmental samples.[24] This is because many of the co-extractives eg. fats and oils encountered in pesticide residue analysis tend to be molecularly larger than most pesticides.

Advantages include easy automation or operation on a manual basis, smaller volumes of solvent (compared to other clean-up procedures) needed, no losses of residue with the process and system can be re-used.

A cross-linked polyvinyl resin (beads), Bio-beads SX – 3 is the most universally used beads for pesticide residue analysis. Solvents commonly used are mixtures of ethylacetate and cyclohexane, Cyclohexane and acetone, hexane and dichloromethane.

The choice of solvent system is essential to the correct functioning of the procedure. The beads swell in organic solvents to a size many times that of their dry state and this condition is vital to the effective operation of the GPC technique.

The set up of basic GPC equipment and apparatus includes:

- A GPC glass column with adjustable plungers and teflon tubing.
- A pump capable of producing a constant flow rate or for semi-automated GPC, a pure nitrogen source to force flow of solvent.
- A sample loop injector or septa closed point that allows manual injection through septa unto packed column. With manually operated ones, the GPC column should have a solvent reservoir with a glass tap for operation under gravity.

2.1.4 SOLID PHASE EXTRACTION METHOD (SPE)

The term solid phase extraction is usually connected with the commercially available, ready-to-use systems, based on pre-packed cartridges such as Bond Elut^{R} and Extract clean^{TM} (alletech). Each cartridge is usually made from an inert material such as polypropylene, packed with either 100mg or 500mg of a particular silica based bonded phase packing. They provide fast and efficient pre-analysis sample cleanup and concentration. There are three types of packing:

REVERSE PHASE: These types of packing include C18 which is the most widely used reversed phase. In addition, C8, C2, Cyclohexyl and Phenyl bonded phases are available. Generally, non-polar to moderately polar compounds in a polar matrix are retained by reversed phases. These packing require conditioning with an organic solvent followed by an aqueous solvent prior to use. Elution of moderately polar compounds is often accomplished with methanol while non-polar compounds require less polar solvents.

NORMAL PHASE : Silica, Florisil, Amino, Cyano, diol and Alumina are available. Normal phases retain polar compounds from non-polar matrices. Conditioning is done with a non-polar solvent. Elution is accomplished with more polar solvents. Basic compounds are readily retained by silica. Very polar compounds can be irreversibly retained on silica. In such a case, a bonded phase like diol or amino is a better choice.

ION EXCHANGE-Anion and cation exchangers, both strong and weak are available. These however are of little use in pesticides residue work.

The nature of the sample and the compound (s) to be isolated determine the proper packing type. Aqueous or polar organic matrices generally require reversed phases while non-polar matrices require a normal phase. The matrix can be modified by adding an appropriate solvent to increase or decrease its polarity. The analyst must also determine whether the compound of interest is to be retained for later elution or whether the goal is to retain the interfering compounds. For example, a non-polar analyte (eg. pesticide, drug) in a polar sample matrix (eg water, urine) would indicate that a reversed phase would be an appropriate packing material to choose. A C18 or C8 packing would preferentially retain non-polar compounds, allowing weakly retained polar compounds to be washed off with a polar solvent prior to the final elution with a non-polar solvent.

The principles of SPE are based on the principles of liquid chromatography. However, how the applications of the principles are different. In liquid-solid chromatography a mobile phase is chosen which allows the sample components to continuously interact with the packing bed as they pass down

the column. Different amounts of interaction (due to differences in structure) cause the various sample components to leave the column at different times, allowing the separation and collection of these components. When using SPE, initial conditions are chosen which, instead of allowing continual interaction, allow for the complete retention of the sample compound. The solvent is then changed to one, which causes complete elution (minimal interaction) from the packing bed. Hence the term "digital" or "on-off" chromatography.

Obtaining optimal results from SPE cartridges requires attention to four factors. These are:

1. **Proper physical and chemical characterization of the sample.** Compounds polarity relative to the matrix, the presence of charged functional groups, solubility, molecular weight, etc. determine how strongly the sample compound is retained by the packing bed.
2. **Selection of proper retention strategy.** Two approaches are possible. The desired compound can be unretained by the packing bed while interfering compounds are retained, thus cleaning up the sample. Conversely, the desired compound is retained by the packing bed while interferences are either unretained or washed off the packing bed prior to eluting the desired compound. This second approach is commonly employed when concentration of the sample is desired.
3. **Selection of the proper packing type and bed size**. The different packing types offer various selectivities that should be exploited to maximise the structural differences between the sample compound and sample interferences. Choosing the packing with the proper selectivity

results in the cleanest extract with the best recovery. Poor sample recovery often occurs when the packing bed size is not optimised. Too large a packing bed results in incomplete elution while too small a bed results in incomplete retention. Both situations result in poorer than expected recoveries.

4. **Selection of suitable conditioning, wash and elution solvents.** It is important that attention is paid to solvent strength relative to the packing material. This determines whether one is doing a reverse or normal phase solvent elution. Conditioning solvents should be chosen such that they do not cause pesticides of interest to be eluted when sample is loaded on the SPE. Buffers should be used to control ionisation of charge compounds. Wash solvents should remove weakly retained interferences but shouldn't be strong enough to elute the desired compound. Elution solvents should be strong enough to completely elute a compound in a small volume (1 – 2ml)

The solubility of a compound in a solvent is an important consideration. A solvent that the sample compound (pesticide) isn't soluble in but sample interferences are would be an excellent wash solvent for separating the unwanted matrix from the pesticide of interest on the SPE column.

2.1.5 SWEEP CODISTILLATION

It involves the introduction of the concentrated sample extract into a continuous stream of nitrogen. This is then transferred to a hot zone where the material is condensed on the large surface of glass wool. The more volatile pesticides are codistilled from the surface of the glass wool by means of a

sweeping flow of volatile solvent, such as hexane, and trapped in a cooler.[37] The interfering material from the plant or animal tissue is then left stack on the glass wool. The method can be applied to the separation of organophosphorus and organochlorine pesticides from plant and animal samples as well as for separating triazine herbicides from soil. Although it was first used for cleaning up of fat samples.[38]

2.2 METHODS OF PESTICIDE RESIDUES ANALYSIS

2.2.1 GAS CHROMATOGRAPHY (GC)

First introduced in 1953[39], it has now become the most versatile tool for analysis of pesticide residues in soil and plant tissues as well as water samples. [40, 41, 42]

The most common form of GC is Gas – Liquid Chromatography (GLC), which consists of a carrier gas, a column (stationary phase being a non-volatile liquid), a detector and a recorder/ electronic integrator. GLC achieves separation of mixtures (components) by partition of components between a mobile gas phase, usually an inert carrier gas (Helium, Nitrogen etc.) and a stationary phase (a non volatile solvent distributed on a solid support). Components are selectively retarded by the solvent according to their distribution coefficient. This causes the components to leave column in the gas stream in a certain order and recorded as a function of time by the detector. Identification is by means of retention times and quantification by peak area or peak height.

Choice of column, to a large extent determines the success of a separation. Two types of column are available; packed and capillary columns. Packed

columns consist of glass or metal columns (I.D 2 – 5mm and length 0.5 – 8m) packed with fine particles of a chemically inert support coated with the stationary phase (weight % between 1-25%). Packed columns are only used where high resolution is not necessary. Capillary columns instead of packed columns are used for the analysis of complex mixtures. They give better resolutions, more rapid analysis time, less decomposition and less adsorption on the column. Generally, the solid support, type and amount of liquid phase, method of packing, length and temperature of column are important factors in obtaining resolution. Some of the capillary columns normally used for pesticide residue analysis include SPB5, 5% SE30, SPE 608 and OV-17. These are mostly dimethyl siloxane polymers. [43]

In this system of analysis, pesticide residue is based on the sensitivity and selectivity of gas chromatographic detector. These detectors measure the amount of components coming from the column. The four most popular selective detectors in pesticide residue analysis are the electron-capture (ECD), the flame photometric (FPD), the alkali flame ionisation (AFID), and the thermionic nitrogen phosphorus (NPD) detectors.

The ECD is used mainly for the detection of organochlorine[43], AFID and NPD for pesticides containing phosphorus and nitrogen. Levels as low as 50fg/s nitrogen in azobenzene and 25fg/s phosphorus in malathion have been reported[44]. The FPD however can be operated either in the sulphur or phosphorus specific mode depending on what one is measuring. Detection limits as low as 0.5pg/s phosphorus and 50pg/s sulphur have been reported[45].

Eluents from columns if containing pesticides are detected by the detector. The detector hooked to an amplifier and a chart recorder or computer data system then magnifies the signal and quantified based on the measurement peak area or height compared to that of peak heights or areas of standards of known concentration or an internal standard added to the sample analysed. The disadvantages being that you need a constant source of electricity, high level of man power training and high cost of gases as well as columns and equipment. Constant servicing of equipment is also needed.

2.2.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Modern high-resolution liquid chromatography, variously known as high pressure, high performance and high speed liquid chromatography are usually referred to as HPLC. Here the basic unit and operation is similar to the GC, but the mobile phase is a liquid instead of the gas for G.C. In comparison with GC, HPLC is not limited in applicability by component volatility or thermal stability. For pesticide residue analysis, the most common stationery phase used in the column is Octadecylsilane (ODS) which is bonded to a silica support via a silyl ether (siloxane) linkage (Fig. 1). Such a packing

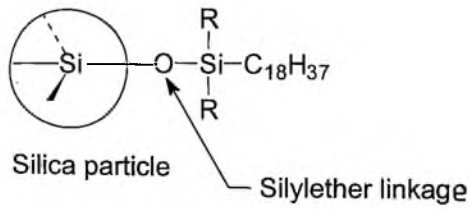


Fig. 1 Octadecylsilane (ODS) chemically-bonded stationary phase.

material is used with a polar eluant, e.g. methanol or methanol – water mixture, an elution technique commonly referred to as a reverse-phase chromatography. Other reverse phase packings used for pesticide analysis have bonded to the support one of the following: C_8 hydrocarbon chains, C_8 groupings containing aromatic moieties, phenyl groups and polar materials such as cyanopropyl and nitropropyl. In HPLC, filtered eluant is drawn from solvent reservoirs, the composition being determined by the proportion of each solvent delivered to the column via a high-pressure pump and a solvent mixing system. The cleaned-up sample mixture is applied to the top of the column (sample loop) and then carried down through the column by the eluant at a rate inversely proportional to their attraction for the packing material. The passage of the pesticide residues from the column is monitored by the detector and the response displayed on either a chart recorder or an integrator connected to a computer system. Retention time, peak height or area is used for identification and quantification when run along with known standards. Detectors are normally located at the exist of the column and may be classified either as those which monitor a specific property of the solute (e.g. UV or Visible spectrophotometer which may be of fixed wavelength or variable wavelength design) or those which detect changes in a bulk property of the column effluent (e.g. refractive index monitor which functions by recording the refractive changes in the eluant as the solute passes through the detector cell. Some pesticides usually analysed using HPLC are the Herbicides (Atrazine, simazine etc.) and organo-phosphates. Some detectors used in GC like flame ionisation [46], flame emission[47] and thermionic emission detector[48] have been developed which are usable with micro-column HPCL systems for residue

analysis. The disadvantages are once again the high cost of equipment, consumption of a lot expensive solvents and requires high level of training. Leakage's on the system and the need for a constant supply of power is another problem.

2.2.3 COLORIMETRIC ANALYSIS

For this kind of analysis to be possible, compound of interest (pesticide residue) should produce a certain colour reaction with a chromogenic reagent, the absorbance of which is related to the concentration of the substance being determined. The relationship between optical absorbance and concentration of substance being linear. The amount of colour for given sample may be measured by several instruments, such as visible comparators, or colorimeter. Present technology, however, requiring the utmost sensitivity for the micro-analysis of pesticide residues, depends on spectrophotometers of high resolving power. Pesticide residue analysis generally requires an effective clean-up process prior to actual determination. Ultra violet methods even require an especially rigorous clean-up to ensure that the final solution is free from any material that will absorb light in the region of the spectrum where the pesticide content is measured. Many colorimetric methods will detect as little as 10μg on a routine basis and lesser amounts with micro methods[49]. Other spectrophotometric methods of analysis include infrared spectrometry (IR). The infrared portion of the spectrum covers the region from approximately 13,300 to 100cm^{-1} (0.75 - 100μ). The region of primary concern is 667cm^{-1} . Vibrations in the molecules cause bands to appear in the infrared region. The position of these bands is typical of the molecule and

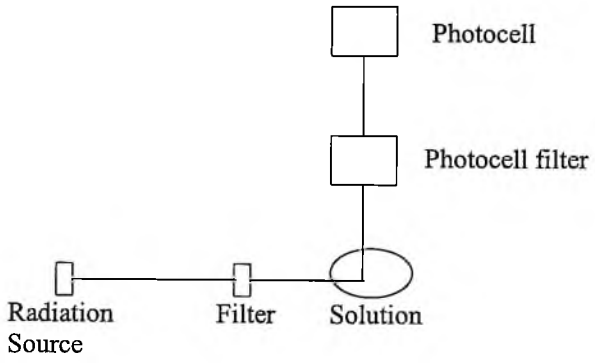
offers a peculiar advantage for identification. The intensity of the bands as in ultraviolet and visible spectra, is proportional to the concentration of the compound. Sample to be analysed can be a liquid, solid or gas. If the material is a solid, its spectrum is determined after grinding it with a solid diluent, such as potassium bromide, and pressing it into a pellet. The common practice, where only very small amounts of material are available, is to determine the spectra of the material in solution. Ordinarily, hydrolytic solvents are not used since they absorb strongly in the infrared region. Some formulated products analysed using this method include α, β, γ, δ-isomers of benzene hexachloride[50, 51], Lindane[52] and 2,6,dichloro-4-nitroaniline[53]. A number of methods have also been described for IR–residue analysis including aldrin and dieldrin from soils [54, 55], Mitox [56] and Thiodan[57] residues from pears. IR methods have certain advantages. It is seldom necessary for compound to undergo chemical reactions in order to be quantitated as with colorimetric procedures. They give a definite indication on the material being determined as the compound of interest. The disadvantages compared to GC and TLC, are that it requires relatively high concentration of the sample in order to obtain the spectrum and extensive clean-up with certain limited solvents that can be used.

2.2.4 FLUORESCENCE

Fluorescence normally occurs when a substance absorbs radiation and is maintained in an excited electronic state for 10^{-8} to 10^{-9} seconds or longer. Substances with absorption bands in the short wavelength ultraviolet region of the spectrum, in general, do not fluoresce. This method of analysis, in the

past, was primarily an analytical tool for compounds that are themselves fluorescent or which can be easily converted to fluorescent compounds. One common use was for the quantitative assay of vitamins, especially thiamine and riboflavin. With time, the requirement for detection and accurate determination of increasingly small quantities of pesticides in animals and plant parts forced residue chemists to use this method which is applicable to submicrogram quantities of material. It measures accurately quantities of material which are only a tenth, a hundredth, or even a thousandth of the smallest amounts measurable by classic colorimetric procedures.

Determinations usually involve the use of a fluorometer with the following basic design:



A suitable radiation source is passed through a filter to isolate the desired excitation band before passing through the solution. Fluorescence is then measured at right angles to the irradiating beams. Based on calibrations from known standards, quantitation can then be made on the sample. The expression relating intensity of fluorescence and intensity of the exciting light absorbed by the sample is given below. This is based on the assumption that Beer's law applies to the absorption.

$$P = KI\,(1 \quad 10^{-abc})$$

Where P = Intensity of the fluorescence.

K = a constant which includes such factors as the area of the solution irradiated, the transmission bands of the photocell filter, and the spectral response of the photocell.

I = the intensity of the exciting radiation

a = the specific absorption coefficient of the exciting radiation.

b = the depth of fluorescing solution through which the light passes.

c = the concentration of fluorescing material.

As the value of abc becomes small and approaches 0.01 or less the expression becomes

$$P = 2.3\,K\,I\,abc \quad \text{or} \quad P = K'\,C$$

Where K' is an overall constant for one particular substance in a given instrument under specific operating conditions.

Actual quantitative residue methods have been worked for a few compounds. These are mainly organophosphorus compounds. These include Co-ral[58] Bayer 22408 [59] (O, O diethyl- O- naphthalimido phosphorothioate.), Guthion[60] and DEF.[61] As with other techniques, the application of the fluorescence method is made in a variety of ways:

a. Direct fluorescence: This is done on compounds which are highly fluorescent.

b. Hydrolysis: here the compound in question is readily converted to a highly fluorescent substance.

The table below shows the advantage of fluorescence methods over classic procedures such as colorimetry

Table III[61] Table comparing the sensitivities of colorimetry and Fluorometry as applied to pesticide residue determination.

COMPOUND	COLORIMETRIC µg [a]	FLUOROMETRIC µg[b]	RATIO
Col-Rad	2.5	0.002	1250
Guthion	1.6	0.01	160
DEF	10	0.02	500

a Micrograms required for absorbance of 0.1 using 2ml of solution in a Beckman DU spectrophotometer.

b Micrograms required for 10% galvanometer reading at maximum sensitivity with 1ml of solution (Slit arrangement #3)

Despite the high sensitivity, this method has the disadvantage of having several factors affecting the results, chief amongst them being solvent and pH. Fluorescence readings are also relative rather than absolute. They cannot be used on pesticides that do not fluoresce and equipment cost is relatively high. Relationship between fluorescence and concentration is usually only linear over a limited concentration range.

2.2.5. THIN LAYER CHROMATOGRAPHY (TLC)

Amongst the numerous analytical methods available for the identification and detection of pesticide residues is TLC. It is analogous to other adsorptive techniques such as column chromatography and gas chromatography. This technique was developed in the early 1950's[63]. This method has been partly used for the analysis of pesticide residues in soils[64, 65, 66], plants and vegetables[67, 68, 69, 70, 71], water [66, 72, 73, 74, 75], urine[76] and tissues[77].

A TLC consists essentially of an adsorbent spread on a plate. The adsorbents normally used are Silica gel, Alumina (alkaline, neutral or acid washed).

Others include Kieselguhr, magnesium silicate, polyamides and activated carbon. Cellulose, ion-exchange resins and ion-exchangers based on modified cellulose are also commercially available. These adsorbents normally contain binders, which allow them to stick on plate. Commercially coated plates are these days available and give uniform coating, hence preferred to home-made ones. In TLC, the components to be separated and identified are spotted on the coated plate. The plate is placed in a developing tank containing a solvent (with tank covered and saturated with solvent) that acts as the mobile phase. Components of the sample mixture are carried along the plate as the solvent migrates. A continuous adsorption-elution process takes place and the most mobile compounds travel farthest, causing complex mixtures to be resolved into a series of spots i.e. resolution of components is based on differential migration. Choice of solvent as mobile phase depends on the degree of polarity required of the solvent to bring about the desired movement and separation.

The location of spots on a chromatogram is an index to the chemical composition and identification of the compounds separated. The migration is usually expressed as an Rf value, which is determined by the ratio of distances:

$$Rf = \frac{\text{Distance of centre of spot from starting point}}{\text{Distance of solvent front from starting point}}$$

Rf values tend to show great variation between laboratories because of factors like temperature and saturation of developing tank. Rf values can be

calculated relative to a marker compound (RRf) with certain peculiar property that is always run with compound for internal quality control.

$$RRf = \frac{\text{Rf of compound}}{\text{Rf of marker}}$$

The Rf values of the marker compounds are used as reference for the RRf values which greatly facilitates the identification of the spots on the plates. The RRf values have the advantage of being relatively constant between laboratories.

Methods employed in quantitation usually involve:

i. Semiquantitative visual estimation from spot appearance; A number of standards are spotted on the plate with the samples. After development and visualisation, the sample spots are visually compared with the standard spots. The concentration of the sample is then taken as that of the standard spot for which the intensities and size are about the same. On the other hand, the diameters of the standard spots can be taken and plot against their concentrations to give a calibration curve from which the concentration of the sample can be estimated.

ii. Colour development on TLC plate, followed by extraction and colorimetry of the coloured material: It involves spraying the plate with a colour developing reagent and treatment to develop the colour. An area around and including the spot is removed, extracted, and the absorbance of the resulting solution is measured at a given wavelength. The concentration is then determined from a

calibration curve of standards of known concentration measured at same wavelength.

iii. Extraction of the material from TLC plate, followed by colour development in solution and colorimetry.

iv. Densitometry (after visualisation): This involves optically scanning the thin layer plate after development and visualisation. The absorbance of the spot on the thin layer is measured as compared to the absorbance of the thin layer plate. The amount of the unknown pesticide is then found from a similar calibration curve obtained out of measurements on known standards. This method offers advantages over visual elution methods, particularly when working in the microgram range.

Advantageous features of TLC method over all the analytical methods reviewed so far include low cost, ease and speed of operation and high resolution. The large number of adsorbents available and the ease of changing conditions give the chemist a considerable number of parameters that can be varied to obtain a desirable separation. It has genuine and general utility for pesticide analysis because; it is applicable to most of the types of analytical problems in which column chromatography, paper chromatography, gas chromatography and electrophoresis can be used; Capable of giving reliable information in screening for parent pesticides and for transformation and degradation products. Also useful for screening important commodities moving in international trade or the domestic food supply, with an unknown history. It is much simpler and faster than these techniques as well as an investment of only a few hundred dollars to set up the equipment and a

technician can learn the technique in a few hours. Samples also do not need a very thorough clean up as required in GC and HPLC analysis. The problem with this technique is that the identification using Rf values depends on several factors including temperature. Notwithstanding, this technique has the advantage of being simple and very cheap, requiring no complicated equipment and giving comparable results to that of other chromatographic techniques.

CHAPTER THREE

EXPERIMENTAL

3.1 EQUIPMENT

TLC basic set, Camag, including application guide, atomiser and developing tank obtained from Camag, sonnenmattstr. 11CH-4132, Muittenz, Switzerland was used as a guide for spotting samples, developing chromatogram and spraying reagents. A semi-automatic gel chromatography system (type KL - SX - 3, operation with constant nitrogen over pressure 0.5 att) obtained from Radamed Bt, Orvosi Müszertechnikai BT. H-3534 Miskolc, Szinyei M. P. U 10 in Hungary was used for the gel permeation clean-up. Homogenizer with 1 litre container was obtained from Waring products div., New Hartford, Conn., U.S.A and used for homogenising plant samples and cow liver. Buchii 461 Rotavapor from Switzerland was used in concentrating extracts. A 25000rpm refrigerated supercentrifuge (Sorvall RC-5B) obtained from Du Pont company, diagnostic and bioresearch systems, sorvall products, Wilmington, Delaware 19898, U. S. A. was used in centrifuging the pig serum, separation of cow liver enzyme from liver material as well as washing off acid from aluminium oxide G. Varian star 3400CX Gas chromatograph from U.S.A was used in determining the pesticide residues in the plant and soil matrices.

3.2 CHEMICALS, REAGENTS AND MATERIALS

Silica gel plates (5721, 5715) , Alumina plates (5713) and all reagents and chemicals were purchased from Merck (E) GmbH, Germany. Solvents were obtained from Fluka Chemie AG., Industriestrasse 25, Ch-9470 Buchs,

Switzerland. The chemicals include; Acetic acid (AR), Hydrochloric acid concentrated (AR), Potassium permanganate GR ACS, 150, Potassium iodide (GR), 4-nitrobenzenediazonium tetrafluoborate for synthesis (AR), Sodium hydroxide anhydrous (AR), O-tolidine (4,4' - Diamino - 3, 3' dimethylbiphernyl) AR, Glycerol (AR), Borax (AR), Nitric acid 60% (AR), Aluminium oxide G, Silver nitrate GR ACS, ISO, Ethanol (AR), Ethylene glycol (AR), p-dimethylamino-benzaldehyde (AR), Potassium nitrate AR ISO, β - naphthyl acetate (AR) for Biochemistry, 2,6 - dichlorophenol - indophenol sodium salt dihydrate GR for determination of Ascorbic acid., Fast blue salt B zinc chloride double salt for microscopy, Bromine (GR), Sodium sulphate anhydrous granulated for organic analysis of traces, S acetylthiocholine iodide for Biochemistry, Tris (hydroxymethyl) aminomethane (Trometamol) GR buffer substance, Di-sodium hydrogen phosphate dihydrate (GR), Sodium bicarbonate GR, Parafin liquid (AR), Benzene (GR), Ethylacetate (AR), Dichloromethane (AR), Cyclohexane (AR), Acetone (pesticide residue grade), Lichrolut Si 60 500mg Cartridges, Bio-Beads S-X3, 200 - 400 mesh, TLC plates 20 x 20cm silica gel 60, TLC plates 20 x 20cm silica gel 60 F-254, TLC plates 10 x 20cm silica gel 60, TLC plates 20 x 20cm aluminium oxide 60 F-254 and TLC plates 20 x 20cm aluminium oxide 60. Pigs blood for separation of serum enzyme and cow liver for liver enzyme were obtained from the University Ghana Agricultural Research Station at Nungua and the Accra Abattoir respectively.

3.2.1 PESTICIDE STANDARDS

Unless otherwise stated, pesticide standards were purchased from DR. Ehrenstorfer Gmbh, Ausburg, Fed. Rep. of Germany. The organochlorines amongst them were: aldicarb (98.7%), aldrin (99.5%), dieldrin (96%), α - endosulfan (99.3%), β - endosulfan (98.2%), γ- HCH (99.4%), λ-cyhalothrin (96%), lindane (99.4%), and o, p' DDT (99.5%). The carbamates were carbaryl (99.5%), carbofuran (99.1%), dioxacarb (97%), benomyl (98%), and propoxur (99.7%). The phosphoric acid derivatives were: chlorpyrifos (98.7%), diazinon (94.0%), dimethoate (98.7%), fenitrothion (98.5%), isazofos (94%), malathion (99.5%), monocrotophos (99.4%), pirimiphos-methyl (98%) and parathion – methyl (98.6%). The others were ametryn (94.0%), atrazine (98.4%), captan (98.4%), dimethametryn (94%), diuron (98.8%), merpan (99%), metobromuron (99%), mirage (98%), nitrofen (98.5%), propanil (99.5%), pyrinex (98%) and thiram (97%)

3.2.2 PREPARATION OF STANDARD SOLUTIONS

Stock solutions of the above listed analytical pesticide standards were prepared by first weighing a clean dry glass stoppered volumetric flask on a previously calibrated analytical balance. Primary standard was then adjusted for purity. Eg. for standard of 98% purity, $\frac{100}{98} \times 0.050g = 0.0510$ gram

0.0510 grams were then weighed by difference into flask and dissolved in acetone (PR grade) and made to the 50ml mark. Standards were properly labelled (substance, solvent, concentration, date of preparation and signature) and stored in a refrigerator until use.

3.3 PREPARATION OF WORKING SOLUTIONS

First, second and in some cases third working standards were prepared out of the stock by carrying out serial dilutions. Each individual dilution factor was not greater than 10 and the minimum volume pipetted was between 2 – 5ml using a calibrated pipette. Suitable mixtures of pesticides, which are separable on the TLC plates and solvents used to develop, were also prepared.

3.4 ELUTION OF PESTICIDES ON TLC PLATES

The Rf values were determined in developing tanks at 24°C and 32 ± 3°C respectively. 5µl of standard stock solutions of all the pesticides was applied to the sorbent layer with a syringe, with a 90° cut needle tip. Standards were applied in spots of the same size, which was achieved, by spotting the same volume, using the same solvent. Spots were applied along the imaginary band on the spotting guide, allowed to dry before applying again until the whole volume had been applied. Care was also taken to include a marker on each plate. The eluent was equilibrated with vapour phase by inserting filter paper in the developing tank and waiting for at least 30 minutes before the plates were placed in the tanks. The eluent was allowed to run to at least 10.5cm from origin. The detection procedures are described in section 4.7.

The Rf values were determined in the following systems:

Layer: Silica gel 60 F-254 0.25mm (Merck No. 5715), activated at 105°C for 30 minutes before use.

Aluminium oxide (Merck No. 5713), activated at 85°C for 45 minutes before use.

Self-made Aluminium oxide, activated at 80°C for 45 minutes before use.

System I: Silica gel 60F – Ethyl acetate

System II: Silica gel 60F – Dichloromethane

System III: Ready made Al_2O_3 - Ethylacetate

System IV: Self-made Al_2O_3 – Ethyl acetate

System V: Ready Made Al_2O_3 – Dichloromethane

System VI: Silica gel 60F – Benzene

System VII: Self Made Al_2O_3 – Dichloromethane

3.5 DETEMINATION OF RF AND RRF VALUES OF THE MARKER COMPOUNDS AND OTHER PESTICIDES.

Standard solutions (5µl) of all the pesticides listed were applied to the sorbent layer with a syringe, with a 90° cut needle tip. Standards were applied in spots of the same size, which was achieved, by spotting the same volume, using the same solvent. Spots were applied along the imaginary band on the spotting guide, allowed to dry before applying again until the whole volume had been applied. Care was also taken to include a marker on each plate.

The appropriate solvent system being used was equilibrated in a covered Camag developing tank by transferring 50ml into the tank, containing a filter paper and waiting for 30 minutes before inserting the plates. A thermometer was also inserted to read the temperature of the solvent. The eluent was allowed to run up in an ascending mode to 10±0.5cm from the origin. At the end of the run, the solvent fronts were marked in a fume hood. An appropriate detection method was then applied.

3.6 THE EFFECT OF TEMPERATURE ON RF AND RRF

All pesticide standards were spotted and run just as described for 3.1.1. The saturated tanks for development were however equilibrated in rooms with temperatures 24°C. and 32°C ± 3°C. The time of run was noted by means of a stop clock. Plate was air dried and appropriate detection method applied. A ruler was then used to measure the distance moved by the spots.

3.7 THE EFFECT OF SATURATED AND UNSATURATED CONDITIONS ON RF VALUES.

Pesticide standards (5µl) were spotted on activated silica gel 60 plates using technique as previously described. Two developing tanks were set up at room temperature. One with a filter paper in tank and allowed to saturate whiles the other had no filter paper and was set up and covered only after inserting the plate. Order of spotting was as shown below, including a marker on each plate.

PLATE 1	PLATE 2	PLATE 3	PLATE 4
ATRAZINE	Dimethametryn	Atrazine	Atrazine
Cabaryl	Dimethoate	Aldrin	Benomyl
Dioxacarb	Chlorpyrifos	λ-cylohalothrin	Ametryn
Diuraon	O'p-DDT	Carbaryl	Dimethametryn
Monocrotophos	Aldicarb	Dioxacarb	Dimethoate
Propanil	Pyrinex	Diuron	Chlorpyrifos
Metabromuron	Mirage	Monocrotophos	O'Pddt
Propoxur	Atrazine	Propanil	Aldicarb
Carbofuran	Lindane	Metabromuron	Pyrinex
Benomyl	Merpan	Propoxur	Mirage
Ametryn	Nitorfen	Carbofuran	Lindane

PLATE 5	PLATE 6
Merpan	Atrazine
	Propoxur
Aldrin	Carbofuran
λ cylohalothrin	Benomyl
Atrazine	Ametryn
Carbaryl	Dimethametryn
Dioxacarb	Chlorphrifos
Diuron	O'PDDT
Monocroptophos	Aldicarb
Propanil	Pyrinex
Metabromuron	Mirage

Two of each plates spotted were used, one put in the saturated tank and the other in the unsaturated. The time of run, length of run and temperature of solvent were noted in the two solvents systems used (Ethylacetate and Dichloromethane).

3.8 DETERMINATION OF MINIMUM DETECTABLE QUANTITY VALUES (MDQ).

Marker compounds and other pesticides were applied on freshly activated Silica gel 60 TLC plates at MDQ Levels and developed in ethyl acetate (3 separate spots of different concentrations on each plate and repeated once for the same compound). MDQ levels detected by using appropriate detection method. Spots detected were repeated using lower concentrations. If not values were reported as MDQ values.

3.9 EFFECT OF SOLVENT ON THE MDQ

In this set up, sample concentrations spotted at the base of plate were also spotted at the top of the plate, where at the end of the run, the eluting solvent did not reach. This was to determine the effect of solvent on detectability after applying the detecting reagent.

3.10 EFFECT OF SAMPLE LOAD ON RF

Tomato samples were extracted and cleaned up as described under 4.9 and 4.10. Volumes containing 300 and 600mg were applied to activated silica gel plates. On top of these extracts were spotted 5µl pesticide standard markers and run at 32 ± 3°C. Rf measurements were then taken at the end of the run.

3.11 DETERMINATION OF BETWEEN AND WITHIN PLATE VARIATION.

Three separate 5µl aliquots of standard solutions at 2 x MDQ Level (Marker Compounds and other pesticides easily separable in silica gel- ethylacetate system) were spotted on freshly activated plates (Silica gel), developed and detected. Experiment was conducted on two separate days using two different detection methods for the same compounds.

From four replicate spots on the same plates were determined the within plate variability of the Rf values. Design was again repeated on four more Silica gel plates to determine the between plate variability.

3.12 LINEARITY OF CONCENTRATION VRS RESPONSE TEST

In order to be able to do some quantitative work this experiment was carried out. 1 – 15 times MDQ concentrations of marker compounds and some of the standard pesticides were spotted on freshly activated Silica gel 60, developed and detected using the various detection methods described. Spotting volume and size was the same for all. Spot diameter, diagonally and across was taken using a ruler and the average found. The average was then plotted as the response against the concentration to determine the linear range.

3.13 TLC DETECTION OF PESTICIDES

3.13.1 METHOD 1 (O - Tolidine + potassium iodide [OTKI]

Reagents

(a) O - tolidine (0.1g) dissolved in 2ml acetic acid

(b) KI (0.04g) dissolved in 2ml distilled water

Solutions (a) and (b) were mixed and made up to 100ml with distilled water. Reagent could be used after a month storage in a refrigerator.

Detection

Developed ready-made silica gel plates were air dried and placed in a tank saturated with chlorine for 30 seconds (a 25ml beaker containing about 1 to 2 g $KMnO_4$ was placed on the bottom of a developing tank, and concentrated HCl was added). Excess chlorine was removed in a well-ventilated fume hood after which the plate was sprayed with the reagent.

Colour reaction

Spots appeared as Blue, lilac on white background. Organochlorine pesticides gave whitish spots.

3.13.2 **METHOD 2 p - Nitrobenzene - fluoroborate [NBFB]**

Reagents

(a) Fluoroborate (0.2g) was dissolved in 2.5ml ethylene glycol and 22.5ml ethanol. This solution was freshly prepared and used within 5 - 10 minutes.

(b) NaOH (15g) was dissolved in 200 ml distilled water, cooled at room temperature and made up to 250ml.

Detection

Developed air-dried silica gel plates were air-dried and sprayed with reagent (b). Plate is later heated at 70°C for 10 minutes in an oven. Cooled to room temperature and sprayed with reagent (a).

Colour reaction

Red, lilac or blue spots appeared on white background. Suitable for use on ready-made silica gel plates.

3.13.3 **METHOD 3 - p - dimethylamino benzaldehyde [pDB]**

Reagent

p-dimethylamino-benzaldehyde (1g) was dissolved in a mixture of 47.5ml ethanol and 2.5ml HCl. Reagent was always prepared fresh before use.

Detection

Air dried aluminium oxide plate was placed in an oven set at 160°C for 25 minutes. Plate sprayed with reagent after cooling to room temperature.

Colour reaction

Compounds appeared after spraying as yellow spots on white background. Suitable for self or ready made aluminium oxide plates.

3.13.4 **METHOD 4a – For self-made aluminium oxide plates**

Reagent

0.15g $AgNO_3$ (0.15g) was dissolved in 15ml freshly prepared distilled water.

Preparation of TLC plates:

Aluminium oxide G adsorbent (45g) was firmly shaken for 15 minutes in a 300ml glass stoppered Erlenmeyer flask with 90ml 0.2% nitric acid. Slurry was transferred into centrifuge tubes and centrifuged at 3500 rpm for 10 minutes. Acid layer decanted and discarded. Aluminium oxide G was washed with 3 x 50ml double distilled water. It was centrifuged at 3500rpm for 10 minutes after each wash. 15ml 1% $AgNO_3$ solution was then added, followed by 15ml double distilled water. Stirred with a glass rod and the homogeneous slurry drawn over 5pcs 20 x 20cm on plates (0.25mm thick) without delay

using a TLC spreader. Good plates were air dried at room temperature for 30 minutes, then 85°C for 45 minutes and stored over activated silica gel in a dessiccator.

Detection

Air dried plates were sprayed with reagent and exposed to sunlight and/UV light.

Colour reaction

Blue, brown and greyish-black spots on white background.

3.13.5 **METHOD 4b – For ready-made Aluminium oxide plates**

Preparation of TLC plates:

50g aluminium oxide G adsorbent (50g) was firmly shaken for 2 minutes in 55ml freshly prepared double distilled water in a 100ml stoppered Erlenmeyer flask.

The homogenous slurry was spread over 5pieces of 20 x 20cm plates with 0.25mm thickness. The plates were dried first at room temperature and then, at 80°C in an oven for 45 minutes. Plates were stored in an activated silica gel in a dessiccator.

Reagent:

$AgNO_3$ (0.2g) was dissolved in 1 ml freshly prepared double distilled water. 20ml phenoxy-ethanol and one drop H_2O_2 were added and made up to 200ml with acetone.

Detection

Air dried plates were sprayed with reagent and exposed to sunlight and/or UV light.

Colour reaction: Blue, brown and greyish - black spots appeared on white background.

3.13.6 **METHOD 4c – For ready-made aluminium oxide plate**

Reagents (Dipping solution)

$AgNO_3$ (2.125g) was dissolved in 12.5ml distilled water. Concentrated ammonia solution (6.25ml) was then added and volume made up to 500 ml with acetone.

Detection

Activated ready-made aluminium oxide plates were dipped in the reagent for 5 seconds and left to dry in the hood. Dried plates were stored in a dark place; and, were usable within one month. The plate was developed, air dried, and exposed to sunlight.

Colour reaction

Most pesticides were visible as bluish black spots with 30 minutes of exposure to sunlight. Dieldrin takes 1 – 2 days depending on intensity of sunlight.

3.13.7 **METHOD 5 - Photosynthesis inhibition (Hill reaction)**

Reagents

(a) Cut *Sporobulus pyramidalis* or Spinach leaves (30g) were weighed into a mortar. 3ml glycerol, 15ml double distilled water and 5g acid washed sand were added. The mixture was ground into a homogenous pulp. The homogenate was transferred into cheese cloth in a funnel and pressed through. The filtrate was protected from sunlight by wrapping in aluminium foil and stored in a refrigerator until use. Fresh filtrate was prepared daily.

(b) **Borax buffer solution:** 350ml of 0.05M borax solution (9.5g Borax dissolved in 500ml water) was mixed with 150ml 0.1 M HCl.

(c) **DCPIP reagent:** 200mg of 2, 6 - dichlorophenol-indophenol Na-Salt dissolved in 500ml borax buffer solution.

Detecting reagent:

Mix 10 ml of *sporobulus sp* leaf pressing with 12 ml DCPIP solution or 10 ml spinach leaf pressing with 13.5 ml of DCPIP solution (using a universal indicator, pH of the mixtures is between 9 and 10). The reagent was always prepared fresh before use.

Detection

(a) Developed air dried ready-made silica gel plates were uniformly sprayed with the detecting reagent. Plates were immediately placed about 20cm below a 60W lamp. The inhibition occurred within 10 minutes and blue spots appeared against a light green background. Spots are however unstable and disappear within a few minutes.

3.13.8 **METHOD 7: Enzyme inhibition with cow liver extract and β-naphthyl – acetate substrate [EβNA]**

(a) Enzyme solution: Cut fresh liver (100g) was weighed into a homogeniser. Double distilled water (900ml) was added and homogenised. Centrifugation was done at 4000 min^{-1} for 10 minutes. Supernatant was collected in 10 or 20ml portions and placed in deep freezer until use. Enzyme concentrate was diluted to 2 times with distilled water before use.

(b) β - naphthyl – acetate: 125mg dissolved in 100ml in ethanol.

(c) Echtblau-salt: 10mg salt dissolved in 16ml double-distilled water. Prepared freshly each time before use.

(d) Substrate solution: Mixture of 10ml β-naphthyl-acetate solution and 16ml Echtblau – salt solution.

Detection

Developed ready-made silica gel plates were air-dried and placed in a tank saturated with bromine vapour for 15 minutes (a 25ml beaker containing about 0.5ml bromine was placed on the bottom of a developing tank, and allowed to saturate covered tank). Excess bromine was removed from plate by keeping it in a well ventilated fume hold for 45 minutes.

Plate was then sprayed with enzyme solution until thoroughly wet and placed in an oven pre-saturated with distilled water at 37°C for 30 minutes. Excess water was removed by blowing a stream of air from a standing fan over plate for 5 minutes after incubation. Plates were later sprayed with substrate solution.

Colour reaction

Most pesticides were visible as white spots over a pink (bluish – red) background.

3.13.9 Method 8: Enzyme inhibition with pig or house blood serum and acetylthiocholine iodide substrate [EAcI]

Reagent:

(a) 2,6 – dichlorophenol – indophenol Na-salt (50mg) dissolved in 50 ml distilled water to give 1mg/ml solution

(b) Tris (hydroxy ethyl) aminomethane (3.04g) was dissolved in 500ml double distilled water to give 0.05 mol tris-buffer

(c) Enzyme solution: Pig's blood was collected from the University of Ghana Agricultural research farm at Nungua, allowed to stand in a cold room for one hour. The serum was then decanted gently into centrifuge tubes and centrifuged at 4000 rpm for 10 minutes. Serum was then collected in 10ml portions and stored in a deep freezer until use. Cholinesterase activity was determined with the Ellman method (See Appendix C) and activity brought down to 140U/l by dilution with tris buffer (reagent b) before use.

(d) Substrate Solution: 45mg of acetyl thiocholine iodide dissolved in 30 ml of distilled water (1.5mg/ml water solution (can be stored at 4°C for 6 weeks)

Detection

Air-dried plate was treated with bromine and enzyme solution as in method 7. After incubation at 37°C for 30 minutes, excess water was removed with air stream. Substrate solution was later sprayed and plate incubated for another 15 min. The reagent solution is then sprayed.

Colour reaction

Spots appeared as blue on white background.

3.14 SENSITIVITY OF TROPICAL LEAVES TO PHOTOSYNTHESIS INHIBITION METHOD

Various serial concentrations (5µl) of all the eight analytical herbicide standards were spotted on a pre-coated silica gel 60F plate (20 x 20cm,

0.25mm thickness), previously activated at 105°C for 30 minutes. The spotted plates were then developed in a tank saturated with ethylacetate at 32° ± 3°C.

A 10mL leaf pressing extract of each of the fourteen plants investigated was obtained by grinding 30g of cut plant leaves with 3ml glycerol, 15ml double distilled water and 5g acid washed sand. This was then filtered through cheese cloth to obtain the pressing extract.

Between 12 and 20mL 2, 6- o – dichlorophenol–indophenol Na –salt [(DCPIP) solution prepared by dissolving 20g of DCPIP in a 500ml 0.05M borax buffer solution, was then added to the leaf pressing and pH of the mixture adjusted to between 9 and 10. Air – dried developed plates were then evenly sprayed with the reagent and immediately placed about 20cm below a 60W electric lamp. Spots became visible within 10 minutes. A ruler was then used to measure the Rf's and the concentrations of spots visible for at least 30 seconds taken as MDQ.

3.15 EXTRACTION PROCEDURE

Extraction with ethylacetate

A 60g sample was homogenised with 60ml ethylacetate in the presence of 50g $NaHCO_3$ and 50g anhydrous sodium sulphate. The solvent was allowed to separate from the solid material by keeping the sample solvent mixture in fume hood for 15 to 30 minutes. The free solution was filtered through a small cotton wool plug into a measuring cylinder to obtain 30ml filtrate. (1ml = 1g sample). The filtrate was evaporated in a rotary evaporator to about 2 – 3 ml and after transfer to a calibrated conical test tube the evaporation was continued with gentle air stream to near dryness. About 3 ml of the solvent

used for the cleanup procedure was added and evaporation repeated two more times. The final volume was adjusted to 2ml (equivalent to 30g sample) with ethylacetate: cyclohexane (1:1).

3.16 CLEAN-UP PROCEDURES

Clean-up on SX-3 gel column

A 5.6g weight SX-3 bead, previously soaked for 6 hours in cyclohexane/ethylacetate (1+1) was packed into a 10mm i.d x 20cm x 1.2mm thick pyrex gel column under the pressure of nitrogen. Solvent cyclohexane/ethylacetate (1+1) was pumped under pressure from a solvent reservoir by means of nitrogen gas through column. Flow rate was adjusted to 1.5ml/min. Column was calibrated by injecting a 200µl standard mixture of atrazine (1.10µg/ml) and Diuron (1.28µg/ml) unto the column and the fraction collected in 1ml portions. Fractions were concentrated to a volume of 100µl by blowing nitrogen gas over them for direct spotting on TLC plates or made up to 2ml before clean up on silica gel cartridges.

Silica gel cartridge

The cartridge was first reconditioned by flushing through two volumes of methanol followed by two volumes of distilled water. The diluted extract was then passed through the reconditioned C-18 solid phase extraction (SPE) column at a flow rate of 2ml/min. The column was later washed with 1ml distilled water. The column was vacuum dried for 15 minutes. Pesticides on dried column were then eluted with 2ml ethylacetate or hexane.

3.17 VALIDATION AND APPLICABILITY OF METHODS

3.17.1 EXPERIMENTAL DESIGN FOR UNDEN 20 ANALYSIS

The experiment was conducted on a 6-hectare cocoa plantation plot situated at Cocoa Research Institute, Tafo.

Four plots, each measuring 15m x 15 m were marked out. The plot that had been earmarked for pesticide treatment, was sprayed with a water emulsion of commercial unden 20 at a dose rate of 210g/56l of water/hectare. The spraying was done from two sides of a tree from the trunk into the canopy, as has been the normal practice (T2 method) to achieve adequate coverage. Application of insecticide was repeated three weeks after the first application. Leave, pod and soil samples were taken before and on day of treatment and subsequently at weekly interval for two months. Soil samples were taken at depths of 0 to 6 inches and 6 to 12 inches.

3.17.2 EXPERIMENTAL DESIGN FOR ATRAZINE ANALYSIS

Atrazine, which is heavily used to control weeds in pineapple, maize and tomato farming in Ghana, was used as the test herbicide. The commercial product investigated was a powdered formulation containing 200g atrazine active ingredient per Kg sample. To every 2 Kg of atrazine powder was added 300l of water. This was used to spray one hectare of plot according to the manufacturer's recommendation.

Top soil samples were taken at the depth of 0 to 6 inches from four demarcated plots in a cocoa plantation of 18 years old, two diagonally treated with atrazine and the other two kept as control. Sampling was done at weekly

intervals for five weeks after the experimental plots were treated with the herbicide.

3.17.3 EXTRACTION AND CLEAN-UP FOR HERBICIDE

Triplicate soil samples (50g), which had been sieved through 2mm mesh, were soxhlet extracted for 5 hours with 250ml methanol. A 50ml aliquot of methanol extract was evaporated to dryness in a rotary evaporator and the residue taken up in 1ml methanol and 2.5ml water mixture. The diluted extract was passed through a reconditioned C-18 solid phase extraction (SPE) column at a flow rate of 2ml/min. The column was then washed with 1ml distilled water. The column was vacuum dried for 15 minutes, following which the pesticide was eluted with 2ml ethylacetate. 1µl was then injected onto the GC.

3.17.4 EXTRACTION AND CLEAN-UP FOR INSECTICIDE

Leaves: Leaf tissue (60g) was homogenised, extracted and cleaned-up as described under sections 3.15 and 3.16

Pods: Skin of the pod (60g) were peeled off and extracted as described for leaves.

Soil: Extraction procedure was as described in 3.17.3. The final extract was however dissolved in 2ml of hexane instead of ethylacetate.

3.17.5 GAS LIQUID CHROMATOGRAPHIC (GLC) ANALYSIS.

The analysis was done on a Varian star 3400CX GLC equipped with a Thermionic specific detector (TSD). The Nitrogen carrier gas flow rate was 1ml/min. using a 15m (5.3µm ID X 1.5µm film) J & W DB-5 capillary column. The run was isothermal with other operating conditions as:

Injector temperature: 230°C, Column temperature: 290°C, Detector temperature: 350°C, Make-up gas: 30ml/min, Hydrogen: 4ml/min (54 Psi) and Air; 175ml/min (6 bar).

3.17.6 THIN LAYER CHROMATOGRAPHY (TLC) ANALYSIS WITHOUT CLEAN-UP

Methanol extract (50ml) from soxhlet extraction of the soil was concentrated to near dryness on a rotary evaporator and dissolved in 2ml of ethylacetate. Cleaned and uncleaned extracts (5µl) were then analysed by TLC using spinach leave in detection method as previously described. Standards of various concentrations were run alongside samples and the diameters taken for calibration curve.

3.18 MARKET SURVEY

Tomatoes, maize, pineapple, were obtained from the open market at Domi and Madina. Each sample was chopped and four separate 60g taken. To each portion was added 60ml ethylacetate and 50g $NaHCO_3$ and homogenised. The mixture was allowed to stand for 30 minutes in a glass-stoppered flask. The resulting mixture was filtered through anhydrous Na_2SO_4 on a pad of cotton wool. 30 ml of organic phase was taken and concentrated on a rotary evaporator to near dryness. A further 3 ml of cyclohexane was added to residue, mixed and dried again. Addition of cyclohexane was repeated two more times and the final volume adjusted to 2 ml with cyclohexane : ethylacetate (1 : 1). 5µl of the extracts were then analysed on TLC plates.

3.19 RESIDUE ANALYSIS FOR MARKET SURVEY

The resulting solution (1ml) from section 3.18 was first cleaned on GPC as described under 3.15. Volume of combined fraction was reduced to 2ml and further cleaned on a preconditioned C-18 SPE as described under 3.16. The other 1ml was cleaned up in the reverse way, by first using SPE followed by GPC before analysis by TLC.

3.20 RECOVERY EXPERIMENTS

Leaves and pods: Each cocoa leaf (60g) and skin of pods were spiked with 2ml of 10μg/ml of propoxur standard. Samples were homogenised, extracted and cleaned-up as described under section 3.15 and 3.16. for GC analysis.

Soils: Pesticide free samples (50g) were air dried and ground to pass through a 2mm-mesh sieve. 2 ml of 10μl of propoxur and atrazine standards were applied to separate soil samples. The soils were then soxhlet extracted for 5 hours with methanol. Extracts were then cleaned up as in 3.17.4 for GC measurements.

Tomatoes, Pineapple and Maize: Replicate samples, 60 g each of pesticide free tomatoes, maize, pineapple and maize fortified with mixed standards of Atrazine and Diuron at 2 and 3 times MDQ were extracted and cleaned-up as described under 3.15. 1ml of resulting final extract in 1: 1 cyclohexane/ethylacetate was cleaned on the GPC. Volumes 6-19ml was collected from GPC concentrated to 1 ml and further cleaned with SPE as described under 3.16.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RF VALUES OF PESTICIDES

Table 1 and 2, show the Rf of 35 pesticides run at two different temperatures in system 1 (ethylacetate-silica gel 60).

Table 1: Rf values of pesticides in silica gel- ethyl acetate system at 24°C

	Rf values of pesticides in silica gel- Ethyl acetate system at 24°C								
		Replicate Measurements							
	Active ingredient	1	2	3	4	5	Average	**CV**	**SD**
1	Aldicarb	0.50	0.50	0.48	0.51	0.50	0.50	0.026	0.013
2	Aldrin	0.68	0.63	0.66	0.70	0.67	0.67	0.045	0.030
3	Ametryn	0.61	0.62	0.61	0.58	0.59	0.61	0.027	0.015
4	Atrazine	0.61	0.61	0.61	0.62	0.61	0.61	0.003	0.002
5	Benomyl	0.42	0.43	0.36	0.31	0.31	0.37	0.158	0.059
5a	Benomyl	0.61	0.65	0.65	0.61	0.61	0.63	0.029	0.019
6	Carbaryl	0.60	0.60	0.60	0.58	0.59	0.59	0.014	0.008
7	Carbofuran	0.58	0.57	0.58	0.57	0.57	0.57	0.011	0.006
8	Captan	0.64	0.65	0.65	0.61	0.65	0.64	0.027	0.017
9	Chlorpyrifos	0.68	0.69	0.66	0.68	0.67	0.67	0.019	0.013
10	λ- cyhalothrin	0.69	0.65	0.69	0.72	0.70	0.69	0.041	0.028
11	Diazinon	0.66	0.66	0.68	0.67	0.67	0.67	0.014	0.010
12	Dieldrin	0.69	0.66	0.75	0.71	0.70	0.70	0.069	0.048
13	Dimethamethryn	0.64	0.64	0.64	0.62	0.58	0.62	0.038	0.023
14	Dimethoate	0.35	0.35	0.33	0.35	0.34	0.34	0.021	0.007
15	Dioxacarb	0.46	045	0.45	0.46	0.46	0.46	0.009	0.004
16	Diuron	0.38	0.36	0.37	0.40	0.40	0.38	0.050	0.019
17	α-Endosulfan	0.69	0.68	0.68	0.66	0.67	0.68	0.017	0.011
18	β- Endosulfan	0.68	0.68	0.66	0.66	0.66	0.67	0.015	0.010
19	Fenitrothion	0.67	0.68	0.69	0.68	0.65	0.68	0.020	0.013
20	Isazofos	0.68	0.70	0.69	0.68	0.68	0.69	0.013	0.010
21	Lindane	0.68	0.68	0.67	0.67	0.79	0.70	0.071	0.050
22	γ- HCH	0.70	0.68	0.68	0.78	0.67	0.69	0.072	0.053
23	Malathion	0.70	0.69	0.70	0.70	0.69	0.70	0.007	0.005
24	Merpan	0.63	0.62	0.61.	0.60	0.61	0.61	0.015	0.009
25	Metobromuron	0.57	0.58	0.56	0.59	0.63	0.59	0.046	0.027
26	Mirage	0.44	0.43	0.40	0.38	0.40	0.41	0.067	0.028
27	Monocrotophos	0.10	0.09	0.08	0,11	0.11	0.10	0.144	0.014
28	Nitrofen	0.68	0.68	0.70	0.68	0.66	0.68	0.019	0.013
29	o,p-DDT	0.70	0.67	0.68	0.67	0.66	0.67	0.015	0.010
30	Parathion-methyl	0.66	0.65	0.68	0.67	0.68	0.67	0.017	0.012
31	Pirimiphos-methyl	0.70	0.71	0.70	0.72	0.71	0.71	0.013	0.010
32	Propanil	0.57	0.58	0.55	0.56	0.59	0.57	0.029	0.016
33	Propoxur	0.57	0.59	0.58	0.59	0.58	0.58	0.011	0.007
34	Pyrinex	0.67	0.68	0.68	0.67	0.66	0.67	0.012	0.008
35	Thiram	0.59	0.60	0.56	0.57	0.59	0.58	0.030	0.019

Table 2 : Rf values of pesticides in Ethyl – acetate system at 32 ± 3° C

	Rf values of pesticides in silica gel -ethylacetate system at 32 ± 3° C								
		Replicate Measurements							
	Active ingredient	**1**	**2**	**3**	**4**	**5**	Average	**CV**	**SD**
1	Aldicarb	0.49	0.52	0.49	0.50	0.48	0.50	0.030	0.015
2	Aldrin	0.70	0.67	0.66	0.67	0.66	0.67	0.024	0.016
3	Ametryn	0.60	0.60	0.61	0.61	0.62	0.61	0.013	0.008
4	Atrazine	0.60	0.57	0.60	0.61	0.59	0.59	0.025	0.015
5	Benomyl	0.37	0.40	0.28	0.25	0.29	0.32	0.200	0.164
5a	Benomyl	0.63	0.61	0.63	0.61	0.52	0.60	0.077	0.046
6	Carbaryl	0.58	0.59	0.59	0.60	0.60	0.59	0.014	0.008
7	Carbofuran	0.59	0.56	0.57	0.59	0.59	0.58	0.024	0.014
8	Captan	0.61	0.65	0.64	0.63	0.65	0.63	0.033	0.021
9	Chlorpyrifos	0.68	0.69	0.68	0.68	0.69	0.68	0.007	0.005
10	λ- cyhalothrin	0.72	0.71	0.69	0.73	0.68	0.70	0.030	0.021
11	Diazinon	0.64	0.67	0.61	0.64	0.62	0.64	0.036	0.023
12	Dieldrin	0.74	0.75	0.77	0.73	0.70	0.74	0.034	0.026
13	Dimethamethryn	0.64	0.63	0.64	0.65	0.62	0.64	0.017	0.011
14	Dimethoate	0.30	0.29	0.29	0.30	0.33	0.30	0.053	0.016
15	Dioxacarb	0.43	0.43	0.5	0.46	0.44	0.44	0.029	0.013
16	Diuron	0.41	0.42	0.41	0.38	0.41	0.41	0.037	0.015
17	α-Endosulfan	0.71	0.69	0.70	0.71	0.68	0.72	0.039	0.029
18	β- Endosulfan	0.73	0.75	0.72	0.70	0.68	0.70	0.039	0.027
19	Fenitrothion	0.65	0.63	0.63	0.65	0.63	0.64	0.017	0.011
20	Isazofos	0.69	0.71	0.70	0.77	0.77	0.73	0.053	0.039
21	Lindane	0.68	0.70	0.71	0.69	0.68	0.69	0.019	0.013
22	γ- HCH	0.67	0.71	0.70	0.68	0.68	0.69	0.018	0.014
23	Malathion	0.70	0.71	0.64	0.64	0.67	0.67	0.049	0.033
24	Merpan	0.61	0.64	0.66	0.65	0.67	0.65	0.053	0.023
25	Metobromuron	0.56	0.57	0.58	0.58	0.58	0.57	0.016	0.009
26	Mirage	0.40	0.34	0.49	0.33	0.45	0.40	0.173	0.060
27	Monocrotophos	0.07	0.05	0.05	0.08	0.05	0.06	0.233	0.014
28	Nitrofen	0.67	0.70	0.71	0.70	0.71	0.70	0.023	0.016
29	o,p-DDT	0.69	0.68	0.71	0.70	0.67	0.69	0.023	0016
30	Parathion-methyl	0.65	0.65	0.66	0.63	0.67	0.65	0.009	0.006
31	Pirimiphos-methyl	0.66	0.65	0.64	0.72	0.72	0.68	0.057	0.039
32	Propanil	0.57	0.59	0.58	0.60	0.56	0.58	0.027	0.016
33	Propoxur	0.59	0.60	0.61	0.58	0.62	0.60	0.020	0.012
34	Pyrinex	0.66	0.60	0.62	0.60	0.68	0.63	0.058	0.036
35	Thiram	0.56	0.55	0.57	0.58	0.54	0.56	0.028	0.015

The Rf's compare favourably, except at very low Rf values where there is some variation. Monocrotophos for example had an Rf value of 0.10 with a coefficient of variation (CV) of 0.144 at 24° whiles it was 0.06 with a CV of 0.233 at 32 ± 3°C. The validation process involved working at 22°C, but

because of the high cost of energy and for easy adoption of method for our laboratory, the method was tried at room temperature (32° ± 3°C). Results show the suitability of the validation at this temperature. The spread of the Rf values for pesticides run at both 24°C and 32°C ± 3° were in the ranges 0.10 and 0.71 and 0.12 and 0.73 respectively. Eluent temperature seems to have an effect on the Rf values but no clear pattern was established within the 10°C range difference. Some researchers[78] however reported an increase in Rf values with increase in temperature while others reported a decrease[79]. With the exception of monocrotophos which had a coefficient of variation (CV) of 0.233 at 32° ± 3°C, all the coefficients of variation was found to be different for both temperatures but were all less than 0.25 for both temperatures as shown in Figure 2 below.

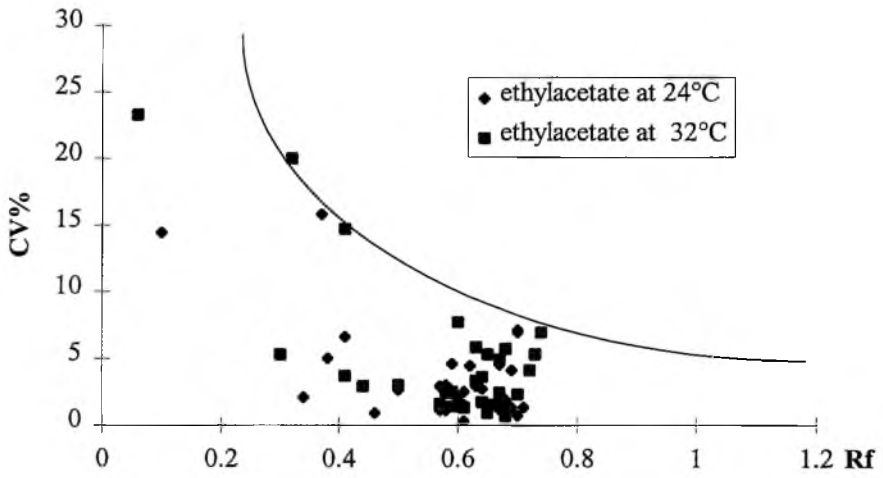


Figure 2: Coefficient of variation (CV) of Rf values of compounds run in ethylacetate as a function of Rf

Comparing Rf values obtained to those of other co-ordinated researchers at the same time in other laboratories (Appendix A), there seems to be some inter-

laboratory variation between Rf's but a closer look at the CV and comparing to those obtained in our laboratory show they are not significant. Rf's for most of the chemicals could not be compared because no Rf' values were available from literature.

The relationship between coefficient of variation (%CV) and Rf values for ethylacetate at 24°C and 32° ± 3°C is also shown in figure 2. For individual pesticides plotted, it is seen from the graph that, the smaller the Rf values the bigger the CV. Compounds of similar average Rf's have different coefficient of variation. Temperature seems to slightly increase the coefficient of variation at lower Rf values whiles it is virtually not significant at higher Rf values. CV values, therefore from Figure 2 seem to be slightly higher for the 32°C run than the 24°C. This can be attributed to the wide variation of the tank temperature in the laboratory, which we had no control over, compared to the 24°C (air-conditioned room), which was easy to maintain and fairly stable. The higher the Rf values however the lower the variation and the less significant the difference in CV of the Rf values of same compounds runs at the different temperatures.

Tables 3 and 4 summarises the Rfs of the pesticides run at 24° and 32 ± 3°C using Dicloromethane as developing solvent.

Table 3: Rf values of pesticides in Silica gel – Dichloromethane system at 24° C

	Rf values of pesticides in Silica gel – Dichloromethane system at 24° C								
		Replicate Measurements							
	Active ingredient	**1**	**2**	**3**	**4**	**5**	Average	**CV**	**SD**
1	Aldicarb	0.10	0.11	0.12	0.12	0.08	0.11	0.158	0.017
2	Aldrin	0.73	0.74	0.73	0.74	0.73	0.74	0.008	0.006
3	Ametryn	0.10	0.09	0.09	0.10	0.09	0.09	0.075	0.007
4	Atrazine	0.05	0.05	0.06	0.06	0.09	0.06	0.206	0.016
5	Benomyl	0.23	0.25	0.17	0.21	0.24	0.22	0.141	0.036
5a	Benomyl	0.70	0.71	0.70	0.70	0.71	0.70	0.008	0.005
6	Carbaryl	0.24	0.26	0.25	0.23	0.19	0.23	0.121	0.028
7	Carbofuran	0.06	0.12	0.12	0.11	0.14	0.11	0.274	0.030
8	Captan	0.40	0.38	0.36	0.36	0.38	0.38	0.050	0.019
9	Chlorpyrifos	0.68	0.68	0.68	0.71	0.71	0.69	0.024	0.016
10	λ- cyhalothrin	0.69	0.68	0.70	0.69	0.69	0.69	0.010	0.007
11	Diazinon	0.03	0.03	0.02	0.02	0.02	0.03	0.175	0.005
12	Dieldrin	0.66	0.66	0.63	065	0.65	0.65	0.025	0.017
13	Dimethamethryn	0.09	0.11	0.10	0.12	0.13	0.11	0.135	0.015
14	Dimethoate	0.01	0.04	0.04	0.03	0,05	0.03	0.492	0.015
15	Dioxacarb	0.02	0.04	0.04	0.04	0.04	0.04	0.218	0.009
16	Diuron	0.09	0.09	0.09	0.10	0.10	0.09	0.053	0.005
17	α-Endosulfan	0.71	0.71	0.68	0.66	0.69	0.68	0.033	0.023
18	β- Endosulfan	0.72	0.71	0.71	0.71	0.70	0.71	0.010	0.007
19	Fenitrothion	0.59	0.58	0.58	0.59	0.59	0.59	0.009	0.005
20	Isazofos	0.35	0.33	0.33	0.32	-	0.33	0.038	0.013
21	Lindane	0.71	0.69	0.71	0.73	0.69	0.71	0.019	0.014
22	γ- HCH	0.68	0.72	0.70	0.69	0.70	0.70	0.021	0.014
23	Malathion	0.27	0.27	0.28	0.30	-	0.28	0.051	0.014
24	Merpan	0.40	0.39	0.41	0.44	0.44	0.42	0.054	0.023
25	Metobromuron	0.24	0.21	0.28	0.29	0.33	0.27	0.174	0.047
26	Mirage	0.04	0.02	0.03	0.02	0.02	0.03	0.300	0.009
27	Monocrotophos	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
28	Nitrofen	0.65	0.67	0.67	0.65	0.67	0.66	0.014	0.009
29	o,p-DDT	0.74	0.72	0.72	0.75	0.73	0.73	0.021	0.015
30	Parathion-methyl	0.57	0.57	0.69			0.58	0.029	0.017
31	Pirimiphos-methyl	0.44	0.43	0.43	0.46		0.44	0.032	0.014
32	Propanil	0.21	0.23	0.21	0.19	0.24	0.21	0.095	0.021
33	Propoxur	0.09	0.15	0.13	0.13	0.16	0.13	0.199	0.026
34	Pyrinex	0.70	0.68	0.68	0.68	0.67	0.68	0.014	0.009
35	Thiram	0.21	0.22	0.25	0.23	0.25	0.23	0.078	0.018

Table 4: Rf values of pesticides in Silica gel- Dichloromethane system at 32 ± 3°C

	Rf values of pesticides in Silica gel_ Dichloromethane system at 32 ± 3°C								
		Replicate Measurements							
	Active ingredient	**1**	**2**	**3**	**4**	**5**	Average	**CV**	**SD**
1	Aldicarb	0.11	0.10	0.11	0.11	0.10	0.11	0.046	0.009
2	Aldrin	0.75	0.74	0.71	0.72	0.74	0.73	0.029	0.023
3	Ametryn	0.09	0.08	0.09	0.10	0.08	0.09	0.092	0.008
4	Atrazine	0.04	0.05	0.06	0.08	0.05	0.06	0.253	0.015
5	Benomyl	0.01	0.01	0.01	0.02	0.02	0.01	0.500	0.005
5a	Benomyl	0.69	0.70	0.67	0.71	0.71	0.70	0.024	0.016
6	Carbaryl	0.31	0.30	0.26	0.27	0.33	0.29	0.099	0.029
7	Carbofuran	0.12	0.12	0.14	0.13	0.13	0.13	0.074	0.010
8	Captan	0.44	0.44	0.41	0.45	0.44	0.44	0.039	0.017
9	Chlorpyrifos	0.69	0.68	0.67	0.66	0.68	0.68	0.017	0.011
10	λ- cyhalothrin	0.66	0.68	0.68	0.67	0.67	0.67	0.012	0.008
11	Diazinon	0.03	0.03	0.04	0.04	0.02	0.04	0.144	0.006
12	Dieldrin	0.66	0.67	0.64	0.65	0.66	0.66	0.017	0.011
13	Dimethamethryn	0.09	0.10	0.11	0.11	0.12	0.11	0.104	0.011
14	Dimethoate	0.04	0.03	0.04	0.04	0.05	0.04	0.177	0.007
15	Dioxacarb	0.05	0.06	0.06	0.08	0.07	0.06	0.195	0.012
16	Diuron	0.08	0.08	0.09	0.09	0.09	0.09	0.061	0.005
17	α-Endosulfan	0.66	0.66	0.68	0.66	0.67	0.67	0.017	0.012
18	β- Endosulfan	0.67	0.68	0.68	0.69	0.67	0.68	0.012	0.008
19	Fenitrothion	0.63	0.63	0.64	0.66	0.64	0.64	0.022	0.014
20	Isazofos	0.37	0.39	0.41	0.45	0.40	0.40	0.074	0.030
21	Lindane	0.73	0.67	0.67	0.70	0.70	0.70	0.036	0.025
22	γ- HCH	0.70	0.69	0.70	0.71	0.72	0.70	0.030	0.020
23	Malathion	0.32	0.33	0.35	0.37	0.34	0.34	0.065	0.022
24	Merpan	0.43	0.42	0.40	0.43	0.44	0.42	0.036	0.015
25	Metobromuron	0.28	0.28	0.27	0.27	0.28	0.28	0.020	0.005
26	Mirage	0.02	0.03	0.02	0.03	0.04	0.03	0.279	0.008
27	Monocrotophos	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000
28	Nitrofen	0.61	0.61	0.62	0.60	0.63	0.61	0.019	0.011
29	o,p-DDT	0.73	0.72	0.70	0.73	0.73	0.72	0.018	0.013
30	Parathion-methyl	0.60	0.58	0.58	0.59	0.58	0.59	0.023	0.012
31	Pirimiphos-methyl	0.53	0.51	0.48	0.48	0.50	0.50	0.049	0.024
32	Propanil	0.22	0.22	0.23	0.23	0.24	0.23	0.036	0.008
33	Propoxur	0.08	0.10	0.09	0.11	0.12	0.10	0.158	0.015
34	Pyrinex	0.61	0.60	0.62	0.61	0.63	0.61	0.019	0.011
35	Thiram	0.12	0.06	0.07	0.07	0.06	0.07	0.339	0.024

Unlike the ethylacetate, the Rf values range between 0.06 and 0.74 for both temperatures. The spread is much wider here than in ethylacetate with CV between 0.00 and 0.0.339 (figure 3).

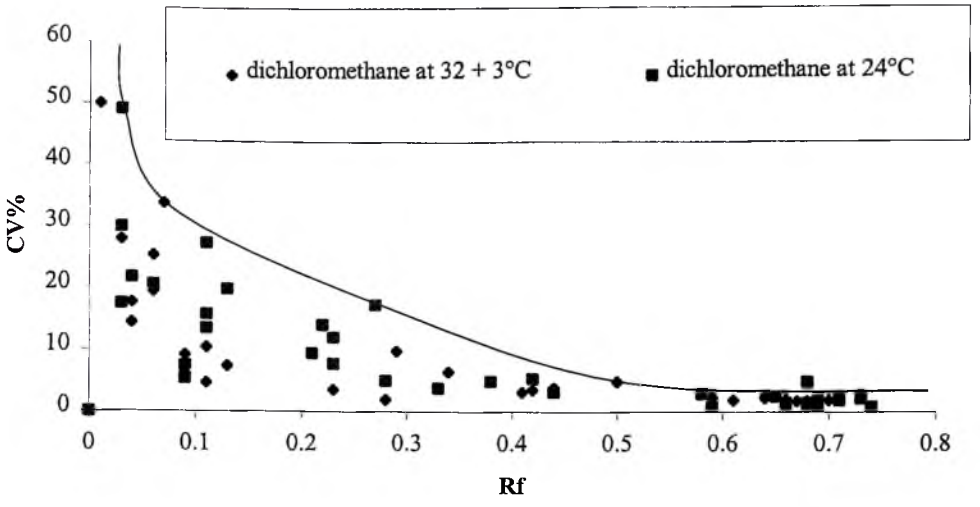


Figure 3: Coefficient of variation (CV) of Rf values of compounds run in dichloromethane as a function of Rf

Once again, no clear pattern was established to confirm earlier observations by some researchers[77] that the higher the temperature, the lower the Rf values within the limits of this experiment. The only cases observed to be affected by this phenomenon were the compounds Benomyl and Thiram, which seem to have breakdown products in them as a result of exposure to the atmosphere. Here the Rf values of breakdown products were lower at 32° ± 3°C compared to 24°C. It can be seen from figure 4, that, the lower the Rf, the wider the CV. Tables 1, 2, 3 and 4 clearly indicate that the Rf's are different in both systems.

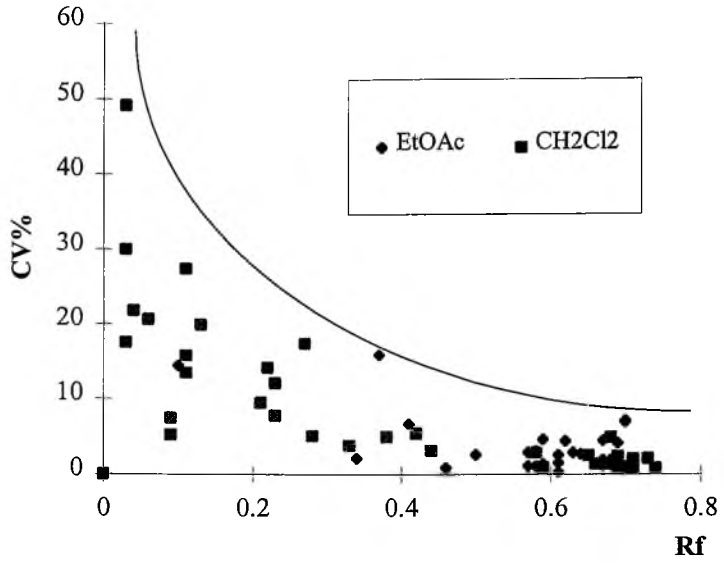


Figure 4: Coefficient of variation of compounds in CH_2CL_2 and EtOAc as a function of Rf

Comparing the CV in ethylacetate (EtOAc) and dichloromethane ($CHCl_2$) at the same temperature, it is seen from figure 4, that, there is a wide variation and distribution of the coefficient of variation in dichloromethane compared to the uniform and less variation in the ethylacetate run. The lower the Rf, the wider the CV. Run time for the solvent dichloromethane is shorter than for ethylacetate, but the solvent front is slightly difficult to determine because of its high volatility. This probably explains the variation and partly due to the fact that Rf values in the $CHCl_2$ are generally low and hence the accompanying variation as already discussed with low Rf values.

The curves in both Figure 2 and 3 show that CV approaches zero as Rf approaches one, indicating that compounds with higher Rf's or runs in solvents that give higher Rf's are likely to be reproducible at various laboratories and have less inter laboratory variation.

Generally ethylacetate was found to be the most suitable solvent for any first line of action for screening pesticide residues by TLC due to less variation and higher Rf values.

In screening for possible pesticide residues or formulations resulting in a given spot on a TLC plate, the least favourable situation has to be considered. This is due to the fact that pesticides having similar RF values show different CV values as mentioned above. The Rf values of pesticides can be generalised, from figure 2, to fall in the range $Rf \pm (2CV)/Rf$. This agrees perfectly well with what other researchers have reported.[80]

The table 5 gives the Rf value of the pesticides in a benzene system.

Table 5: Rf value of pesticides in Silica gel- Benzene system at 32 ± 3°C

	Rf value of pesticides in Silica gel- Benzene system at 32 ± 3°C								
		Replicate Measurements							
	Active ingredient	**1**	**2**	**3**	**4**	**5**	Average	**CV**	**SD**
1	Aldicarb	0.03	0.02	0.03	0.03	0.02	0.03	0.183	0.005
2	Aldrin	0.69	0.67	0.69	0.67	0.68	0.68	0.017	0.012
3	Ametryn	0.03	0.03	0.03	0.03	0.03	0.03	0.018	0.001
4	Atrazine	0.02	0.01	0.02	0.02	0.02	0.02	0.200	0.004
5	Benomyl	0.04	0.05	0.04	0.05	0.04	0.04	0.104	0.004
5a	Benomyl	0.05	0.06	0.05	0.06	0.06	0.06	0.096	0.006
6	Carbaryl	0.19	0.20	0.21	0.21	0.20	0.20	0.048	0.010
7	Carbofuran	0.02	0.03	0.01	0.02	0.02	0.02	0.25	0.005
8	Captan	0.16	0.16	0.15	0.16	0.15	0.16	0.034	0.005
9	Chlorpyrifos	0.60	0.62	0.60	0.60	0.62	0.61	0.018	0.011
10	λ- cyhalothrin								
11	Diazinon	0.09	0.09	0.10	0.11		0.10	0.096	0.010
12	Dieldrin								
13	Dimethamethryn	0.05	0.04	0.05	0.04	0.05	0.04	0.125	0.005
14	Dimethoate	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.000
15	Dioxacarb								
16	Diuron	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.00
17	α-Endosulfan								
18	β- Endosulfan								
19	Fenitrothion	0.45	0.45	0.46	0.46	0.46	0.46	0.013	0.006
20	Isazofos	0.17	0.17	0.19	0.17		0.18	0.056	0.010
21	Lindane								
22	γ- HCH								
23	Malathion	0.12	0.12	0.13	0.13	0.12	0.12	0.046	0.005
24	Merpan	0.15	0.15	0.15	0.15	0.16	0.15	0.028	0.004
25	Metobromuron	0.09	0.08	0.08	0.08	0.09	0.09	0.082	0.007
26	Mirage								
27	Monocrotophos								
28	Nitrofen	0.59	0.55	0.59	0.59	0.55	0.58	0.036	0.021
29	o,p-DDT	0.69	0.67	0.68	0.68	0.67	0.68	0.013	0.009
30	Parathion-methyl	0.44	0.41	0.44	0.46	0.41	0.43	0.045	0.019
31	Pirimiphos-methyl	0.31	0.31	0.33	0.32	0.30	0.31	0.037	0.011
32	Propanil	0.06					0.06		
33	Propoxur	0.02	0.04				0.03		
34	Pyrinex	0.62	0.60	0.61	0.61		0.61	0.014	0.009
35	Thiram	0.38	0.38	0.38	0.38	0.38	0.38	0.000	0.000

Pattern of Rf in this eluent was similar to dichloromethane. Because of the environmental and health concerns, it is not recommended for use, since dichloromethane, can act as a substitute.

The Rf of the 35 pesticides run on ready made Al_2O_3 at 24°C and 32°C ± 3°C using elthylacetate as eluting solvent is presented in Tables 6 and 7.

Table 6: Rf values of pesticides in ready-made Alumina–Ethyl acetate system at 24° C

	Rf values of pesticides in ready- made Alumina–Ethyl acetate system at 24° C								
		Replicate Measurements							
	Active ingredient	**1**	**2**	**3**	**4**	**5**	Average	**CV**	**SD**
1	Aldicarb	0.64	0.65	0.61	0.69	0.62	0.64	0.049	0.031
2	Aldrin	0.80	0.80	0.81	0.85	0.84	0.82	0.029	0.023
3	Ametryn	0.73	0.75	0.75	0.74	0.73	0.74	0.014	0.010
4	Atrazine	0.74	0.76	0.73	0.75	0.74	0.74	0.015	0.011
5	Benomyl								
5a	Benomyl	0.79	0.80	0.82	0.74	0.79	0.79	0.043	0.034
6	Carbaryl	0.74	0.72	0.71	0.71	0.72	0.72	0.017	0.012
7	Carbofuran	0.68	0.70	0.72	0.80	0.71	0.70	0.029	0.021
8	Captan	0.76	0.77	0.76	0.77	0.77	0.77	0.007	0.005
9	Chlorpyrifos	0.80	0.80	0.86	0.84	0.85	0.83	0.034	0.027
10	λ- cyhalothrin	0.80	0.81	0.81	0.83	0.85	0.82	0.024	0.020
11	Diazinon	0.82	0.82	0.82	0.79	0.81	0.81	0.016	0.013
12	Dieldrin	0.77	0.83	0.84	0.85	0.85	0.83	0.040	0.030.
13	Dimethamethryn	0.74	0.81	0.82	0.84	0.73	0.79	0.063	0.050
14	Dimethoate	0.54	0.53	0.55	0.52	0.54	0.54	0.021	0.011
15	Dioxacarb	0.61	0.64	0.61	0.50	0.62	0.61	0.030	0.018
16	Diuron	0.61	0.66	0.65	0.62	0.65	0.64	0.034	0.022
17	α-Endosulfan	0.76	0.84	0.83	0.85	0.85	0.83	0.046	0.038
18	β- Endosulfan	0.76	0.78	0.82	0.83	0.83	0.77	0.040	0.032
19	Fenitrothion	0.81	0.76	0.84					
20	Isazofos	0.82	0.81	0.86	0.83		0.83	0.026	0.022
21	Lindane	0.79	0.78	0.83	0.83	0.83	0.81	0.030	0.025
22	γ- HCH	0.83	0.83	0.83	0.79	0.78	0.81	0.030	0.025
23	Malathion	0.82	0.80	0.82	0.76	0.79	0.79	0.032	0.025
24	Merpan	0.76	0.77				0.78	0.041	0.032
25	Metobromuron	0.71	0.73	0.72	0.72		0.72	0.020	0.014
26	Mirage	0.66	0.66	0.65	0.65		0.66	0.009	0.006
27	Monocrotophos	0.26	0.28						
28	Nitrofen	0.81	0.77	0.82	0.84	0.85	0.80	0.033	0.026
29	o,p-DDT	0.80	0.80	0.80	0.84	0.84	0.82	0.027	0.022
30	Parathion-methyl	0.83	0.86	0.82	0.81	0.82	0.83	0.023	0.019
31	Pirimiphos-methyl	0.83	0.87	0.71	0.82	0.82	0.81	0.074	0.060
32	Propanil	0.68	0.70	0.69	0.70		0.69	0.014	0.010
33	Propoxur	0.70	0.71	0.73	0.74		0.71	0.022	0.015
34	Pyrinex	0.79	0.81	0.79	0.86	0.86	0.80	0.014	0.012
35	Thiram	0.73	0.76	0.75	0.70		0.73		

Table 7: Rf values of pesticides in Ready-made alumina-ethyl acetate system at $32 \pm 3^{0}C$

	Rf values of pesticides in ready-made alumina - ethyl acetate system at $32 \pm 3^{0}C$								
		Replicate Measurements							
	Active ingredient	**1**	**2**	**3**	**4**	**5**	Average	**CV**	**SD**
1	Aldicarb	0.69	0.69	0.67	0.69	0.62	0.67	0.049	0.033
2	Aldrin	0.78	0.83	0.81	0.83		0.81	0.029	0.024
3	Ametryn	0.79	0.76	0.79	0.81	0.81	0.79	0.026	0.020
4	Atrazine	0.79	0.73	0.76	0.78	0.79	0.77	0.033	0.025
5	Benomyl	0.35	0.35	0.36	0.35		0.36	0.016	0.006
5a	Benomyl	0.67	0.68	0.67	0.68		0.68	0.008	0.006
6	Carbaryl	0.75	0.71	0.76	0.72	0.76	0.74	.032	0.023
7	Carbofuran	0.75	0.75	0.77	0.77	0.77	0.76	0.014	0.011
8	Captan	0.77	0.75	0.74			0.75	0.020	0.015
9	Chlorpyrifos	0.78	0.78	0.77	0.81	0.84	0.80	0.036	0.029
10	λ- cyhalothrin	0.79	0.82	0.79	0.80	0.81	0.80	0.022	0.017
11	Diazinon	0.83					0.83		
12	Dieldrin	0.77	0.81	0.79	0.80	0.83	0.80	0.027	0.022
13	Dimethamethryn	0.80	0.82	0.77	0.78	0.82	0.80	0.029	0.023
14	Dimethoate	0.60	0.55	0.56	0.56	0.49	0.55	0.072	0.040
15	Dioxacarb	0.68	0.70	0.68	0.65	0.68	0.68	0.026	0.018
16	Diuron	0.71	0.73	0.73	0.70	0.68	0.71	0.030	0.021
17	α-Endosulfan	0.77	0.82	0.82	0.82	0.82	0.81	0.028	0.022
18	β- Endosulfan	0.76	0.79	0.80	0.79	0.81	0.79	0.024	0.019
19	Fenitrothion	0.82					0.82		
20	Isazofos	0.84					0.84		
21	Lindane	0.77	0.77	0.83	0.83	0.80	0.79	0.044	0.035
22	γ- HCH	0.76	0.77	0.77	0.76	0.77	0.77	0.007	0.006
23	Malathion	0.83					0.83		
24	Merpan	0.78	0.77	0.77	0.76	0.79	0.77	0.015	0.011
25	Metobromuron	0.76	0.75	0.74	0.78		0.76	0.022	0.017
26	Mirage	0.63	0.64	0.59	0.63	0.64	0.63	0.033	0.021
27	Monocrotophos								
28	Nitrofen	0.83	0.82	0.84	0.83	0.82	0.83	0.010	0.008
29	O,p-DDT	0.78	0.74	0.78	0.77	0.81	0.78	0.032	0.025
30	Parathion-methyl	0.79	0.76	0.76			0.77	0.022	0.017
31	Pirimiphos-methyl	0.83					0.83		
32	Propanil	0.78	0.70	0.72	0.74		0.74	0.046	0.034
33	Propoxur	0.72	0.78	0.78	0.78	0.75	0.76	0.035	0.027
34	Pyrinex	0.79	0.77	0.80	0.78	0.85	0.80	0.039	0.031
35	Thiram	0.54	0.53	0.52			0.53	0.019	0.010

Silica and alumina, as absorbents have similar general chromatographic properties and therefore separate according to polarities. In addition, alumina

has a higher adsorption affinity for carbon-carbon double bonds and better selectivity towards hydrocarbons and their derivatives. This is reflected in the Rf values obtained in the Tables (6 & 7).

In table 6, the Rf range was between 0.37 and 0.81 compared to that of silica gel (Table 1) of 0.10 and 0.71. Table 7 also gave a range of 0.36 and 0.85 compared to that of silica (Table 2) of 0.12 and 0.73. In both cases it can be seen that the Rf values were higher for alumina compared to silica. Since higher Rf values gives less percentage Coefficient of variation (%CV), alumina would probably be the adsorbent of choice for uniformity of Rf values. With the exception of detection methods three and four, however, it was found to have a high background level when used with all the detection methods, making visualisation and quantification difficult. It is only suitable for organochlorine compounds. Once again, a general trend with respect to temperature change could not be established. Table 8 similarly presents the Rf values of pesticides on ready made alumina in dichloromethane at 24°C.

Table 8: Rf values of pesticides in Ready-made alumina -dichloromethane system at 24°C

	Rf values of pesticides in Ready-made alumina- Dichloromethane system at 24°C								
		Replicate Measurements							
	Active ingredient	**1**	**2**	**3**	**4**	**5**	Average	**CV**	**SD**
1	Aldicarb	0.55	0.47	0.43	0.60		0.48	0.118	0.057
2	Aldrin	0.81	0.78	0.84	0.86	0.88	0.84	0.023	0.019
3	Ametryn	0.55	0.61	0.60			0.61	0.20	0.012
4	Atrazine	0.39	0.42	0.42	0.43	0.56	0.54	0.070	0.038
5	Benomyl								
5a	Benomyl								
6	Carbaryl	0.45	0.52	0.53	0.53	0.49	0.60	0.082	0.049
7	Carbofuran	0.60	0.54	0.55			0.56	0.059	0.033
8	Captan	0.68					0.68		
9	Chlorpyrifos	0.87	0.83	0.87			0.86	0.026	0.022
10	λ- cyhalothrin	0.79	0.82				0.81	0.025	0.020
11	Diazinon								
12	Dieldrin	0.80					0.80		
13	Dimethamethryn	0.69	0.63	0.64			0.66	0.048	0.032
14	Dimethoate	0.37	0.32				0.35	0.086	0.030
15	Dioxacarb	0.44	0.41				0.42	0.050	0.021
16	Diuron	0.59	0.44	0.55			0.52	0.146	0.076
17	α-Endosulfan								
18	β- Endosulfan								
19	Fenitrothion								
20	Isazofos								
21	Lindane	0.86	0.80				0.83	0.048	0.040
22	γ- HCH								
23	Malathion								
24	Merpan	0.78	0.67	0.73			0.73	0.073	0.053
25	Metobromuron	0.74	0.56	0.66			0.65	0.135	0.088
26	Mirage	0.39	0.31	0.29			0.33	0.152	0.050
27	Monocrotophos								
28	Nitrofen	0.84	0.77	0.80			0.80	0.041	0.033
29	o,p-DDT	0.55					0.55		
30	Parathion-methyl								
31	Pirimiphos-methyl								
32	Propanil	0.66	0.60	0.52			0.50	0.115	0.068
33	Propoxur	0.57	0.53	0.54	0.55		0.55	0.033	0.018
34	Pyrinex	0.87	0.82	0.86			0.85	0.029	0.025
35	Thiram	0.57					0.57		

The range for Table 8 being between 0.33 and 0.86. These values are quite high and depart from what we expect from a dichloromethane system as seen

for the silica in Table 4. This once again can be attributed to the adsorbent. The CV here is very small, hence a good adsorbent to use.

The Rf values for the self-made plates (Table 9) compare favourably with the factory made ones.

Table 9: Rf values of pesticides in Self- made alumina- ethyl acetate system at 32 ± 3°C

	Rf values of pesticides in Self- made alumina- ethyl acetate system at 32 ± 3°C								
		Replicate Measurements							
	Active ingredient	**1**	**2**	**3**	**4**	**5**	Average	**CV**	**SD**
1	Aldicarb	0.67	0.69	0.74	0.74	0.691	0.71	0.045	0.032
2	Aldrin	0.78	0.83	0.83	0.81	0.83	0.82	0.029	0.024
3	Ametryn	0.81	0.79	0.81	0.80	0.76	0.79	0.026	0.021
4	Atrazine	0.79	0.79	0.78	0.76	0.77	0.78	0.017	0.013
5	Benomyl	0.36	0.35	0.36	0.35		0.36	0.016	0.006
5a	Benomyl	0.67	0.68	0.67	0.68		0.68	0.008	0.006
6	Carbaryl	0.77	0.76	0.76	0.80	0.80	0.78	0.026	0.021
7	Carbofuran	0.75	0.77	0.77	0.64	0.78	0.74	0.078	0.058
8	Captan	0.77	0.75	0.74	0.78	0.76	0.77	0.020	0.015
9	Chlorpyrifos	0.83	0.86	0.77	0.84	0.81	0.82	0.041	0.034
10	λ- cyhalothrin	0.82	0.79	0.80			0.81	0.026	0.021
11	Diazinon	0.83	0.81	0.84	0.83	0.85	0.83	0.017	0.014
12	Dieldrin	0.81	0.79	0.82	0.84	0.85	0.82	0.029	0.023
13	Dimethamethryn	0.77	0.78	0.82	0.73	0.82	0.78	0.048	0.038
14	Dimethoate	0.49	0.49	0.43	0.55		0.49	0.100	0.049
15	Dioxacarb	0.68	0.65	0.64	0.65	0.68	0.66	0.028	0.019
16	Diuron	0.73	0.70	0.68	0.73	0.68	0.70	0.036	0.025
17	α-Endosulfan	0.87	0.86	0.87	0.88	0.87	0.87	0.007	0.006
18	β- Endosulfan	0.89	0.86	0.86	0.89	0.88	0.88	0.019	0.015
19	Fenitrothion	0.66					0.66		
20	Isazofos	0.84					0.84		
21	Lindane	0.77	0.77	0.79	0.80	0.79	0.78	0.016	0.013
22	γ- HCH	0.80	0.79	0.78	0.76	0.81	0.79	0.019	0.015
23	Malathion	0.83					0.83		
24	Merpan	0.77	0.77	0.76	0.79	0.69	0.76	0.051	0.038
25	Metobromuron	0.78	0.75	0.74	0.76	0.76	0.76	0.027	0.021
26	Mirage	0.63	0.64	0.63	0.64	0.69	0.63	0.033	0.021
27	Monocrotophos								
28	Nitrofen	0.78	0.82	0.82	0.83	0.84	0.82	0.028	0.023
29	o,p-DDT	0.74	0.81	0.77	0.78	0.78	0.78	0.037	0.029
30	Parathion-methyl	0.76	0.86	0.82	0.87	0.87	0.84	0.056	0.047
31	Pirimiphos-methyl	0.83	0.85	0.82	0.83	0.84	0.83	0.013	0.011
32	Propanil	0.74	0.74	0.70	0.78		0.74	0.046	0.034
33	Propoxur	0.75	0.78	0.70	0.78	0.72	0.75	0.048	0.036
34	Pyrinex	0.87	0.85	0.87	0.85	0.80	0.85	0.034	0.029
35	Thiram	0.54	0.53				0.54	0.013	0.007

Similarly the CV and statements previously made for the factory made ones apply. They however have the added advantage of being very sensitive to method 4, when silver nitrate is incorporated during preparation. Factory made ones, however do not have silver nitrate and adding to the spraying reagent creates quite a bit of background, making visualisation and quantification difficult, hence increasing the MDQ values. Method four has the added advantage of developing the spots under sunlight, which is abundant in Ghana, instead of an ultraviolet lamp.

4.2 EFFECT OF SAMPLE LOAD ON RF'S

Studies carried out on effect of sample load on Rf values using detection methods 1 and 5 are shown on tables 10 and 11. Sample load did not seem to have any significant effect on the absolute Rf values.

Table 10: Effect of sample load on Rf using photosynthesis inhibition on silica gel-ethylacetate system at 32 ± 3°C

MARKER COMPOUND: ATRAZINE					MARKER COMPOUND: ATRAZINE				
NO.	AMOUNT(ng)		Rf	Rrfatr	NO.	AMOUNT(ng)		Rf	RRfatr
1	MDQ	0.25	0.61	1.00	1	MDQ	0.25	0.60	0.98
2	2MDQ	0.50	0.62	0.98	2	2MDQ	0.50	0.61	1.00
3	4MDQ	1.0	0.61	1.00	3	4MDQ	1.0	0.61	1.00
4	6MDQ	1.5	0.61	1.00	4	6MDQ	1.5	0.61	1.00
MARKER COMPOUND: DIURON					MARKER COMPOUND: DIURON				
1	MDQ	1.0	0.40	0.65	1	MDQ	0.25	0.40	0.65
2	2MDQ	2.0	0.39	0.64	2	2MDQ	0.50	0.40	0.65
3	4MDQ	4.0	0.40	0.65	3	4MDQ	1.0	0.41	0.67
4	6MDQ	6.0	0.40	0.65	4	6MDQ	1.5	0.41	0.67
LOADED WITH 300mg OF TOMATOES					LOADED WITH 600mg OF TOMATOES				
MARKER COMPOUND: ATRAZINE					MARKER COMPOUND: ATRAZINE				
1	MDQ	0.25	0.61	1.00	1	MDQ	0.25	0.61	1.00
2	2MDQ	0.50	0.61	1.00	2	2MDQ	0.50	0.62	1.02
3	4MDQ	1.0	0.62	1.02	3	4MDQ	1.0	0.61	1.00
4	6MDQ	1.5	0.62	1.02	4	6MDQ	1.5	0.63	1.03
MARKER COMPOUND: DIURON					MARKER COMPOUND: DIURON				
1	MDQ	1.0	0.41	0.67	1	MDQ	0.25	0.41	0.67
2	2MDQ	2.0	0.40	0.65	2	2MDQ	0.50	0.41	0.67
3	4MDQ	4.0	0.40	0.65	3	4MDQ	1.0	0.40	0.65
4	6MDQ	6.0	0.41	0.67	4	6MDQ	1.5	0.42	0.69

Table 11: Effect of sample load on Rf using o-Tolidine + potassium iodide on silica gel-ethylacetate system at 32 ± 3°C

MARKER COMPOUND: ATRAZINE					MARKER COMPOUND: ATRAZINE				
NO.	AMOUNT(ng)		Rf	RRfatr	NO.	AMOUNT(ng)		Rf	RRfatr
1	MDQ	25	0.60	0.98	1	MDQ	25	0.61	1.00
2	2MDQ	50	0.61	1.00	2	2MDQ	50	0.61	1.00
3	4MDQ	100	0.61	1.00	3	4MDQ	100	0.61	1.00
4	6MDQ	150	0.61	1.00	4	6MDQ	150	0.61	1.00
MARKER COMPOUND: DIURON					MARKER COMPOUND: DIURON				
1	MDQ	30	0.39	0.64	1	MDQ	30	0.40	0.65
2	2MDQ	60	0.39	0.64	2	2MDQ	60	0.40	0.65
3	4MDQ	120	0.39	0.64	3	4MDQ	120	0.39	0.64
4	6MDQ	180	0.40	0.65	4	6MDQ	180	0.40	0.65
LOADED WITH 300mg OF TOMATOES					LOADED WITH 600mg OF TOMATOES				
MARKER COMPOUND: ATRAZINE					MARKER COMPOUND: ATRAZINE				
1	MDQ	25	0.61	1.00	1	MDQ	25	0.61	1.00
2	2MDQ	50	0.61	1.00	2	2MDQ	50	0.62	1.02
3	4MDQ	100	0.60	0.98	3	4MDQ	100	0.61	1.00
4	6MDQ	150	0.61	1.00	4	6MDQ	150	0.62	1.02
MARKER COMPOUND: DIURON					MARKER COMPOUND: DIURON				
1	MDQ	30	0.41	0.67	1	MDQ	30	0.42	0.69
2	2MDQ	60	0.40	0.65	2	2MDQ	60	0.41	0.67
3	4MDQ	120	0.40	0.65	3	4MDQ	120	0.40	0.65
4	6MDQ	180	0.40	0.65	4	6MDQ	180	0.39	0.64

Presented in tables 12 and 13 are the within and inter-plate variation of some of the pesticides on silica gel plates, when analytical standards were applied.

Table 12: Within–plate variation of Rf using silica gel ethyl acetate system.

	RF VALUES				AVE		
CHEMICAL	1	2	3	4	RF	SD	CV
ALDICARB	0.51	0.51	0.51	0.51	0.51	0.000	0.000
ATRAZINE	0.61	0.61	0.62	0.61	0.61	0.004	0.007
BENOMYL	0.33	0.33	0.32	0.33	0.33	0.005	0.015
CARBARYL	0.60	0.59	0.58	0.60	0.59	0.009	0.016
CARBOFURAN	0.60	0.58	0.57	0.57	0.58	0.012	0.021
CHLOROPYRIFOS	0.68	0.67	0.69	0.68	0.68	0.008	0.012
DIMETHOATE	0.31	0.31	0.32	0.30	0.31	0.010	0.030
DIOXACARB	0.45	0.45	0.45	0.46	0.45	0.005	0.011
DIURON	0.41	0.41	0.40	0.40	0.41	0.005	0.013
METOBROMURON	0.59	0.58	0.57	0.58	0.58	0.008	0.014
MONOCROTOPHOS	0.08	0.08	0.08	0.09	0.08	0.004	0.050

Table 13: Inter–plate variation of Rf using silica gel ethyl acetate system.

	RF VALUES				AVE		
CHEMICAL	1	2	3	4	RF	SD	CV
ALDICARB	0.51	0.49	0.50	0.51	0.50	0.010	0.019
ATRAZINE	0.61	0.62	0.62	0.62	0.62	0.005	0.008
BENOMYL	0.34	0.35	0.32	0.33	0.34	0.012	0.038
CARBARYL	0.59	0.61	0.63	0.60	0.61	0.017	0.028
CARBOFURAN	0.61	0.62	0.60	0.57	0.60	0.021	0.036
CHLOROPYRIFOS	0.70	0.67	0.68	0.68	0.69	0.014	0.020
DIMETHOATE	0.31	0.34	0.33	0.31	0.32	0.015	0.047
DIOXACARB	0.45	0.47	0.50	0.46	0.47	0.021	0.046
DIURON	0.41	0.42	0.43	0.42	0.42	0.008	0.019
METOBROMURON	0.54	0.55	0.57	0.58	0.56	0.018	0.032
MONOCROTOPHOS	0.05	0.06	0.05	0.06	0.09	0.006	0.096

The variability of Rf within the plates as measured by the coefficient of variation as expected was smaller than between the plates. However, in both cases, Rf variability within and between the plates was less than 5%. This pattern was clearly observed in the sample load on Rf experiment.

4.3 EFFECT OF UNSATURATION ON RF'S

The average Rf values found in the unsaturated system of ethylacetate is shown in table 14.

Table 14: EFFECT OF TANK UNSATURATION ON RF VALUES OF PESTICIDES RUN IN A SILICA-GEL-ETHYLACETATE SYSTEM

	ACTIVE INGREDIENT	SATURATED	UNSATURATED	CHANGE IN RF
1	Aldicarb	0.49	0.81	0.32
2	Aldrin	0.67	0.96	0.31
3	Ametryn	0.59	0.94	0.35
4	Atrazine	0.61	0.95	0.34
5	Benomyl	0.36	0.72	0.36
6	Benomyl	.061	0.95	0.34
7	Carbaryl	0.58	0.94	0.36
8	Carbofuran	0.57	0.92	0.35
9	Captan	0.61	0.95	0.34
10	Chloropyrifos	0.66	0.96	0.30
11	λ-cylohalothrin	0.67	0.98	0.31
12	Dieldrin	0.65	0.97	0.32
13	Dimethametryn	0.62	0.95	0.33
14	Dimethoate	0.30	0.59	0.29
15	Dioxacarb	0.44	0.80	0.36
16	Diuron	0.40	0.75	0.35
17	α-endosulfan	0.67	0.99	0.32
18	β-endosulfan	0.66	0.97	0.31
19	Lindane	0.65	0.97	0.32
20	γ-HCH	0.64	0.97	0.33
21	Merpan	0.62	0.96	0.34
22	Metobromuron	0.56	0.94	0.38
23	Mirage	0.38	0.76	0.38
24	Monocrotophos	0.10	0.19	0.09
25	Nitrofen	0.67	0.98	0.31
26	O, P' DDT	0.66	0.98	0.32
27	Pirimiphos-methyl			
28	Parathion-methyl	0.68	0.97	0.29
29	Propanil	0.55	0.93	0.38
30	Propoxur	0.58	0.94	0.36
31	Pyrinex	0.66	0.98	0.32
32	Thiram	0.31	0.62	0.31
33	Thiram	0.55	0.94	0.39

Unsaturation seems to affect the Rf values equally, raising them by a value of between 0.30 to 0.40. Monocrotophos, however with a very low Rf value of

0.10 deviated from the norm. It can be inferred from the data that when the Rf is greater or equal to 0.30, the change in Rf due to unsaturation is greater than 0.30 units. Despite the change in Rf due to unsaturation, the RRf remains constant (Table 15) and can therefore be used as a means of identification of spots detected on plates, instead of the Rf.

Table 15: EFFECT OF TANK UNSATURATION ON RRF VALUES OF PESTICIDES RUN IN A SILICA-GEL-ETHYLACETATE SYSTEM

	ACTIVE INGREDIENT	UNSATURATED Rf	RRFatrazine
1	**Aldicarb**	**0.81**	**0.85**
2	**Aldrin**	**0.96**	**1.01**
3	**Ametryn**	**0.94**	**0.99**
4	**Atrazine**	**0.95**	**1.00**
5	**Benomyl**	**0.72**	**0.76**
6	**Benomyl**	**0.95**	**1.00**
7	**Carbaryl**	**0.94**	**0.99**
8	**Carbofuran**	**0.92**	**0.97**
9	**Captan**	**0.95**	**1.00**
10	**Chloropyrifos**	**0.96**	**1.01**
11	**λ-cylohalothrin**	**0.98**	**1.03**
12	**Dieldrin**	**0.97**	**1.02**
13	**Dimethametryn**	**0.95**	**1.00**
14	**Dimethoate**	**0.59**	**0.62**
15	**Dioxacarb**	**0.80**	**0.84**
16	**Diuron**	**0.75**	**0.79**
17	**α-endosulfan**	**0.99**	**1.04**
18	**β-endosulfan**	**0.97**	**1.02**
19	**Lindane**	**0.97**	**1.02**
20	**γ-HCH**	**0.97**	**1.02**
21	**Merpan**	**0.96**	**1.01**
22	**Metobromuron**	**0.94**	**0.99**
23	**Mirage**	**0.76**	**0.80**
24	**Monocrotophos**	**0.19**	**0.20**
25	**Nitrofen**	**0.98**	**1.03**
26	**O, P' DDT**	**0.98**	**1.03**
27	**Pirimiphos-methyl**		
28	**Parathion-methyl**	**0.97**	**1.02**
29	**Propanil**	**0.93**	**0.98**
30	**Propoxur**	**0.94**	**0.99**
31	**Pyrinex**	**0.98**	**1.03**
32	**Thiram**	**0.62**	**0.65**
33	**Thiram**	**0.94**	**0.99**

4.4 MINIMUM DETECTABLE QUANTITIES OF PESTICIDES

Table 16 summaries the result of detectability of pesticides using the various detection methods.

Table 16: Detectability of pesticides (in ng) with various detection methods

		0-TKI	NBFB	$AgNO_3$	Hill	EBNA	EAcI	ρDB
1	Aldicarb	100				500	3	
2	Aldrin	2000						
3	Ametryn	20			0.25			
4	Atrazine	25		400	0.25		1500	
5	Benomyl	300		100			500	
6	Carbaryl	100	37.5			2	10	
7	Carbofuran	500	30			500	100	
8	Captan	5000		100		500	500	
9	Chlorpyrifos	660		250		1	50	
10	λ- cyhalothrin							
11	Diazinon	300				300	0.2	
12	Dieldrin			75				
13	Dimethamethryn				0.13			
14	Dimethoate	100		500		500	1000	
15	Dioxacarb	25	100			500	100	
16	Diuron	30		100	0.25			50
17	α-Endosulfan	300		50				
18	β- Endosulfan	300		50				
19	Fenitrothion					15	5	150
20	Isazofos	3000					20	
21	Lindane	500		5				
22	γ- HCH	500						
23	Malathion	1500				10	10	
24	Merpan			300	500			
25	Metobromuron	200			0.50			50
26	Mirage				50			
27	Monocrotophos	300				250	5	
28	Nitrofen				0.25			150
29	o,p-DDT							
30	Parathion-methyl	5000				40	4	150
31	Pirimiphos-methyl	500				200	8	
32	Propanil	50				3		250
33	Propoxur	250	>300	300		200	5 * 100	
34	Pyrinex	500			0.50			
35	Thiram							

* MDQ without bromination

The choice of method depends on the application. For greater sensitivity, that is detecting small amounts, the Hill method is recommended. This method detects mainly herbicides to as low as 0.13ng/5µL. The minimum detectable quantity is defined as the minimum amount of analytical standard, expressed in nanogram, spotted on the plate, which gives clearly visible spots after elution under average chromatographic conditions[81]

The selectivity of the detection methods varies. The ortho-tolidine + KI (method 1) was found to act as a general screening method, having medium sensitivity for several compounds. It could detect up to 20ng/5µL for ametryn and then as high as 3000ng for others. The organochlorines tend to give white spots, making visualisation a bit difficult for the untrained eye.

Similarly, nitrobenzene-fluoroborate method was of medium sensitivity, but could detect reasonably only the carbamate pesticides. It therefore has limited use and can be used for confirmation of identity of residues. Alumina plates were not suitable for use with this detecting reagent. The MDQ values here ranged between 30 and 300ng .

Detection with p-dimethylaminobenzaldehyde was of limited use, detecting between 50 and 250ng of a few pesticides, mainly those pesticides that can be hydrolysed to primary amines, like the urea herbicide metobromuron. Compounds that were visible after heating disappeared after spraying of reagent. The compounds Thiram, Parathion-Methyl, Fenitrothion and Nitrofen were observed to be clearly visible after heating at 160°C, but

disappear on application of the spray reagent and hence had to be quantified before applying the reagent.

The enzyme inhibition methods (7 and 8) were very sensitive to the carbamate and organophosphate pesticides with MDQ values between 0.2 and 2000ng. Depending on the type of carbamate or organophosphate, the sensitivities for the two reagents differ. For carbaryl, the MDQ for method 7 was 10ng whiles it was 2ng for method 8. On the other hand it was 200 and 100ng for dioxacarb respectively. They therefore complement each other and must be available in a screening laboratory. Both methods were found to be unsuitable for use on an alumina plate.

The silver nitrate method was the only one found to be most suitable for detecting the organochlorine pesticides. Lindane was the most sensitive to this reagent and had an MDQ value of 5ng/5µL. This method was suitable for use on only alumina plates, both self and factory made ones. Detection was also possible under sunlight, in the absence of a strong UV light source.

4.5 DETECTION OF HERBICIDES USING LOCAL PLANTS

As part of a search for an alternative to wheat leaves recommended for use for herbicide detection[81], thirteen local plants and grasses were tested on a 20 x 20 silica gel 60F plate developed in ethylacetate at 32° ± 3°C and minimum detectable quantities (MDQ's) reported. (Table 17)

TABLE 17: Detectability (in ng) of pesticides using various leaves in photosynthesis inhibition reaction.

Leaf	Atrazine	Ametryn	Dimethametryn	Diuron	Nitrofen	Propanil	Merpan	Metobromuron
Panicum maximum	0.5	0.25	0.5	1	1	50	1000	1
Spinach	0.25	0.20	0.125	0.25	0.25	0.5	250	0.5
Sporobulus pyramidalis	0.25	0.20	0.125	0.25	0.25	0.5	250	0.5
Cyperus rotundus	1000	1000	300	1000	1000	3000	3000	N.D
Zea mays l	0.5	0.5	>0.25	0.50	0.5	1	N.D	3
Manihot esculenta	100	100	5	N.D	5	100	3000	300
Lettuce	N.D	300	300	N.D	100	N.D	N.D	N.D
Cabbage	N.D	300	100	N.D	50	1000	N.D	N.D
Azadirachta indica	3	2	1	3	2	30	N.D	N.D
Launaea taraxacifolia	300	300	300	N.D	N.D	N.D	3000	N.D
Bamboo	0.5	0.8	0.5	0.5	0.25	0.5	3000	3
Amaranthus hybridus	1	1	0.25	1	5	0.5	3000	3
Xanthosoma mafaffa	100	300	300	100	N.D	300	N.D	300
Wheat leaves*	1	-	-	1				

N.D.- No detection.

*Reference.(Ambrus,1996)

Sporobulus pyramidalis and spinach were found to be the most sensitive to all the herbicides listed and the MDQ values are far lower than the reference values quoted using wheat leaves (Table 17). The other leaves also gave comparatively good results for some of the herbicides. It can also be seen from the table that narrow leafed plants generally responded better than the broad-leafed ones. *Cyperus rotundus*, a narrow-leafed grass however did not respond any better than the broad leaves. The order of sensitivity of the leaves tested was as follows: *Sporobulus pyramidalis* = Spinach > *Panicum maximum* > *Zea mays l* > *Bamboo* > *Amaranthus hybridus* > *Manihot esculenta* = *Azadirachta indica* > *Xanthosoma mafaffa* = Lettuce = Cabbage > *Launaea taraxacifolia* > *Cyperus rotundus*.

In preparing the detecting reagent, we found that the volume of DCPIP recommended[81] for mixing with the 20 – 25ml of wheat pressing to obtain a bluish-green colour (pH 9 – 10) did not work. In all cases of leaves tried, we found that the amount of DCPIP needed to attain pH 9 to 10 was 1 to 3 times the volume of leaf pressing used. Leaves that generally responded better used 1 to 2 times whilst the broad leaves needed 2 to 3 times. *Cyperus rotundus*, needed between 1 to 2 times of DCPIP supporting the fact that it is a narrow-leafed plant but surprisingly did not give good results. This could probably be due to insufficient chlorophyll in the extract as observation of the leaf smash showed a faint green colour unlike the others, which were distinctly green. We inferred from our results that leaves that generally require more of DCPIP would not be good as detecting reagents.

Xanthosoma mafaffa, a broad-leafed plant had an extra problem of being slightly slimy, a property which caused non-uniformity of its spray on plates and raised limits of detection. This observation goes to discourage the use of such slimy leaf extracts in TLC detection of these herbicides.

4.6 APPLICATION OF MARKER COMPOUNDS FOR INTERNAL QUALITY CONTROL

Summarised in Tables 18 and 19 are the Rf values of the pesticides relative to certain marker compounds in ethylacetate and dichloromethane respectively.

Table 18: RRf values of marker compounds in System 1 (Silica gel- Ethyl Acetate system)

	Active ingredient	Rf	$RRf_{Atrazine}$	$RRf_{Carbaryl}$	RRf_{Captan}	$RRf_{parathion-methyl}$
1	Aldicarb	0.50	0.82	0.85	0.78	0.75
2	Aldrin	0.67	1.10	1.14	1.05	1.00
3	Ametryn	0.60	0.98	1.02	0.94	0.90
4	Atrazine	0.61	1.00	1.03	0.95	0.91
5	Benomyl	0.37	0.61	0.63	0.58	0.55
5a	Benomyl	0.63	1.03	1.07	0.98	0.94
6	Carbaryl	0.59	0.97	1.00	0.93	0.88
7	Carbofuran	0.57	0.93	0.97	0.89	0.85
8	Captan	0.64	1.05	0.09	1.00	0.96
9	Chlorpyrifos	0.67	1.10	1.14	1.05	1.00
10	λ- cyhalothrin	0.69	1.13	1.17	1.08	1.03
11	Diazinon	0.67	1.10	1.14	1.05	1.00
12	Dieldrin	0.70	1.15	1.19	1.09	0.99
13	Dimethamethryn	0.62	1.02	1.05	0.97	0.93
14	Dimethoate	0.34	0.56	0.58	0.53	0.51
15	Dioxacarb	0.46	0.75	0.78	0.72	0.68
16	Diuron	0.38	0.63	0.64	0.14	0.57
17	α-Endosulfan	0.68	1.12	1,15	1.06	1.02
18	β- Endosulfan	0.67	1.10	1.14	1.05	1.00
19	Fenitrothion	0.64	1.05	1.08	1.00	0.95
20	Isazofos	0.69	1.13	1.17	1.08	1.03
21	Lindane	0.70	1.15	1.19	1.09	1.05
22	γ- HCH	0.69	1.13	1.17	1.08	1.03
23	Malathion	0.70	1.15	1.19	1.09	1.05
24	Merpan	0.61	1.00	1.03	0.95	0.91
25	Metobromuron	0.59	0.97	1.00	0.92	0.88
26	Mirage	0.41	0.67	0.70	0.64	0.61
27	Monocrotophos	0.10	0.16	0.17	0.16	0.15
28	Nitrofen	0.68	1.12	1.15	1.06	1.02
29	O,p-DDT	0.67	1.10	1.14	1.05	1.00
30	Parathion-methyl	0.67	1.16	1.20	1.11	1.00
31	Pirimiphos-methyl	0.71	1.10	1.14	1.05	1.06
32	Propanil	0.57	0.93	0.97	0.89	0.85
33	Propoxur	0.58	0.95	0.98	0.91	0.87
34	Pyrinex	0.67	1.10	1.14	1.05	1.00
35	Thiram	0.58	0.95	0.98	0.91	0.87

Table 19: RRf values of marker compounds in System 2 (Dichloromethane system)

	Active ingredient	Rf	$RRf_{Atrazine}$	$RRf_{Carbaryl}$	RRf_{Captan}	$RRf_{parathion-methyl}$
1	Aldicarb	0.11	1.83	0.48	0.29	0.19
2	Aldrin	0.74	12.33	3.22	1.95	1.28
3	Ametryn	0.09	1.50	0.39	0.24	0.16
4	Atrazine	0.06	1.00	0.26	0.16	0.10
5	Benomyl	0.22	3.67	0.96	0.58	0.38
5a	Benomyl	0.70	11.67	3.04	2.33	1.59
6	Carbaryl	0.23	3.83	1.00	0.61	0.40
7	Carbofuran	0.11	1.83	0.48	0.29	0.19
8	Captan	0.38	6.33	1.65	1.00	0.66
9	Chlorpyrifos	0.69	11.50	3.00	1.82	1.90
10	λ- cyhalothrin	0.69	11.50	3.00	1.82	1.90
11	Diazinon	0.03	0.50	1.30	0.08	0.05
12	Dieldrin	0.65	10.83	2.83	1.71	1.21
13	Dimethamethryn	0.11	1.83	0.48	0.29	0.19
14	Dimethoate	0.03	0.50	0.13	0.08	0.05
15	Dioxacarb	0.04	0.67	0.17	0.11	0.07
16	Diuron	0.09	1.50	0.39	0.24	0.16
17	α-Endosulfan	0.68	11.33	2.96	1.79	1.17
18	β- Endosulfan	0.68	11.33	2.96	1.79	1.17
19	Fenitrothion	0.59	9.83	2.57	1.55	1.02
20	Isazofos	0.33	5.50	1.44	0.87	0.57
21	Lindane	0.71	11.83	3.09	1.87	1.22
22	γ- HCH	0.70	11.67	3.04	2.33	1.59
23	Malathion	0.28	4.67	1.22	0.74	0.48
24	Merpan	0.42	7.00	1.83	1.11	0.72
25	Metobromuron	0.27	4.50	1.17	0.71	0.47
26	Mirage	0.03	0.50	0.13	0.08	0.05
27	Monocrotophos	0.00	0.00	0.00	0.00	0.00
28	Nitrofen	0.66	11.00	2.87	1.73	1.14
29	o,p-DDT	0.73	12.17	3.17	1.92	1.26
30	Parathion-methyl	0.44	7.33	1.91	1.16	0.76
31	Pirimiphos-methyl	0.58	9.67	2.52	1.53	1.00
32	Propanil	0.21	3.50	0.91	0.55	0.36
33	Propoxur	0.13	2.17	0.57	0.34	0.22
34	Pyrinex	0.68	11.33	2.96	1,79	1.17
35	Thiram					

The marker compounds were added to check the proper elution and detection conditions for greater control of the TLC conditions. These compounds selected were based on the relative stability of compounds in standard

solutions, sensitivity for the detection conditions (not appearing on the plate if the conditions are not optimal) and have a reproducible Rf values.[81] These were always run on plate with pesticides.

The results indicate that, as with any relative retention data in chromatography, the TLC RRf can be better used for identification of compounds than the Rf's. Unlike the Rf, which is influenced by many factors like variation in temperature, unsaturation in tank etc., the RRf was found to remain virtually constant, provided the system was working well and can be used for guidance. This is confirmed in table 14, where the calculated $RRf_{atrazine}$ for the unsaturated system is virtually the same as the saturated one presented in table 18.

4.7 APPLICABILITY OF EXTRACTION PROCEDURE

The colouring spots occurring when 75mg ethylacetate sample extracts from tomatoes, pineapple and maize without clean-up were spotted and developed in ethylacetate-Silica gel system are shown in table 20. Spots were easy to clean and suggest the suitability of the ethylacetate extraction method.

Table 20: Co-extractives detected with methods 1 and 5 from Tomatoes, pineapple and maize samples using ethylacetate extraction.

DETECTION METHOD 1			DETECTION METHOD 5		
Tomatoes	Pineapple	Maize	Tomatoes	Pineapple	Maize
0.33	0.06		0.40		
0.43	0.31	-	0.58		
0.51	0.75	-	0.61		
0.61	0.77	-			

Results clearly indicates that with the exception of maize, all the other material needs to be cleaned up after extraction to avoid any interference's in residue analysis. These colouring spots are easily cleaned on the GPC, making the ethylacetate extraction suitable.

4.8 EXPECTABLE LIMITS OF DETERMINATION (LOD) OF PESTICIDE RESIDUES

Ethylacetate extraction and clean up on both Gel permeation chromatography and SPE cartridges gave recoveries of greater than 79.9%. With a sample load of 300mg the expectable limits of determination (LOD mg/Kg) are calculated and summarised in table 21.

Table 21: Detectability of pesticides with various detection methods applying cleanup extracts equivalent to 300mg sample

Active ingredient	Limit of determination, LOD mg/Kg			
	O-TKI	AgUV	Hill	EacI pig
Aldicarb	0.20			
Atrazine	0.08		0.00	
Benomyl	0.72	0.30		1.04
Captan	1.04	0.32		1.04
Carbaryl	0,24			0.04
Carbofuran	1.04			0.21
Chloropyrifos	1.38			0,1
Diazinon				0.00
Diuron	0.12		0.00	
Dimethoate	0.24	1.04		2.40
α-Endosulfan	0.78	0.12		
Fenitrothion				0.01
Lindane	1.04	0.01		
Malathion	3.45			0.02
Parathion-methyl	10.4	2.38		0,01

LOD gives an idea about the lowest practical concentration of a pesticide residue or contaminant that can be quantitatively measured and identified in the specified food commodity or animal feedstuff with an acceptable degree of certainty by current regulatory methods of analysis. The results suggest the suitability of the methods in screening pesticide residues.

4.9 RATING THE APPLICABILITY OF DETECTION METHODS

Applicability of detection methods tried for residue analysis based on their selectivity, sensitivity and clean-up requirements is as follows:

Method 1.	O-tolidine + potassium iodide [o-TKI]	++
Method 2.	P-nitrobenzene-fluoroborate [NBFB]	++
Method 3:	p-dimethylamino benzaldehyde [pDB]	+
Method 4:	Silver nitrate + UV exposition [AgUV]	+
Method 5	Photosynthesis inhibition (Hill reation)	+++++
Method 7	Enzyme inhibition with cow liver extract and β-naphthyl-acetate substrate [EβNA]	++++
Method 8	Enzyme inhibition with pig serum and Acethylthiocholine iodide substrate [EacI]	++++

Where greater positive signs show greater selectivity, and sensitivity.

4.10 QUANTITATIVE DETERMINATION OF PESTICIDE RESIDUES

4.10.1 LINEARITY OF CONCENTRATION VERSUS RESPONSE

Shown in Table 22 and Figures 5, 6, 7, 8 and 9 below are the linear range of some of the pesticides relative to the detection methods used.

Table 22: LINEAR RESPONSE RANGE OF SOME OF THE PESTICIDES

	LINEAR RANGE (in ng)	DETECTION METHOD
CHEMICAL		
ATRAZINE	25 - 200	1
ATRAZINE	0.25 – 10	5
CARBOFURAN	50 - 400	1
CARBARYL	250 – 1000	1
DIURON	30 - 100	1
DIURON	0.25 – 15ng	5
DIOXACARB	25 - 250	1
ENDOSULFAN	50 - 800	4
PROPOXUR	300 800	4
PROPOXUR	3.6 140	8
LINDANE	5 - 50	4
MONOCORTOPHOS	100 - 500	1

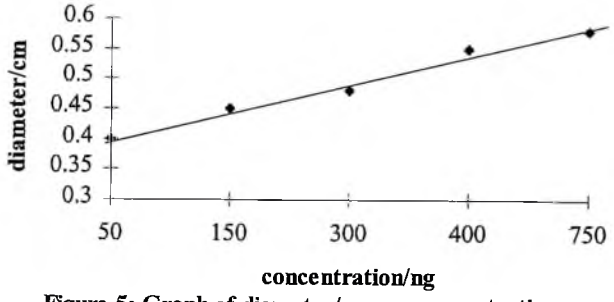


Figure 5: Graph of diameter/cm vrs concentration for carbofuran using method 1

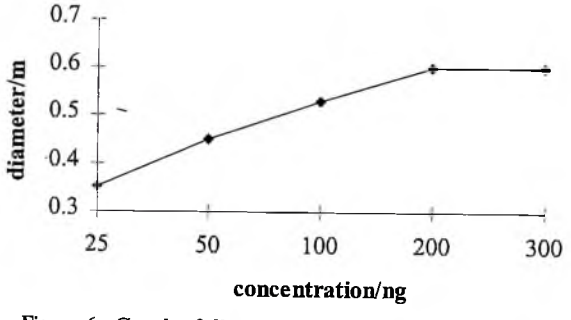


Figure 6 : Graph of diameter/cm vrs concentration/ng for atrazine using method 1

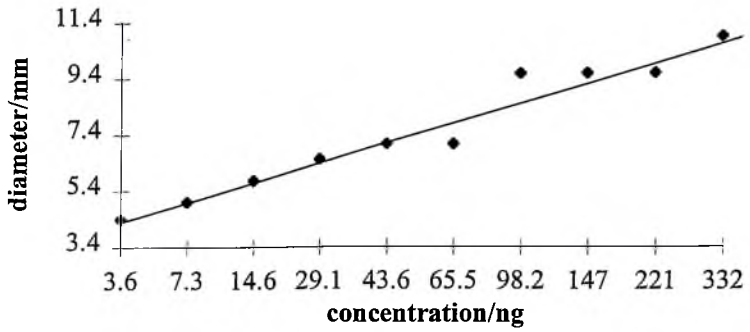


Figure 7: Graph of response against concentration/ng for Propoxur using method 8

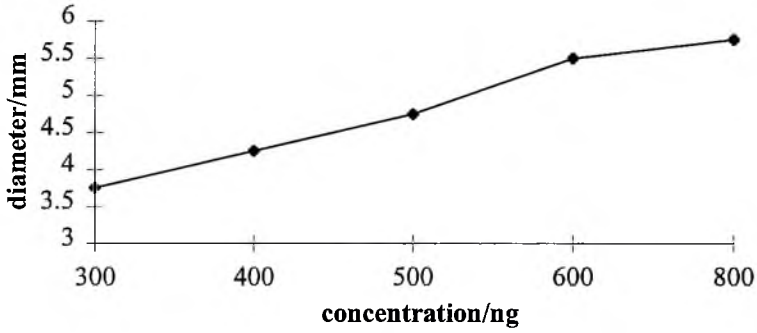


Figure 8: A graph of response against concentration (ng) for propoxur using method 4

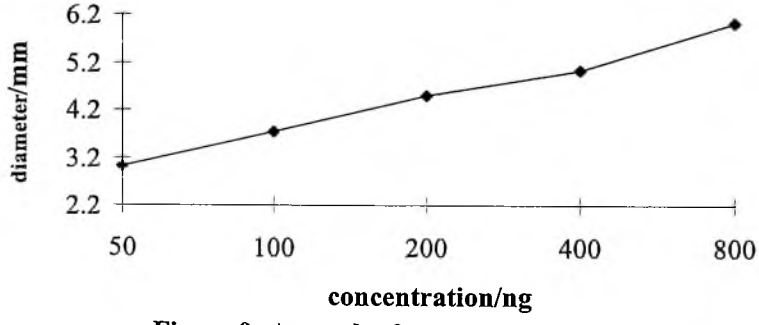


Figure 9: A graph of response against concentration (ng) for endosulfan using method 4

Results indicate that the linear range of a particular chemical depends on the method of detection used. Whereas the range was between 300 and 800ng for propoxur using method 4 it was between 3.6 to 140 using method 8. In the case of Atrazine it was between 25 and 200ng for method 1 whiles it was between 0.25 and 10ng using method 5. The linear range was found to be wider for chemicals that had very low limits of detection with particular methods.

4.10.2 ELUTION CHARACTERISTICES OF DIURON, ATRAZINE DIAZINON AND PROPOXUR FROM GPC

Results indicate that the pesticides were eluted in the volumes 7 to 16ml with the recoveries being between 80 to 90%. Figure 10 is a bar chart showing the percentage of pesticide in each fraction.

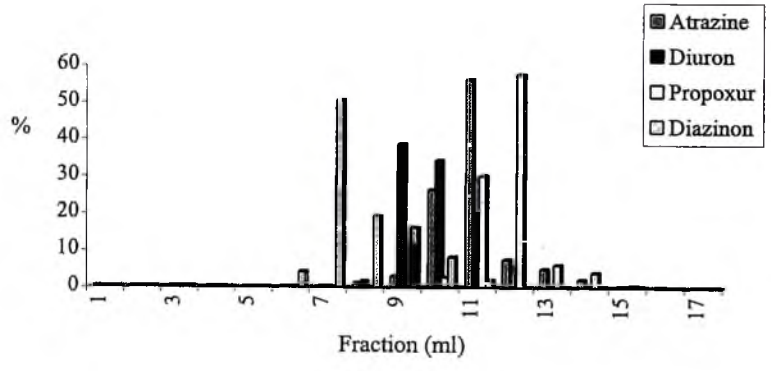


Figure 10 : GPC elution profile on small column

The elution profile shows a slight difference in the elution volumes of the various pesticides used. This could be explained in terms of the difficulty in setting the solvent flux and keeping it constant. As nitrogen gas from the cylinder is being used the pressure begins to fall, causing the flux of the solvent to change slightly.

4.11 VALIDATION AND APPLICABILITY OF THE METHODS

4.11.1 EXPERIMENTAL FARM PLOTS

The atrazine residue levels obtained from cleaned and uncleaned extracts from the soil samples using GLC and TLC analyses were compared. Results show atrazine is lost from the top soil either by degradation or leaching within five weeks table 23.

Table23: Residue levels of atrazine in soil as determined by GLC and TLC.

	MEAN RESIDUE LEVELS (µg/g)		
DAYOF SAMPLING	TLC**	TLC***	GLC
Pretreatment	N.D	N.D	N.D
7th day of treatment	27.2 ± 0.4	22.9 ± 0.5	25.31 ± 0.08
21st day of treatment	9.72 ± 0.8	5.67 ± 0.6	6.48 ± 0.09
28th day of treatment	0.049 ± 0.08	0.029 ± 0.07	0.024 ± 0.005
35th day of treatment	N.D	N.D	N.D

TLC** - Results before clean-up.
TLC***- Results after clean-up.
N.D. No detection.

The herbicide concentration in the soil was below detection level as revealed by both methods after 5 weeks. Comparing the results from the TLC with that of the GLC, it can be said that the method of estimation by taking the diameter

of the spots on the TLC achieves an accuracy and reproducibility in the range 10-20%. This degree of accuracy is adequate in most situations in environmental monitoring.

4.11.2 PROPOXUR RESIDUE IN SOIL, COCOA LEAF AND POD

The average of the results of soil and cocoa pod for plots treated with propoxur are shown in tables 24 and 25.

Table 24: Residue levels of propoxur in soil as determined by GLC and TLC.

		MEAN RESIDUE LEVELS (μg/g)		
DAY OF SAMPLING	DEPTH SAMPLED (INCHES)	TLC**	TLC***	GLC
Pretreatment	0 – 6	N.D	N.D	N.D
	6 – 12	N.D	N.D	N.D
Day of Treatment	0 – 6	0.79 ± 0.21	0.70 ± 0.09	0.76 ± 0.12
	6 – 12	N.D	N.D	N.D
7 days after treatment	0 – 6	0.21 ± 0.09	0.16 ± 0.05	0.18 ± 0.04
	6 – 12	0.81 ± 0.04	0.58 ± 0.18	0.64 ± 0.01
14 days after treatment	0 – 6	0.53 ± 0.11	0.79 ± 0.16	0.46 ± 0.03
	6 – 12	0.98 ± 0.05	0.90 ± 0.08	0.10 ± 0.05
21days after treatment	0 – 6	N.D	N.D	N.D
	6 – 12	0.13 ± 0.09	0.11 ± 0.07	0.12 ± 0.06
7 days after 2nd treatment	0 – 6	0.69 ± 0.10	0.51 ± 0.09	0.68 ± 0.03
	6 – 12	N.D	N.D	N.D
14 days after 2nd treatment	0 – 6	0.46 ± 0.11	0.28 ± 0.08	0.41 ± 0.04
	6 – 12	N.D	N.D	N.D
21days after 2nd treatment	0 – 6	N.D	N.D	N.D
	6 – 12	N.D	N.D	N.D

TLC** - Results before clean-up.
TLC***- Results after clean-up.
N.D. No detection.

Table 25: Residue levels of propoxur in cocoa pod as determined by GLC and TLC.

	MEAN RESIDUE LEVELS (μg/g)	
DAY OF SAMPLING	TLC***	GLC
Pretreatment	N.D	N.D
Day of Treatment	0.92 ± 0.17	0.86 ± 0.14
7 days after treatment	0.26 ± 0.08	0.33 ± 0.11
14days after treatment	0.19 ± 0.06	0.15 ± 0.03
21 days after treatment	0.09 ± 0.04	0.20 ± 0.10
7 days after 2nd treatment	0.25 ± 0.06	0.38 ± 0.02
14 days after 2nd treatment	0.07 ± 0.03	0.09 ± 0.01
21 days after 2nd treatment	N.D	N.D

TLC***- Results after clean-up.
N.D. No detection.

The residue levels were found to have decreased rapidly and by the 21st day none was detected in the topsoil (0 – 6 ins). The amount left in the top soil after the first seven days were 27%, 23% and 24% as determined by the TLC without clean-up, TLC with clean-up and GLC respectively. The results of the analysis from the various methods give very good correlation. The residue however was not detected in the deep soil (6 – 12ins) on the day of treatment.

It started accumulating and reached a maximum within 14 days, thereafter decreasing till none was detected 21 days after the first treatment. The initial accumulation could be attributed to leaching from the topsoil considering the heavy rainfall within the period of the survey. The fast dissipation however could be attributed to the high temperature within the period. Increasing temperature is known to increase the rate of loss of pesticides from soils[82] as well as bacterial degradation, all of which are temperature dependent.[82]

About 38% of residue detected on the pod on the day of treatment remained seven (7) days after treatment. The residue detected on the pod on the day of treatment was higher than that for the soil. This is expected because the application of the insecticide was to the plant with the amount in the soil giving an indication of the inefficiency of the spraying.

The average of the results of leaf analysis obtained from the four subplots for the cocoa trees are shown on table 26.

Table 26: Residue levels of propoxur in cocoa leaves as determined by GLC and TLC.

	MEAN RESIDUE LEVELS (μg/g)		
DAY OF SAMPLING	TLC***	GLC	PERCENTAGE OF RESIDUE LEFT
Pretreatment	N.D	N.D	N.D
Day of Treatment	1.67 ± 0.42	1.75 ± 0.12	100.0
7 days after treatment	0.65 ± 0.33	0.76 ± 0.20	43.4
14 days after treatment	0.07 ± 0.01	0.05 ± 0.01	2.9
21 days after treatment	N.D	0.03 ± 0.01	1.7
7 days after 2nd treatment	0.93 ± 0.06	1.02 ± 0.11	58.3
14 days after 2nd treatment	0.39 ± 0.10	0.46 ± 0.15	26.3
21 days after 2nd treatment	0.05 ± 0.02	0.08 ± 0.01	2.9
28 days after 2nd treatment	N.D	0.01 ± 0.01	0.6

TLC***- Results after clean-up.
N.D. No detection.

The initial residue found on the leaf was far higher than for the pod and the soil. 45% of the initial propoxur residue detected on the day of treatment remained 7 days after treatment. This decreased rapidly to 1.7% in 21 days

compared to 16% for the soil and 23% for the pod. The dissipation is therefore higher in the leaves. This can be attributed to the fact that the leaves are more exposed to the direct sunlight, wind and rainfall which cause dissipation and washing of surface easily compared to the soil which was virtually shaded from the sun by the canopy of the trees. The pods, also hiding in the canopy are somehow shielded. The TLC methodology could however not detect any residue on the 21st day after the first treatment for the leaf because it was below its detecting limit. TLC of uncleaned extracts of pod and leaf gave other spots that could interfere with identification and quantification and hence clean up recommended. The soil samples however do not need any clean up. The results as presented show the potential of using TLC to cut down cost.

4.12 METEOROLOGICAL DATA

The monthly total rainfall and average monthly temperature figures for the area from February to May, 1999 is shown in table 27.

Table 27: February to May monthly total rainfall and average temperature.

MONTH	FEB.	MARCH	APRIL	MAY
TOTAL RAINFALL (mm)	98	252.7	86.2	224.6
AVE. TEMPERATURE (°C)	27.2	27.8	27.8	27.0

Rainfall was generally very high with the month of March having the highest of 252.7mm and February a minimum of 98mm. Average temperature over the period was also high (27.4°C).

4.13 RECOVERY DATA

The recovery data of the two pesticides in the soil, cocoa leaves and pods are shown in table 28. The recoveries ranged between 83.5 to 95.5%

Table 28: Recovery Data

PESTICIDE	MEDIUM	%RECOVERY	S.D
	Soils	91.3	0.4
Propoxur	Cocoa leaves	93.0	0.6
	Cocoa pod	85.5	0.5
Atrazine	Soils	95.5	0.7

The accuracy of a residue analysis from a clean up is a measure of its closeness to the true value and is obtained from the mean value of recovery from laboratory spiked samples. Mean percentage recoveries of pesticide residues between 80 to 110% of the true value are said to be adequate.[35] The range obtained justifies the use of the extraction method for the survey. The average percentage recoveries of two pesticides after clean up with SPE followed by GPC and GPC followed by SPE are shown in tables 29 and 30 respectively.

Table 29: Recovery Data from SPE cleanup followed by GPC.

PESTICIDE	MEDIUM	LEVEL OF SPIKING	%RECOVERY	S.D
	Tomatoes	2 X MDQ	90.0	1.6
Atrazine	Tomatoes	3 X MDQ	83.2	3
	Pineapples	2 X MDQ	78.0	2.5
	Pineapples	3 X MDQ	80.5	5
	Maize	2 X MDQ	91.2	0.8
	Maize	3 X MDQ	92.0	0.9
	Tomatoes	2 X MDQ	89.5	4.2
	Tomatoes	3 X MDQ	88.0	6
Diuron	Pineapple	2 X MDQ	89.5	4
	Pineapple	3 X MDQ	79.5	2.6
	Maize	2 X MDQ	89.9	1.2
	Maize	3 X MDQ	87.0	2.0

Table 30: Recovery Data from GPC clean-up followed by SPE.

PESTICIDE	MEDIUM	LEVEL OF SPIKING	%RECOVERY	S.D
	Tomatoes	2 X MDQ	91.0	1.2
Atrazine	Tomatoes	3 X MDQ	84.2	3.2
	Pineapples	2 X MDQ	80.0	3.5
	Pineapples	3 X MDQ	79.5	4.5
	Maize	2 X MDQ	91.2	1.0
	Maize	3 X MDQ	92.0	1.5
	Tomatoes	2 X MDQ	89.0	2.2
	Tomatoes	3 X MDQ	90.0	0.9
Diuron	Pineapple	2 X MDQ	89.5	3.3
	Pineapple	3 X MDQ	79.5	0.6
	Maize	2 X MDQ	89.0	0.9
	Maize	3 X MDQ	89.1	1.5

The average of the recoveries from both methods compare favourably. Whiles the range for the clean up by SPE followed by GPC was between 79.5% to 92%, that for the reverse was between 80% to 92%. The standard deviation values however indicate that it would be better to use the latter since it gives less deviation, an indication of more consistency.

4.14 MARKET SURVEY

With method 4 nothing was detected for tomatoes, maize and pineapple samples, but with methods 1 and 5, four spots appeared with the Rf of one from methods 1 and 5 corresponding to atrazine for the tomatoes. All four spots were visible just after the run before the application of the detecting reagent. With both methods 1 and 8, treatment with the chlorine and bromine caused these spots to be invisible but appeared on application of spray reagent. Clean up was needed for proper quantification. Upon GPC clean up followed by SPE, nothing was detected for all the samples. Maize extracts did not need any further clean up for detection using method 8.

4.15 CONCLUSION

Based on the above discussions, the following conclusions can be drawn:

TLC detection methods tested in combination with cleanup on GPC and SPE provided an alternative cost effective analytical procedure for screening residues in plant and soil matrices.

Database of Rf and RRf of 35 pesticide active ingredients which are used in priority crops in Ghana, have been established. The detectability of the active ingredients (where appropriate) was also determined using appropriate detection methods. The database indicates the suitability of carrying out TLC procedure at room temperature. Detection methods 1 and 4 are most suitable for general screening of residues whiles method 7 and 8 are for sensitively

screening phosphoric acid and carbamate type insecticides. On the other hand methods 2 and 3 worked well for the confirmation of identity of residues. Method 5 however tends to detect herbicides. It has also been shown that narrow-leaves are more sensitive to the photosynthesis inhibition method of detection than broad leaves tested. Spinach and the grass *Sporobulus pyramidalis* being the most sensitive out of the thirteen (13) tested, used as an alternative to wheat, which is not available in the tropic. As low as 0.001mg/Kg limit of detection was achieved for some organophosphates, 0.04mg/Kg for carbamates and 0.12mg/Kg for organochlorines. The database developed through the methods described have been successfully used for screening and semi-quantitative determination of a range of pesticide residues in tomatoes, leaves, soil and cocoa pod.

The methods developed are recommended for laboratories where irregular supply of electricity, lack of services and limited budget do not allow the continuous use of GLC and HPLC.

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APPENDIX A

TABLE OF RF VALUES OF SOME PESTICIDES ON SILICA GEL – ETHYLACETATE SYSTEM FROM VARIOUS COUNTRIES

	Active ingredient	1	2	3	4	5	6	7	SD
1	Aldicarb	0.50	-	-	-	-	-	0.68	0.09
2	Aldrin	0.67	-	-	0.64		0.70		0.02
3	Ametryn	0.61	-						
4	Atrazine	0.61	0.65	0.61		0.74	0.60		0.05
5	Benomyl	0.37		-	0.31	0.39	0.33	0.32	0.03
5a	Benomyl	0.63	0.66						0.02
6	Carbaryl	0.59	0.61	0.61	0.61	0.74	0.62	0.67	0.05
7	Carbofuran	0.57	-	0.59	0.59	0.78		0.66	0.08
8	Captan	0.64		0.64	0.65	0.81	0.68	0.69	0.06
9	Chlorpyrifos	0.67	-	0.67	0.67		0.75	0.74	0.04
10	λ- cyhalothrin	0.69					-		
11	Diazinon	0.67	-	0.66	0.66	0.75	0.68	0.68	0.03
12	Dieldrin	0.70	-	0.68	0.68	0.81	-	0.78	0.05
13	Dimethamethryn	0.62	-	-	-	-			
14	Dimethoate	0.34	-	0.29	-		0.29	0.39	0.04
15	Dioxacarb	0.46		-					
16	Diuron	0.38		0.38	0.37	0.60	0.42	0.48	0.08
17	α-Endosulfan	0.68				0.77	-	0.75	0.04
18	β- Endosulfan	0.67		-		0.73	-	0.73	0.03
19	Fenitrothion	0.68	-	0.65		0.37	0.79	0.71	0.14
20	Isazofos	0.69		0.64					0.03
21	Lindane	0.70		0.70					0.0
22	γ- HCH	0.70							
23	Malathion	0.70				0.62		0.73	0.05
24	Merpan	0.61					-		
25	Metobromuron	0.59		0.57					0.01
26	Mirage	0.41	-						
27	Monocrotophos	0.10		0.08				0.12	0.02
28	Nitrofen	0.68							
29	o,p-DDT	0.67	-				-		
30	Parathion-methyl	0.67	0.72	0.68		0.80	0.70	0.74	0.04
31	Pirimiphos-methyl	0.71	-	-	-		-		
32	Propanil	0.57	-	0.55	-		-		0.01
33	Propoxur	0.58		-			-		
34	Pyrinex	0.67	-		-				
35	Thiram	0.58							

1. Ghana
2. Lithuania
3. Nigeria
4. Yangon, Myanmar
5. Romania
6. Argentina
7. Thailand
8. Kenya

APPENDIX B

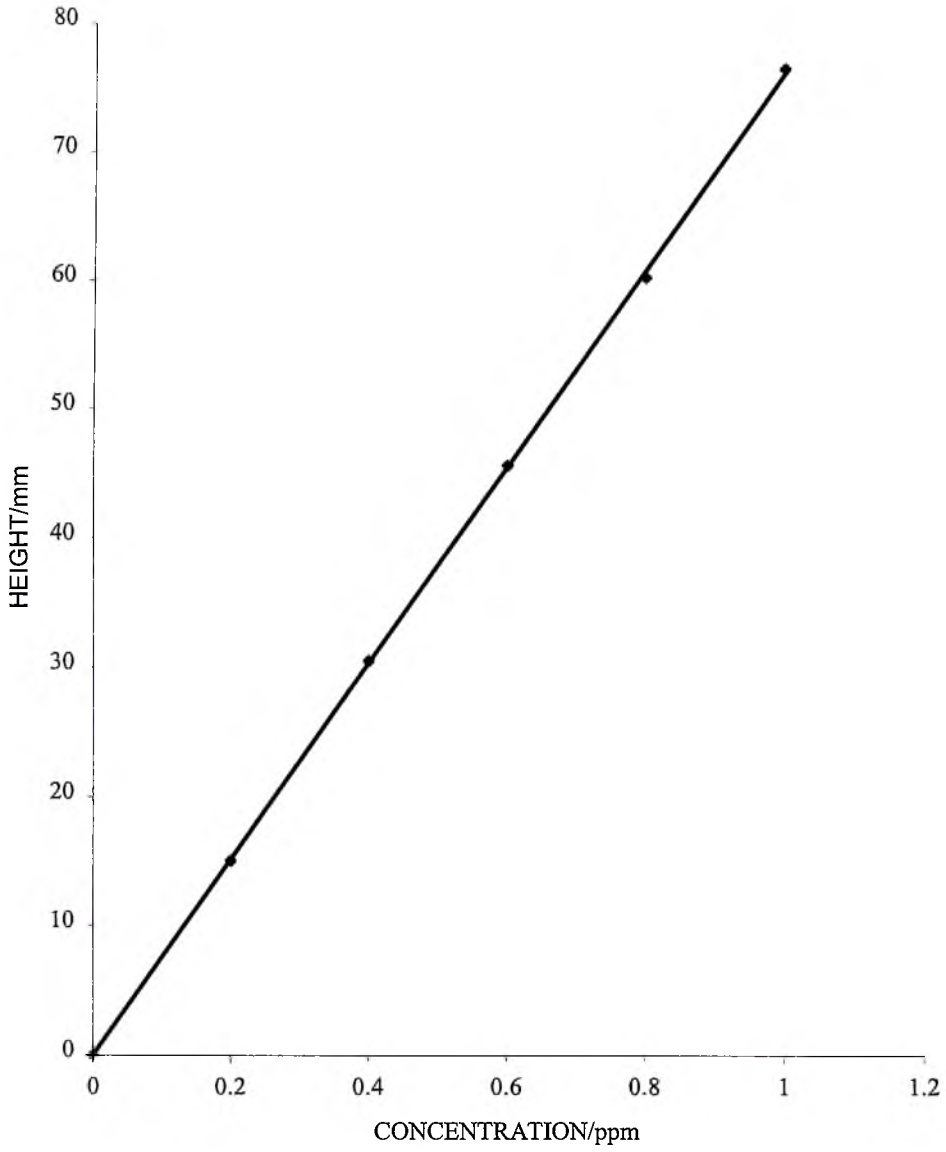

FIG. 11: CALIBRATION CURVE FOR DETERMINATION OF ATRAZINE USING GLC

APPENDIX C

Ellman Test

Principle

Acetylthiocholine + H_20 + cholinesterase thiocoline + acetate thiocoline + dithiobis (nitrobenzoate) → 2 – nitro – mercapto – benzoate.

Reagents

1. Phosphate buffer, 50 mmol/1 (pH 7.2)

   210mg KH_2PO_4 x $2H_2O$ and 500 mg Na_2HPO_4 x $2H_2O$ dissolved in 100 ml double distilled water.

2. 5,5 – dithiobis (nitobenzoic) acid (DTNB)

   solution 1: 10 mg DTNB dissolved in 100 ml phosphate buffer (reagent 1), 0.25 mmol/l.

3. Acetylthiocoline iodide

   Solution 2: 135 mg acetylthiocoline iodide is dissolved in 3ml bi-distilled water, 5mmol/l.

   Note: The solution 1 and 2 are stable for 6 weeks if they are stored in dark at + 4°C.

Sample material

Centrifuged blood serum

Procedure

Photometer: Wave length: 405nm (400 –440)

Cuvelte: 1cm light pass

Temperature: 25°C

Measure against air

NOTE: Reduce initial input sensitivity of the photometer if the initial absorbency exceeds O.500 and the instrument does not compensate automatically pipette into cuvette 300ml solution 1 (25°), 0.02ml sample and 0.1ml solution 2. Mix, read initial absorbance and start stopwatch at the same time. Repeat reading after exactly 30, 60 and 90 sec.

Determine the mean absorbance change per 30sec (Δ A/30sec) and use it for the calculation.

If the absorbance change Δ A/30sec exceeds 0.200 for measurements taken over the space of 90sec dilute 0.10ml 0.9% NaCl solution and repeat the assay (result x 5)

Note: Use 1.5ml solution 1, 10ml sample and 50ml solution 2 or LKB spectrophotometer.

Calculation : Obtain the acuity of cholinesterase in the sample from the table of values or calculate as follows:

V/l (25^0c) = 23460 x Δ A 05/30sec.

Δ A/30sec	**ΔU/L**	Δ A/30sec	**U/L**	Δ A/30sec	**U/L**
0.010	235	0.075	1759	0.140	3284
0.015	352	0.080	1877	0.145	3402
0.020	469	0.085	1994	0.150	3519
0.025	586	0.090	2111	0.155	3636
0.030	704	0.095	2229	0.160	3753
0.035	281	0.100	2346	0.165	3871
0.040	938	0.105	2463	0.170	3988
0.045	1056	0.110	2580	0.175	4105
0.050	1173	0.115	2698	0.180	4223
0.055	1290	0.120	2815	0.185	4340
0.060	1408	0.125	2932	0.190	4457
0.065	1525	0.130	3050	0.195	4575
0.070	1642	0.135	3167	0.20	4692

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