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DETERMINATION OF PESTICIDES AND CHLOROANISOLES IN WATER SAMPLES USING GAS CHROMATOGRAPHY - MASS SPECTROMETRY

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ABSTRACT

Traditionally, pesticide residue analysis methods have focused on the analysis of a single pesticide or a group of chemically related pesticides, but this does not reflect the true varied distribution of residues within the environment. Initially, in this project multi-pesticide residue analysis methods were developed for capillary gas chromatographs using electron capture detection. Variation in temperature conditions, ramp rates and carrier gas flow rates were made to optimise both the resolution and speed of analysis for mixtures of carbaryl (a carbamate), lindane (an organochlorine), chlorpyrifos and chlorpyrifos-methyl (both organophosphorus compounds) and simazine (a triazine herbicide). These methods were then applied to the determination of pesticides spiked into surface and drinking water samples and the recoveries measured.

The same procedure was applied to the chromatographic determination of chloroanisoles, which are believed to result from biotic O-methylation of phenols within the environment. Methods were optimised for the analysis of eleven out of the possible nineteen chloroanisoles available.

Traditionally, such chromatography would have been preceded by solvent extraction of residues, followed by concentration of the resulting organic extract. These methods were compared with the use of the more recently developed Solid Phase Extraction (SPE) cartridges. These allow the extraction of organic materials from large volumes of aqueous samples onto a cartridge followed by subsequent elution using a second solvent. Preliminary work was done on SPE in order to find suitable solvent for both steps so that the analytes could be selectively retained and subsequently released from the cartridges.

The next stage involved the extraction using SPE methods from water samples from rivers around Sheffield. Samples were spiked with a range of pesticides or chloroanisoles prior to extraction and the extracts analysed by gas chromatography-mass spectrometry. Recoveries were found to vary between compounds. Lindane, one of the pesticides used to spike the samples was found to be already present in samples taken from the River Rother at low part per billion levels. Possible sources for this finding are discussed. The presence of chloroanisoles which had previously been reported in this river was not observed.

DECLARATION

No portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this at any other institution of learning.

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CHAPTER ONE - Introduction

1.0 INTRODUCTION

Trace organic analysis involves three main steps, these are sampling, sample clean up and sample analysis. This chapter explains the importance of correct sampling procedures and factors which affect them. Some of the methods used for sample preparation are also discussed. The chapter continues with a description of the use of gas chromatography-mass spectrometry (GC-MS) as an analytical technique for sample analysis.

1.1 SAMPLE PREPARATION FOR CHROMATOGRAPHIC SEPARATIONS

1.1.1 Introduction

Chromatography is one of the techniques that dominate the field of organic analysis. However, sometimes the samples are too complex, too dilute or incompatible with the chromatographic system. Hence, in order to be able to analyse the sample, a preliminary sample preparation technique is required to obtain a sub-fraction of the original sample which has been enriched in all substances of analytical interest. This is mainly to ensure effective separation, detection and system compatibility in the final chromatographic determination.

1.1.2 Sampling

The choice of sampling technique is crucial to succesful analysis. The effects of the sampling technique chosen must be closely monitored, as it is to some extent, reflected in the whole analysis. Incorrect sampling may result in large and inconsistent variations in the data obtained, or simply in no data due to loss of substances of analytical interest. A few factors which must be taken into account during sampling include heterogeneity, storage and preparation of sample and the time factor.

Heterogeneity

Almost all samples are heterogeneous[1] in that the composition of a small portion of a sample does not correspond to the average composition of the whole material being analysed. Hence, in order to obtain accurate results, the whole sample should be analysed. However, as acquiring the whole sample is quite impossible, often representative sub-samples are taken and analysed. Therefore, in order to have meaningful analytical data, a workable plan for acquiring samples has to be implemented and validated by statistical techniques.

Storage and Preparation of Samples

The storage of sample prior to analysis is a very important factor. The interaction between the sample and the container, itself, may result in error. Another source of error is the variation of sample composition over the period of storage and preparation. Therefore, different samples

require different storage procedures in order to reduce this problem to a minimum.

Time Factor

The importance of the time at which samples are taken can be seen in various analyses, and can produce significant variations. For example, in environmental analysis, the time factor must be taken into account in any sampling programme. This affects the results since at different times, particularly different seasons, the sample taken from the same location may contain different level of analytes of interest.

1.1.3 Sample Cleanup

Isolation and Concentration Techniques Using Physical Methods

One of the techniques that have been used for the isolation of volatile compounds from liquid samples or the soluble portions of solid samples is distillation.[2] In this technique, the physical basis of separation depends on the distribution of constituents between the liquid mixture and the vapour in equilibrium with that mixture. In general, the effectiveness of the separation is very much dependent on not only the physical properties of the compounds but also on the method of distillation and the equipment used. The most widely used distillation techniques include simple distillation, fractional distillation and steam distillation. However, in areas involving pesticide residues, assisted or sweep co-distillation is

used to isolate the volatile compounds in animal and vegetable fats and oils at very low concentration.[3]

Assisted sweep co-distillation involves an injection of fluid sample by syringe through a septum injector into an all-glass fractionating tube designated to fit within a multi-port heating block. The fractionating tube, which is either packed with silanised glass beads in the annulus between the centre tube and the glass wall or is fabricated to provide a close fit between the inert and outer tubes is designed so as to allow the sample to pass down the central tube along with the carrier gas prior to coming out and up onto the glass tube wall. The stripping of the volatile pesticides which are collected in a trap containing sodium sulphate and partially deactivated Florisil (magnesium silicate), is facilitated by the high temperature and the high flow of the carrier gas through the tube. In general, the recovery of pesticides depends on the volatility of each pesticide and its resistance to thermal or catalytic decomposition.

Apart from the isolation process, the pre-concentration of samples is also a very important step in sample cleanup procedure. There are several methods which can be used to reduce the sample size. Some are listed below;

1. Sublimation : this is a direct vaporisation and condensation of a solid without passing through a liquid phase. This method is useful for any

compounds that can be sublimed at reasonable temperatures, e.g: polycyclic aromatic hydrocarbon.

2. Freeze concentration : a method used for the concentration of aqueous solutions of organic volatile and substances that are heat labile.

3. Solvent sublation : this method is generally used for the isolation and concentration of surface-active materials by combining foam fractionation with liquid-liquid extraction. The efficiency of this method depends on several factors including bubble size, gas flow-rate and column height.

4. Reverse osmosis : a separation technique suitable for pre concentrating from relatively large volumes of dilute solutions such as drinking water. The method makes use of membranes with small pore diameters with the operating pressure higher than the natural osmotic pressure for the system resulting in the movement of solvent, usually water, from solution of high anal;yte concentration to that of low analyte concentration. As the rate of permeation of organic solutes through the membrane depends on the chemical compatibility of the membrane and analyte, instead of sieving, it can therefore be used to separate solutes of similar size.[4]

Isolation and Concentration Techniques Using Solvent Extraction

Solvent extraction may also be used for the isolation and concentration of a sample. This technique has some advantages over physical methods which include the choice of a large selection of pure solvents to give a wide range of solubility and selectivity, simple equipment and also

solution-phase samples that are convenient and compatible with the sample requirements of chromatographic instruments, that may be employed, subsequently in the analysis.

1. Liquid-liquid Extraction

Liquid-liquid extraction (LLE) can be either a continuous or discontinuous process. Discontinuous liquid extraction involves the attainment of equilibrium between two immiscible phases whereas under continuous conditions, equilibrium is not necessarily obtained. An example of discontinuous extraction is liquid-liquid extraction which can either be used for sample isolation or sample cleanup. Examples of continuous extraction include Soxhlet extractors and countercurrent separators such as centrifugal disk devices. In both extraction procedures, the efficiency of extraction solvents depends on the affinity of the solute for this solvent, (as measured by the efficiency coefficient) phase ratio and the number of extraction steps. The difference in partition coefficients between solutes gives a measure of selectivity of the extraction procedure.

Continuous liquid extraction techniques are normally used in the case where sample volume is large, the extraction rate is slow or when the distribution constant is small. Generally, the extraction can be done in several hours resulting in concentration gains by a factor of up to 10^5.[5] In the case of solid samples such as plant material, some of the principle methods for liquid extraction include shake-flask methods and Soxhlet

extraction. It is generally observed that between the existing methods, Soxhlet extraction gives higher recovery of many analytes from diverse matrices.[5] The basis of this method is that a suitable solvent is vaporised, condensed and allowed to percolate through the solid sample contained in an extraction thimble. The solvent is then returned discontinuously to the boiling flask via a siphon arrangement which increases the contact time of the sample with the solvent. The main disadvantages of this extraction are that the extraction time is generally long and the extracted compounds have to be stable at the boiling point of the extraction solvent as they are eventually accumulated in the boiling flask. In general, such conventional liquid extraction results in the dilution of the sample in a large volume of solvent in comparison to the volume to be used for chromatographic analysis. Hence, a concentration procedure is required. The methods used for the concentration of solvent extracts are listed and described in Table 1[6].

Table 1 : Methods used for solvent reduction

Method	Principle	Disadvantages
Rotary evaporator	Solvent is removed at reduced pressure by mechanically rotating a flask in a controlled temperature water bath.	Volatile compounds may be lost. Recovery of the less volatile compounds due to the entrainment of sample in the solvent vapour. Uncontrolled expulsion from the flask due to uneven evaporation.
Kuderna-Danish evaporative concentrator	The evaporator is operated at atmospheric pressure under partial reflux conditions. The concentrator is mounted with the boiling flask in a controlled-temperature water bath and the final solvent is concentrated into a collection tube of small volume compared with the boiling flask	Slower rate of evaporation with slightly higher recovery of trace organic compounds compared to a rotary evaporator. Method does not allow the reduction of sample to less than 1 ml in a single apparatus
Automated evaporative concentrator	Solvent from a pressure-equalised reservoir is fed at a controlled rate into a concentration chamber where the solvent is vaporised through a short distillation column. The concentration process is completed by nitrogen gas in the absence of heat.	Method requires a boiling point difference of approximately 50^{o}C between the solvent and the analyte for high recovery.
Gas blow-down	A gentle stream of pure gas is passed over the surface of the extract contained in a conical-tipped vessel or culture tube partially immersed in a water bath.	In general, method is limited to sample of less than 25ml. Too high a gas flow-rate may cause sample losses by nebulisation. Gas may contaminate the sample. Carryover of aqueous and high-boiling solvents may be difficult to remove by evaporation.

2. Solid-phase Extraction

Solid-phase extraction is another sample preparation technique for either matrix simplification or trace enrichment.[7] The advantages of using this

method are that it offers lower costs with shorter processing times, simpler processing procedures and it also use less solvent. In addition, the technique can also be automated using special-purpose flow processing units which extract and prepare samples for automatic injections. It is a convenient sample preparation technique for gas, liquid and thin-layer chromatography.

Some of the solid-phase adsorbents available include the bonded-phase silanised silica materials, non-polar and ion-exchange macro reticular resins and some common inorganic adsorbents like alumina and Florisil. These materials are packed into plastic cartridges formed from highly purified polyethylene and sandwiched between two polyethylene frits. The bottom end of most of the cartridges is terminated in a Luer fitting for simple connection to a sampling manifold. Generally, the flow-rate of solution through the cartridge is controlled by vacuum suction. The sample volume that can be processed depends on the flow-rate, concentration of the matrix and the breakthrough volume of the analyte. Generally, solid-phase extraction largely eliminates interfering coextractants from the chromatogram and further gives a more reliable baseline which makes quantitation easier. A description of common adsorbents used for the solid-phase extraction along with typical applications are given in Table 2[8].

Table 2 : Characteristics of silica based bonded-phase sorbents for fractionation and trace enrichment

Sorbent type	Sample type	Applications
Octadecyl	Reversed-phase extraction of non-polar compounds	Drugs, essential oils, food preservatives, vitamins, plasticizers, pesticides and hydrocarbons.
Octyl	Reversed-phase extraction of moderately polar compounds or compounds sorbed too strongly by octadecyl.	Priority pollutants and pesticides.
Silica gel	Adsorption of polar compounds	Drugs, alkaloids, mycotoxins, amino acid, flavinoids, heterocyclic compounds, lipids, steroids, organic acids, terpenes and vitamins.
Aminopropyl	Weak anion-exchange extraction.	Carbohydrates, peptides, nucleotides, steroid, vitamins.
Aromatic sulfonic	Strong cation-exchange and reversed-phase extraction.	Amino acids, catecholamines, nucleosides, nucleic bases.
Quaternary amine	Strong anionic-exchange extraction.	Antibiotics, nucleotides, nucleic acids.
Phenyl	Reversed-phase extraction of non-polar compounds.	Provide less retention of hydrophobic compounds. Not widely used
Dimethylamino propyl	Weak anion-exchange extraction	Amino acids
Diol functionality	Normal-phase extraction of polar compounds.	Protein, peptides and surfactant.
Cyanopropyl	Normal-phased extraction of polar compounds.	Amines, alcohol, dyes, vitamins and phenols.

1.2 GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

1.2.1 Introduction

Conventional analytical techniques which employ classical and wet procedures remain in use in many laboratories. However, nowadays most analysis is performed by instrumental methods. Many analytical chemists

prefer to use efficient analytical instrumentation techniques because they offer high sensitivity and selectivity for analyses that are otherwise tedious or time consuming. Instrumental techniques offer rapid analyses and lower costs compared to classical methods, which are generally labour intensive and hence expensive. One of the instrumental techniques that is widely in use today is gas chromatography.

Chromatography is a physical method of separation in which components to be separated are distributed between two phases, the stationary and mobile phases. Chromatographic separation occurs as a result of repeated sorption and desorption during the movement of the sample components along the stationary phase bed. The separation is due to the differences in the distribution constants of the individual sample components. Chromatographic techniques are characterised by the introduction of small volumes of the sample to be analysed into the mobile phase and the observation of the various components of the sample as they leave the column in the form of concentration bands separated in time.

In any chromatographic experiments, data obtained is generally of the form of a chromatogram which is a record of the concentration or mass profile of the sample components as a function of the movement of the mobile phase. Some of the information readily extracted from a chromatogram includes an indication of column performance, quantitative

assessment of the relative amount of each peak, qualitative identification of sample components based on the accurate determination of peak position and also an indication of sample complexity based on the number of observed peaks.

Chromatographic Column Efficiency

Information on column performance can be obtained from a chromatogram where the sharpness of a peak in that chromatogram is a property of a column and is described as column efficiency. The efficiency of chromatographic columns is measured using an equation which relates the number of theoretical plates, N, and plate height, H (or height equivalent of a theoretical plate, HETP). This is given as

$$N = \frac{L}{HETP} \qquad \textbf{(Equation 1)}$$

where L=length of the column used.

From the equation, the smaller the value of H, the higher is the column efficiency which means that the column should be better at separating the components in a mixture. This is because as the theoretical plate height decreases, the number of equilibrium steps in the column increases. In general, the efficiency of chromatographic columns vary depending on their types (packed or capillary) and the identity of mobile and stationary phases they contain. In a chromatogram, the column efficiency can be

measured from the peak profile. One possible expression for the measurement is

$$N = 5.54\left(\frac{t_R}{W_{1/2}}\right)^2 \qquad \text{(Equation 2)}$$

where t_r = uncorrected retention time

$W_{1/2}$ = peak width in time units at half peak height

Band broadening effects on column efficiency

Band broadening is the consequence of the finite rate at which several mass-transfer processes occur during the migration of a component down a column. As the magnitude of the kinetic effects on column efficiency depend on the length of time the mobile phase is in contact with the stationary phase, it in turn also depends on the flow rate of the mobile phase. Hence, the efficiency of a column has generally been determined by measuring the plate height, H, as a function of mobile phase velocity. This is given as

$$H = \left(\frac{1}{A} + \frac{1}{C_m\bar{\mu}}\right)^{-1} + \frac{B}{\bar{\mu}} + C_s\bar{\mu} + C_{sm}\bar{\mu} \qquad \text{(Equation 3)}$$

where H = plate height

A, B, C = Coefficients of eddy diffusion, longitudinal diffusion and mass transfer respectively

$\bar{\mu}$ = average linear velocity of the mobile phase

Interpretation of this equation enables the prediction of the effects of these parameters on column performance in order to minimise the plate

height, H. Generally, high column efficiency can be obtained by using thin liquid films (in GC), small diameter particles as column packing and a mobile phase of low viscosity.

In gas chromatography, **Equation 3** can be simplified as the mass transfer terms relating to of the mobile phase are of negligible proportions. Hence,

$$H = A + \frac{B}{\bar{\mu}} + C_s\bar{\mu} \qquad \textbf{(Equation 4)}$$

This abbreviated form of equation is referred to as the van Deemter equation. (refer to Figure 3 in Appendix A for diagram) In general, the variations of the terms as a function of mobile phase velocity, μ, suggests that an optimum flow rate exists at which the plate height is a minimum and the separation efficiency is a maximum. For the capillary columns used within this work, **Equation 4** can be further simplified. Since there are no particles within these columns, the eddy diffusion term within the mobile phase should be zero. Hence the equation becomes

$$H = \frac{B}{\bar{\mu}} + C_s\bar{\mu} \qquad \textbf{(Equation 5)}$$

As the name implies, gas chromatography (GC) is a term used to describe all chromatographic techniques which use gas as the mobile phase. The separation of the injected sample mixtures is a result of the interaction of the vaporised sample in the gaseous mobile phase with a liquid or solid stationary phase.

Although GC on its own is a good technique for separating the components of a mixture, it does not allow certain identification of the components. The only criterion that it provides for the identification of an eluting compound is the time the compound takes to pass through the chromatograph (retention time). Since any number of different compounds can have the same retention time, the chromatograph therefore needs to be linked to an instrument capable of giving structural information. One such combination is gas chromatography-mass spectrometry (GC-MS).

In gas chromatography-mass spectrometry, the mass spectrometer can be used as a detector to identify unknown analytes as they are eluted. It is designed to perform three basic functions which are to vaporise compounds of varying volatility, to produce ions from the resulting gas-phase molecules, to separate ions according to their mass-to-charge ratios and subsequently detect and record them. Mass selective detector (MSD) also allows the possibility of identification of unknowns.

Both scan and selected ion monitoring (SIM) have been used in this work. In scan mode, the MSD continually scans across a pre-set mass range. The scanning is performed by a mass filter which changes the m/z values at a set rate over a set period of time. The full mass spectra for each component is then collected by the MSD as each is eluted off the

column. These mass spectra can then be manipulated by computer software to obtain background subtraction or averaged spectra. The corrected mass spectra can subsequently be used for library search in order to identify the compound of interest. In SIM mode, on the contrary, the analyser is set to switch between selected masses and hence improve its sensitivity by spending more time to collect ions of the selected masses.

1.2.2 Main Components of Gas Chromatographs

A gas chromatograph consists of a few essential elements which include a regulated supply of carrier gas, an injection port for sample vaporisation, a thermostated oven in which a column is housed, an on-line detector and a device for recording/ data processing.

1.2.2.1 The carrier gas

The mobile phase or carrier gas can be hydrogen, nitrogen, helium or argon. The choice of carrier gas for use with packed columns is not as essential as with open tubular capillary columns where the most appropriate carrier gas has to be chosen in order to optimise the separation. Some of the most efficient separations achieved for each carrier gas have been reviewed.[9]

An ideal carrier gas should be one which is non-reactive towards the analyte, non-toxic, non-flammable and cheap as it will eventually be

vented at the end of the instrument. However, since the physical properties of the carrier gas has a very small effect on the quality of a particular separation, the choice of gas is usually controlled by the compatibility of the gas with the detector. Nevertheless, if the gas contains impurities, noisy baselines will be seen as a result of the signal produced by the detector corresponding to concentrations of the impurities fluctuating with changes in conditions.

Carrier gas can be obtained commercially in pressurised cylinders. Each of the cylinders is fitted with a two-stage pressure regulator for coarse pressure and flow control. The gas flow is directed through a molecular sieve trap to remove moisture and possibly oxygen. The gas then enters a section in the gas chromatograph where flow controller and pressure regulators are contained. In order to maintain a constant flow of both carrier gas and detector gas at a fixed pressure, a combination of a precision pressure regulator and a needle valve is used. The actual flow rate of the carrier gas, however, is normally measured using a soap-film meter.

1.2.2.2 Sample Introduction Systems

Sample injection is very critical in gas chromatography in that poor injection technique can reduce column resolution and the quality of the quantitative results. Several methods have been developed to introduce samples onto the GC column. These are summarised in Table 3.[10]

Table 3 : Summary of methods used for sample introduction by syringe

Injection technique	Comments
Filled Needle	Sample is taken up into the syringe needle without entering the barrel. Injection is made by placing the syringe needle into the injection zone. No mechanical movement of the plunger is involved and the sample leaves the needle by evaporation.
Cold Needle	Sample is drawn into the syringe barrel so that an empty syringe needle is inserted into the injection zone. Immediately, the sample is injected by depressing the plunger giving no time for the needle to equilibrate to the injection port temperature. Sample remaining in the syringe needle leaves by evaporation.
Hot Needle	Sample is drawn into the syringe barrel as in cold needle. Prior to depressing the plunger, the needle is allowed to equilibrate to the injection port temperature.
Solvent Flush	A solvent plug is drawn up by the syringe ahead of the sample. The solvent and the sample may or may not be separated by an air barrier. The injection is made as in the cold needle method. The solvent is used to push the sample out of the syringe.
Air Flush	Method is similar to solvent flush except that an air plug is used instead of solvent plug. However, with this method, some sample is always retained in the syringe as a result of back pressure caused by rapid vaporisation of sample

A common problem encountered when using vaporising injectors is sample discrimination. The sample leaves the syringe and enters the vaporiser as a stream of droplets, formed by the movement of the plunger and the vaporisation of the remaining sample from the syringe needle. At the evaporation stage, the solvent and the more volatile sample components distill from the syringe needle at a greater rate than the less volatile components, resulting in the sample reaching the column not

identical in composition with the original sample solution. Hence, hot needle and solvent flush techniques are preferred as they are effective in reducing the discrimination. However, for the purpose of this work, the cold needle technique was used.

1.2.2.3 Septa

The septum is used as a sealing object for the injection port while allowing sample introduction through a syringe. Septa can create problems through two processes called coring and bleeding. Coring is the tendency for the needle to punch out a small piece of the septum which is consequently being deposited either in the injection port or into the column. Septum bleed, on the other hand, is a result of diffusion of unpolymerised oil and solutes from the materials which make up the septum. Two important consequences of septum bleeding are deterioration in the column efficiency and a high, unsteady detector baseline resulting in a reduced signal-to-noise ratio. This problem can be reduced the use of either low bleed septa or a septum purge device in which a portion of the carrier gas is allowed to flow across the face of the septum and exits via an adjacent orifice. However, for the purpose of this work, the problem is avoided by changing the septum every few weeks.

1.2.2.4 Sample Inlet Systems

Good sampling and injection techniques are important and crucial for the production of high-efficiency separations and highly reproducible,

accurate and representative quantitative results from very small sample sizes. In order to achieve these qualities, the sampling system has to meet certain requirements[11] which include quantitative and reproducible sample recovery for both trace and major sample components, absence of discrimination effects, no sample decomposition during the vaporisation process and also the sampling system, itself, should introduce only a negligible loss in column efficiency.

Sample inlet systems involving the evaporation of the injected sample are currently contain interchangeable glass liners in the inlet. These liners are variable depending upon the injection techniques and sample types. Their presence is mainly to provide proper mixing between sample vapour and carrier gas, to efficiently transfer heat to the injected sample and also to prevent non-volatile material from reaching the column. In addition, the liner, having a more inert surface than metals, reduce the possibility of catalytic sample decomposition.

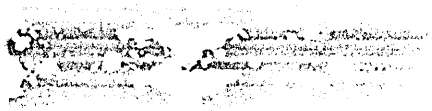
The choice of sample inlet systems in open tubular capillary columns is essential to prevent overloading and hence has resulted in the evolution of various injection systems. The most common is the split/splitless injector. In split mode, the injector system works by venting a proportion of the injected sample instead of allowing it onto the column. This is achieved by having a flow of gas over the heated injector liner.

In splitless mode, the sample and solvent are allowed to enter the column by stopping the flow by means of a valve. The column temperature is set at a lower temperature than that of the solvent's boiling point in order to allow the formation of analyte bands behind the condensed solvent at the column entrance. After a set time, the flow is returned resulting in the solvent and sample being flushed out of the liner into the column. This mode allows almost all of the sample onto the column.

The difference between the two modes is the quantity of sample that can be loaded onto the column. The method used in this work is the splitless mode. This is because the analytes investigated are thought likely to be found at trace levels and hence it is a requirement that a reasonable amount of sample is injected onto the column.

1.2.2.5 Detectors

Although chromatography has been defined in term of separation process, on-line detection still form an integral part of a GC. The detector is used to monitor the column effluent and produces an electrical signal that is proportional to the amount of analyte being eluted. The resulting output signal is then recorded as a continuous trace of signal intensity against time. Peak areas or height can then subsequently be measured either electronically using an integrator or manually from a chart recorder.



The operation and applicability of different detectors can be compared against several performance criteria such as sensitivity, noise, selectivity and minimum detectable quantity. Other than these parameters, detectors can also be classified in other ways. One of the classifications distinguishes between mass-sensitive and concentration-sensitive detectors[12, 13]. In concentration-sensitive detectors, the response has been found to be proportional to the relative concentration of analyte in the carrier gas whereas mass-sensitive detectors produce a signal proportional to the absolute mass of solute vapour reaching the detector per unit time. The latter, in contrast to the former, is independent of detector volume. Another classification is based on destructive and non-destructive detectors[14]. The basic difference between the two detectors is due to the chemical changes in the analyte. While the original chemical form of the analyte persists throughout the detection process in non-destructive detectors, in destructive detectors the detection process involves an irreversible chemical change in the analyte. The non-destructive detectors, hence, is more suitable for further analysis of the analyte.

Detector Requirements

Sensitivity

The signal produced by a detector is its most important characteristic. Sensitivity is defined as a measure of the magnitude of the signal

generated by the detector for a given amount or concentration of analyte. It refers to the change in detector response as a function of the change in amount or concentration of the analyte, i.e

$$S=dR/dC$$

or $$S=dR/dQ$$

where S is sensitivity, R is detector response, C is concentration of the analyte and Q is the total quantity of analyte in the detector.

Detector sensitivity should ideally be a constant independent of the analyte concentration or quantity. This, therefore should give a linear calibration graph of the detector response versus analyte concentration or quantity. Practically, the sensitivity is constant over a range of concentrations or amounts before falling off at some value depending on the particular detector. The range over which the sensitivity of the detector is constant to within 5% is called the linear dynamic range. Within this range, the detector is said to operate with its greatest precision. There are two limits of the dynamic range, the upper and the lower limits. The upper limit of the dynamic range is determined when detector sensitivity falls to zero while the lower limit occurs at the detection limit. The lower limit of the dynamic range not only is a function of detector sensitivity but also of the detector noise.

Noise

Noise is a random perturbation in the signal produced by the detector in the absence of any sample. It is defined as the standard deviation of the detector response when no sample is present and is referred to as the root-mean-square noise, Nrms[15]. There are two types of noise , short-term and long-term. Short-term noise is the maximum amplitude for all random variations of detector signal of a frequency greater than one cycle per minute. Long-term noise, on the other hand, is the maximum detector response for all random variations of the detector signal of frequencies between 6 and 60 cycles per hour[16]. Noise becomes more important as its magnitude approaches that of the analyte signal, it is hence more useful to describe the detector performance using the signal to noise ratio.

Generally, noise can be associated with various sources. Environmental noise is commonly due to the conductor in an instrument behaving as an antenna which picks up electromagnetic radiation from the surroundings and converts it to an electrical signal. Another source of noise is the detector response to the elution of sample contaminants which may obscure the 'true' baseline. However both of these types of noise can be eliminated or minimised. 'Pick-up noise' can be reduced with better shielding and chemical interference by better sample clean-up or greater separation efficiency. This is in contrast to the noise from the electronic components which arises as a result of fundamental, intrinsic properties

of the system and hence can only be minimised and not eliminated by proper circuit design. Noise, in general, has to be kept to a minimum as it restricts the minimum signal that can be detected.

Minimum Detectable Quantity or Detection Limit

Detection limit is defined as the minimum quantity of sample for which the detector gives a detectable response. This is usually three times the signal-to-noise ratio, S/N. Detection limit(DL), noise(Nrms) and sensitivity(S) are related by a simple equation[15]

$$DL=3Nrms/S$$

The limit of quantitation is usually defined as the amount of analyte that gives a peak with a height of at least ten times that of the background noise level.

Response Time, Rt

Significant distortions of peak shape is a reflection of the fluctuation in analyte concentration. Since the signal of the detector is the change of analyte concentration as a function of time, a detector is therefore usually evaluated by its time response behaviour. The response time is a measure of the speed of response of a detector and is defined as the time the detector takes to reach 98%[15] of the final value following a sudden change of signal.

Selectivity

A selective detector should respond to a certain type of compound in comparison to other compounds by a factor higher than 10.[17] This type of detector is useful for the analysis of complex mixtures where the selectivity may greatly simplify the resulting chromatogram and is generally helpful for the screening of a sample of unknown composition.

The selectivity of a given compound over a potentially interfering compound can be measured by the ratio of the detector sensitivities. Selectivity is commonly reported in term of relative molar response (RMR). For selectivity greater than three orders of magnitude of the interfering compounds, a detector is usually referred to as specific for that compound or class of compounds.

Types of Detectors

Flame Ionisation Detector (FID)

The fid, a universal detector for carbon-containing compounds, has been recognised as the most commonly used detector in the analysis of trace level of organic compounds. It generally gives high sensitivity and linearity and is relatively stable towards changes in flow rate, pressure and temperature of the detector gases. Because of these properties, FID has not only has been widely used for the applications involving both

packed and open tubular capillary columns, but also in supercritical fluid chromatography.

A FID consists of a stainless steel jet constructed so that the carrier gas exiting the column flows through this jet, mixes with hydrogen gas and flows to a micro burner tip, which is them swept by a high flow of air, for combustion. The ions produced by this combustion are then collected at a pair of polarised electrodes constituting a small background current which is the signal. As the eluting compound enters the detector, it is combusted resulting in the increased production of ions. The current produced is then amplified and sent to a recording device.[18]

A mass flow detector, FID utilises three gases which are the carrier gas, hydrogen and air which is used for the combustion process. The flow ratios of these gases have to be correctly adjusted in order to obtain optimum detector response. The detector response increases with air flow followed by a plateau in which the working region is recommended.[16] However, comparing the three gases, the flow of air is the least critical factor.

Although the FID has many useful features, it also has some limitations. As it is non-selective, determination and quantitation of small quantities of desired components in a larger sample matrix can be difficult. Another limitation is imposed by the column where the upper end of the effective

linear range in the case of capillary GC-FID is limited by the column capacity.

Electron Capture Detector (ECD)

The ECD, the most commonly used selective detector, has been used mainly in the analysis of compounds of environmental interest. This is attributed to its high sensitivity to a wide range of toxic and biologically active organohalogen compounds present in the environment.

The detector is made up of a chamber containing a radioactive source which is commonly ^{63}Ni. This chamber is constructed as either a parallel plate design or the more common concentric tube design.[16] The latter permits a lower dead volume detector cell and because of its shape optimises the electron capture process. The mechanisms which result in the detection of analyte have been discussed.[15,16, 19, 20] An electron from β-emitter causes ionization of a carrier gas (which is often a nitrogen gas) and the production of a burst of electrons. In the absence of organic species, a constant standing current between a pair of electrodes results from this ionization process. However, in the presence of organic molecules that tend to capture electrons, the current decreases. The resulting response is non-linear unless the potential across the detector is pulsed.

Nitrogen-Phosphorus Detector (NPD)

The nitrogen-phosphorus detector, like the FID and ECD, is an ionisation type of detector for gas chromatography. It is classified as an element-specific detector due to its chemical response to nitrogen and phosphorus atoms with ionisation level of greater than 10,000 times that being produced by comparable amounts of hydrocarbon compounds.[15] Evolved from a detector known as the alkali flame ionisation detector (AFID), the NPD is similar to the FID as it also requires the use of detector gas environment containing both hydrogen and air. In fact, the NPD has also been designed to mount on the existing FID-type detector base on the gas chromatograph.

The NPD is a device consisting of the sample inlet, the thermionic ionisation source and the ion collector while the associated electronics comprise heating current and polarisation supplies for the thermionic source and electrometer respectively. It operates in a detector gas environment composed of a dilute mixture of hydrogen and air. The nitrogen-phosphorus responses in the NPD only appear when the temperature of the thermionic ionisation source has been sufficiently raised to ignite the hydrogen-air mixture. Ignition occurs when enough thermal energy is present to dissociate hydrogen molecules into reactive hydrogen atoms which further react with oxygen molecules in a series of chain reactions that produce a highly reactive chemical environment containing H^+, $OH^{\cdot}$ and water in addition to hydrogen and air.

The detailed mechanism whereby the sample composition products become ionised is still incompletely understood but two different mechanisms have been proposed; the first one involves gas phase ionisation occurring in the boundary layer immediately adjacent to the hot thermionic sources[21-23] and the second involves a surface ionisation occurring on the hot surface of the thermionic source.[21, 24]

The detectors described so far make use of the ionisation as the principle method of detection. There are other less common detectors which use bulk physical, optical or electrochemical properties as the basis of detection. These include the electrolytic conductivity detector[25-27], atomic plasma emission detector[15], mass spectrometric detector[28-30]. and thermal conductivity detector[31,32] The choice of detector for use with analytes depends very much on the factors described earlier.

1.2.2.6 Columns

In GC, five types of columns have been commonly used. These are the classical packed column with internal diameter greater than 2 mm, micro packed columns with diameter less than 1 mm, packed capillary columns having diameter less than 0.5 mm, support-coated open tubular columns (SCOT) in which the liquid phase is coated on a surface covered with a layer of solid support material leaving an open passageway through the

centre of the column and wall-coated open tubular columns (WCOT) in which the liquid phase is coated directly onto the chemically etched column wall.[17,18,33,34] However, in general, these columns can be classified into two main types, packed[35-45] and open tubular capillary columns.[46-54]

In GC, packed columns are those in which the separation medium is a coarse powder coated with a liquid phase. These columns have been used for many of the theoretical and experimental development of GC and have some advantages over the open tubular capillary columns. Apart from being cheaper and easier to use, packed columns are better suited to isolating preparative-scale quantities and can tolerate samples containing thermally labile or involatile components. In addition, unlike in open tubular columns, the large number of phases available in packed columns gives a wider range of selectivity values and hence many components can be separated more efficiently. However, open tubular capillary columns have been used in this work because they give higher resolution, greater sensitivity and shorter analysis times.

1.2.3 The Mass Spectrometer (MS)

The components of a mass spectrometer are the inlet system, ion source, mass analyser detector and an output/storage device. A schematic representation of the systems is shown in the appendix A - Figure 2.[55]

1.2.3.1 Inlet System

In mass spectrometer, there are several types of sample inlet systems of which the choice of used depends on several factors which include the volatility and nature of the samples and the method of ionisation. Some of these inlets are summarised in table 4.[55]

Table 4 : Sample inlet systems in mass spectrometer

Inlet system	Comments
Cold Inlets	The inlet allows the 'leakage' of a gas or volatile compound into the MS through a glass sinter which led along a glass tube to the ion source.
Hot Inlets	These are similar to the cold inlets except that they can be heated to volatilize compounds prior to leading them to a heated line to the ion source.
Direct Insertion Probe	These inlets are directly inserted into the ion source through a vacuum lock and are used for compounds which are not sufficiently volatile.The probe can be heated by radiation from the ion source or heated independently of the source.

The GC- MS Interface

The interface used for the GC-MS in this work is direct link from the end of the chromatographic column to the mass spectrometer.[55] This is also known as direct coupling where the GC capillary column is fed through a gas-thight heated sheath into the ion source with the column end positioned just short of the ionisation region. (refer Appendix A-figure 2)

In this direct coupling arrangement, the carrier gas is passed into the ion source itself. This is usually a highly diffusable gas such as helium. Modern mass spectrometers are mostly fitted with efficient pumping systems which can therefore cope with the low flow rates of the carrier gas that emanates from the capillary column while maintaining an adequate vacuum in the ion source. Also, in this arrangement, the end of the column is no longer at atmospheric pressure. The vacuum outlet conditions in the GC-MS change the gas flow characteristics in the column and subsequently decreases the chromatographic resolution. However, the resulting losses in efficiency of both ionisation and separation power are slight and rarely important in an analysis. The advantages of the direct coupling interface in GC-MS include; (i) all of the sample is transferred into the ion source, hence maintaining good limits of detection (ii) dead volume which would otherwise degrade the resolution is not present (iii) the components only come into contact with the stationary liquid phase coated on the inside wall of the column itself, thus avoiding possible decomposition.

1.2.3.2 Ion Source

The ion source may be defined as the region usually enclosed in a small ion chamber in which ionisation occurs. The resulting ions produced are then propelled out of the chamber towards an exit slit by a low positive potential applied to a repeller plate. Upon leaving the chamber, the ions are then accelerated through a high potential and subsequently passed into the analyser for separation according to the mass-to-charge ratio.

1.2.3.3 Ion Separation (Analyser)

The separation of ions according to their mass-to-charge ratio can be achieved in a number of ways. Some of these are listed in Table 5 below.

Table 5 : Types of Mass Analyser [55]

Mass Analyser	Comments
Double-focusing	These are the combination of electric and magnetic sectors which provide both energy and angular focusing. The energy-focusing electric sector allows the operation of the instrument at high resolving power and accurate mass measurements, may be performed on this instrument.
Single-focusing	There is no electric sector in this kind of analyser and therefore the instrument produces low resolution spectra. This is due to the fact that ions of same mass but with different translational energy are not brought to focus a point .
Quadrupole Mass Filter	Quadrupole analysers consist of four parallel rods. The ions from the ion source are being propelled into the quadrupole analyser by a small accelerating voltage and then follow complex trajectories under the influence of the combination of electric fields. This type of mass analyser was used throughout this project.

1.2.3.4 Ion Detection

In mass spectrometer, the detection of ions and their generation from an electric current that is proportional to their abundance can be done by several methods which include photomultiplier, Faraday Cup detector and electron multiplier. The mass spectrometer used for this project utilised an electron multiplier based detection system

The electron multiplier is the most common detector used with the MS, it consists of a series of electrodes (dynodes). A rapidly moving ion impinges on the first dynode causing the release of a shower of electrons which are subsequently accelerated by an electric potential to the second dynode. The cascading effect continues through the whole series of dynodes and each time provides gain in electric current. The signal obtained from the detector is then subjected to conventional electrical amplification.

CHAPTER TWO - Analysis of Water Samples

2.0 PESTICIDES IN ENVIRONMENTAL WATER

2.0.1 Introduction

Over the years, the need for increased agricultural productivity throughout the world has led to the use of pesticides. However, the positive results from their usage have been accompanied by various negative aspects such as food contamination and water pollution. It has become apparent that our water systems are being contaminated by different waste disposal activities such as landfills and waste water treatment. In addition, contamination can also be the direct or indirect results of agricultural activities such as spilling, spraying or leaching which are commonly associated with the widespread use of pesticides.

Throughout the years, hundreds of different compounds have been used as pesticides with each compound differing in its degree of toxicicity. Their residues remain on the produce causing a potential health risk to consumer especially if they are persistent and do not degrade quickly. The report by the U.S Environmental Protection Agency showed that over 71% of a survey population had quantifiable amounts of pentachlorophenol in their urine[56].

Pesticides are generally designed to specifically interfere with the normal metabolism of either animals or plants. They are biologically active and are therefore hazardous chemicals.

The mode of action of pesticides can be divided into two main categories, systemic and non-systemic pesticides. Systemic pesticides are those which can effectively penetrate the plant cuticle and move throughout the plant by means of its vascular system. Non-systemic pesticides, also known as surface or contact pesticides, by contrast cannot penetrate plant tissues to the same extent and therefore must exert their effects at or near the point of contact. Most of the earliest pesticides fall into this latter category which leaves them open to removal by and susceptible to the effects of weathering. In contrast, modern pesticides tend to be systemic and hence are only slightly affected by weather. As systemic pesticides confer 'all-over' immunity against insects for all plant growth, they are generally more effective.

Most modern pesticides are synthetic. They are manufactured and developed for their toxic qualities and properties. They are mixed with other chemicals, usually a solvent and sometimes wetting agents, to make up the formulated, ready to use commercial product.[57] Other pesticides are based on naturally occurring micro-organisms (e.g. a toxin from *bacillus thurigeniesis*) and chemical extracts from plants (e.g. pyrethrum)[57]. This type of pesticide is however very rare. Pesticides can be classified into groups depending upon their function. In addition, they may be grouped according to the main chemical functional group or the main heteroatom in their structures. These include nitrogen,

phosphorus, oxygen and chlorine. Some representative examples are shown in Appendix A (figure 1).

The presence of pesticides residues has been monitored since 1960's and more recently Maximum Residue Levels (MRLs) have been established by each country. In general, triazine and organophosphorus pesticides are currently the major types used in agricultural activities as most organochlorine pesticides have been withdrawn from use due to their toxicity, persistence and bioaccumulation in the environment. As such, methods suitable for extracting and detecting these compounds in the water systems are very much needed.

Several methods have been developed by the US Environmental Protection Agency, (US-EPA). The philosophy behind these methods consists of three main parts which are to develop and evaluate analytical methods for organic contaminants in water, to determine the response of aquatic organisms to water quality and to develop quality assurance programmes to support the achievement of data quality objectives. Most of the methods are conventional involving solvent partition[58,59] and are generally time consuming and expensive as they involve the usage of large amount of solvents, some of which are banned under the Montreal Protocol on ozone depleting solvents, e.g. CCl_4. In addition, the methods also suffer from problems caused by the formation of emulsions and variable extraction efficiencies for different compounds. Therefore, in

order to minimise the analysis time and costs in multi-residue determination, other methods such as solid-phase extraction (SPE) and membrane extraction have been introduced[60] and subsequently improved[61-63]. There are various solid-supports available for solid-phase extraction of organic components from aqueous solution, but the most frequently used is octadecyl-bonded silica[64-67]. The availability of these solid supports in inexpensive cartridges has contributed to the large increase in the application of solid-phase extraction methods. However, during the development of procedures using this method, it has been found[68] that the use of gas chromatography with high sensitivity electron capture detector (ECD) has resulted in the detection of a number of extraneous peaks. These peaks varied depending upon the solvent used to rinse the cartridges, the supplier of the cartridges and are the results of interference caused by the plastics used to house the cartridges, the frits and also the solid supports themselves. Junk et. al [68] have also demonstrated that these contaminants limit the practical limits of detection obtainable for the components of interest. This problem, however, can be reduced by conditioning the cartridges prior to usage with the final eluting solvent. In conjunction with the determination of pesticides from water, the US-EPA have divided their methods into three main groups which are,

(1) those which use GC with selective detection (ECD or NPD)

(2) those which use gas chromatography-mass spectrometry (GC-MS)

(3) those which use liquid chromatography (HPLC)

When using GC with selective detection, it must be supplemented by confirmatory techniques so as to avoid false positive results. The confirmation is usually achieved by injecting the sample extract onto a column of different polarity and comparing the retention times of the components in the samples. Another more reliable method is by applying a two-dimensional capillary GC where two columns of different selectivity are combined in such a way that a fraction of the eluate can be directly transferred from one column to the other. The different aspects of the instrumentation have been reviewed[34]. Apart from these, confirmation can also be achieved by the chemical derivatisation of the pesticide. This results in the disappearance of the original pesticide peak and the appearance of the derivatised peak.

However, the simplest method for the confirmation can be achieved by GC-MS which requires the use of only one column.

2.0.2 Solid-Phase Extraction (SPE) for Sample Preparation

SPE has been widely used for trace enrichment of very dilute solutions where large sample volumes may have to be processed to yield concentration of analyte sufficient for detection. One area where the use of SPE has been reviewed is the extraction of pesticides from water samples.[75]

Pesticides form a large group of compound with widely differing structures and biological activities. Hence multi-residue methods are often required to provide rapid screening of residues possibly present, identification and quantification of as many different pesticides as possible at the required sensitivity limit. The limits over which the residues can be detected in turn depend on the purpose of the analysis. The proper application of multi-residue methods, therefore, requires knowledge of various aspects such as the extractability of the different compounds from various types of samples, the elution patterns in column chromatographic systems, the sensitivity of the detection and the also the distribution properties of the pesticides in solvent systems of different polarity.

In solid-phase extraction, several multi-residue procedures have been proposed for the determination of different groups of pesticides in water[68].

Mechanism of SPE

The retention of analytes on solid supports involves two types of mechanism. These are partition and adsorption.

Pesticides have some affinity for binding to solid surfaces such as charcoal and porous polymers. The adsorption capacity of a pesticide on a particular sorbent has been found to depend on the treatment

conditions and on the composition of the sorbent. Desorption of the compounds from the cartridge is mainly preferred with a small volume of solvent. In this case, the partition coefficient in a given adsorbent-eluent system should favour the shift of the pesticides towards the eluent. The extent to which the partition is achieved is measured by the fraction of the analyte that is transferred from the solid support to the eluent.

Factors affecting SPE

The extraction of pesticides from water samples depend on various factors which include pH of the water, type of water and the sorbent treatment.

1. pH of water

The effects of pH on the retention of the compounds on a solid phase has been found to be significant only with stable and non-ionic pesticides[69,71-73]. In some cases[74-76] where an appropriate form of the compound is required to achieve a more efficient retention, the pH of the water may be adjusted. Previous studies have shown that pH values between 2 and 8 give efficient retention on the solid phase whereas extreme pH values tend to damage the nature of the bonded phases[77]

2. Volume of water sample

The effect of sample volume on SPE recovery is important for samples of environmental interest. The maximum sample volume where 100%

recovery can be achieved and beyond which the solute of interest begins to elute from the cartridge is called the breakthrough volume. This volume is determined by the capacity factor of the solute in the sample volume which is the sample solvent strength. In the case of reversed-phase sorbents, this volume is a function of the hydrophobicity of the solute and the mass of sorbent used[78,79]. In general, it was found that the recovery drops with the sample volumes.

3. Treatment of sorbent

Treatment of sorbent involves three major steps which are the wetting process, the washing of the bonded phases and the elution of the concentrated pesticides.

(a) The activation of the sorbent

The activation of sorbent achieved by wetting the surface with an organic-water miscible solvent ensures complete permeation of the water sample into the pores of the sorbent. This is essential as effective adsorption requires mutual contact between the solid and the liquid phases.

(b) Washing

Washing is done after the extraction of the sample in order to remove the potential interferents. This is done using solvents of various strength. In the case of non-polar phases, water is used to remove polar constituents of water samples without eluting the pesticides whereas weak solution of

methanol or acetonitrile in water can be used to remove less polar contaminant[77]. However, preliminary analyses are required so as to ensure no analytes are lost during washing.

(c) Desorption

Desorption of pesticides from the SPE column after extraction is done by passing a small volume of solvent, for which the partition coefficent in a given solid phase- eluent system favours the eluent, through the column[80-82].

4. Type of water

The type of water used in an experiment affects the recovery of the pesticides. Samples with low ionic strength and free from colloidal particles such as distilled, de-ionised and tap water generally give high percentage recovery whereas samples with high contents of organic matter such as surface and ground water have been found to experience significant loss of recovery. This observation is due to the competition for the active sites of the adsorbent between the chlorinated hydrocarbons and other hydrophobic groups present in the sample[71, 83, 84].

In addition, sample water containing high concentrations of detergents has been found to diminish the retention of the pesticides in the solid phase. This is thought to be the result of the increase of solubility of the pesticides in water[84]. In other observations, it was found that an

increase in ionic strength of aqueous samples weaken the interaction between the undissociated molecules and water resulting in a higher efficiency[70,85-87]. However, in contrast with this observation, it was found that no significant effect can be observed for a wide range of organic compounds on a C_{18} bonded phase.

2.0.3 Methods for Analysis of Residual Pesticides

Many methods have been developed for use in the analysis of pesticides residues, in both chemical and biological disciplines. Some of the methods include enzyme-linked immunoassays, size exclusion chromatography and high performance liquid chromatography. However, the most common method which has been used for this purpose is gas liquid chromatography. The advantages of using this technique lies in its sensitive and specific detector systems and also in its ability to separate complex mixtures of analytes on the column. In earlier years, GC for the analysis of pesticide residues has mainly concentrated on the use of packed columns containing a wide variety of liquid phases and supports with either the FID, FPD or ECD. In conjunction to this, the wide range of volatility and responses of the pesticides has resulted in the various analytical conditions used to chromatograph several classes of pesticides simultaneously in a single sample. These experimental conditions have been compiled by the US Food and Drug Administration in a document called 'PESTDATA'[70]. However, as many pesticides tend to be either

polar or labile, they are not all suitable for analysis by packed column GLC. One possible solution for this is to use capillary gas chromatography (CGC).

CGC has been used in the analysis of wide variety of pesticides in environmental samples [88-93]. In addition, it has also been used to separate individual compounds within several classes of pesticides. The increase in the use of CGC is mainly due to the improvements in instrumentation; which have included the introduction of new detectors and injection techniques. In addition, advanced column technology has also resulted in more flexible and easily manipulated CGC columns with bonded liquid phases that give the possibility of higher resolution of the analytes and also that provide more inert surfaces so that on-column decomposition or adsorption can be prevented.

2.1 CHLOROANISOLES IN ENVIRONMENTAL WATER

2.1.1 Introduction

Chloroanisoles (chloromethoxybenzenes) are closely related to pesticides. They do not naturally occur in the environment. Instead, they are formed via the biomethylation of the corresponding chlorophenols. Chlorophenols, which represent a major class of contaminants in the environment, include nineteen different compounds with mono-, di-, tri- and tetrachloro- isomers and one pentachlorophenol (PCP). These

compounds have been shown [94,95] to have some degree of accumulation in our food chain. Although health risks resulting from phenols and chlorophenols in water have not yet been established, they are known to give an adverse effect to the taste and odour of drinking water and food products even at trace levels [96]. Various authors have mentioned the finding of phenols in environmental water samples. For example, Sethole and Williams [97] have reported phenols in Canadian drinking water. Similarly, chlorophenols have been frequently found and reported in surface water [98,99] and municipal and industrial discharges [100,101]. The impacts of these compounds on the purity of ground and city drinking water have been studied by Figler and Drevenkar [102]. In addition, phenols have also been found to accumulate in fish [103,104].

Chlorophenols are released into the environment by several sources which include leaching or vaporising from wooden items treated with preservatives, synthesis during routine chlorination of drinking water in treatment plants, releases from factories into air and water and through the incineration of waste materials. Apart from these, chlorophenols particularly PCP, have been widely used as biocides or as precursors in the synthesis of other pesticides and hence are directly released into the environment through adsorption onto the soil. The compounds are subjected to microbial degradation [105-108] and partial chlorination or conversion to substituted benzene-1,2-diols (catechols). Under some circumstances, these phenolic compounds and chlorocatechols undergo

O-methylation to yield chloroanisoles and chloroveratroles (See Appendix A - figures 1a and 4 for structures) respectively. The metabolites could be more toxic than the parent compounds due to their greater lipophilicity and also possess a greater potential for bioaccumulation. Chloroanisoles and thus possibly the related chloroveratroles have been suggested to give rise to the 'musty odour' of contaminated water[109] and therefore reduce water quality and acceptability. Chloroanisoles are reported to be several orders of magnitude more odorous than the corresponding phenol compounds in contributing to the 'off- flavouring' of the water.

2.1.2 Solid Phase Extraction (SPE) For Sample Preparation In The Determination Of Chloroanisoles

Unlike in the case of pesticides, there is no published method on the solid-phase extraction of any type of anisole. Therefore work on the SPE cartridges should be performed as to determine the best conditions of use for the cartridges. Some preliminary work has been carried out within this laboratory. The experiments made use of a suppliers (J.T Baker) published method for phenols [110] since phenols are closely related to anisoles.

This method for phenol extraction using a C_{18} cartridge is as follows. The cartridge is first conditioned by passing through it 10 mL acetone, 10 mL methanol and followed by 10 mL distilled water which has been acidified to pH 2 with concentrated phosphoric acid. The samples were prepared

by adding 20 grams sodium chloride for every 100 mL of water used. The solutions were then acidified to pH 2 using the same acid. The resulting solutions were extracted using C_{18} (3 mL) SPE cartridges. Prior to drying, the cartridges were washed with 10 mL acidified water. Finally, the phenols were eluted with 10 mL methanol.

The original method for phenol was modified in four different ways;

(a) instead of phenols, chloroanisoles were used.

(b) using chloroanisoles, the pH of the sample was adjusted to pH 2, but no salt was added.

(c) using chloroanisoles, sodium chloride was added but no pH adjustment was made.

(d) using chloroanisoles, no salt was added and no pH adjustment was made. .

The results from the work of King [110] within this laboratory showed that no pH adjustment or addition of sodium chloride was required for the extraction of chloroanisoles. Unlike chloroanisoles, chlorophenols can be dissociated easily. The lowering of pH by added acid creates an environment which discourages the dissociation process and allows the undissociated neutral phenols to bond more efficiently to the SPE cartridge.

CHAPTER THREE - Experimental Work

3.0 Development Of Methods For The Separation Of Pesticide Standards By Gas Chromatography

Prior to analysing the real samples, work was carried out to determine the optimum conditions for the separation of a mixture of pesticide standards. Variations in temperature conditions, ramp rate and column head pressure were made to optimise both the resolution and speed of the analysis. Standard solutions of mixed pesticides (10*ug*/mL) was used for this purpose.

3.0.1 Effect Of Ramp Rate On Resolution

With an initial temperature setting of 60^0C and a final temperature of 250^0C, injections of mixed standard pesticides were performed at three different ramp rates; 5^0C min^{-1}, 20^0C min^{-1} and 30^0C min^{-1}.

3.0.2 Effect Of Column Head Pressure

The column head pressure was adjusted to three different settings which are at 50 kPA, 70 kPA and 80 kPA.

3.0.3 Effect Of Detector Temperature On Resolution

Using a ramped programme of 20^0C min-1 between 60^0C and 250^0C, injections of pesticides standard were made at three detector oven temperatures of 250^0C, 300^0C and 345^0C.

3.0.4 Effect Of Oven Temperatures

Using detector temperature of 300^0C and injection temperature of 250^0C, the oven temperatures were varied. Two isothermal conditions were chosen; 140^0C and 180^0C. Similarly the oven temperatures were set at two temperature programming which are listed as follows;

Oven programme :-

		(A)	(B)
Initial temperature	:-	50^0C	50^0C
Initial time	:-	3 min	3 min
Rate	:-	30^0C/min	5^0C/min
Temperature	:-	150^0C	200^0C
Rate	:-	5^0C/min	20^0C/min
Final temperature	:-	260^0C	260^0C
Final time	:-	5 min	10 min

3.1 Standard Preparation Of Glassware

All glassware used in this experiment was washed with soap and rinsed with tap water followed by distilled water prior to draining. They were then rinsed in acetone and the appropriate solvents before being left to air dry.

3.2 Analysis of pesticides

3.2.1 Chemicals and Reagents

List of pesticides used in the experiments :

Names	Molecular Weight	Molecular Formula
Chlorpyrifos-methyl	322.5	$C_7H_7Cl_3NO_3PS$
Chlorpyrifos	350.6	$C_9H_{11}Cl_3NO_3PS$
Carbaryl	201.2	$C_{12}H_{11}NO_2$
Lindane	290.8	$C_6H_6Cl_6$
Simazine	201.0	$C_7H_{12}ClN_5$

Pesticides used for the experiment were commercial products either given by Dow-Elanco or purchased from Lancaster with purity greater than 97% and were used without further purification. Standard solutions were prepared in acetonitrile of HPLC grade which was purchased from Aldrich (Gillingham, Dorset, UK). The solvent was used without in-house re-distillation and procedural blanks were analysed by capillary gas chromatography (CGC) equipped with electron capture detector (ECD) before sample analyses. The stock standard solutions contained 100 mg/L of each pesticide and were stored in screw-capped amber glass vial at 4^0C in the refrigerator. These standards were used to make working standard solutions by appropriate dilution.

3.2.2 Apparatus

An HP 5880 chromatograph from Hewlett-Packard (Avondale, PA, USA) with electron capture detector was used and the data were collected and

integrated by HP 5880 Level Four integrator. A 20 metre x 0.32 mm I.D DB-5 methylphenylsiloxane capillary column of 0.25 *um* film thickness supplied by J & W Scientific and nitrogen gas were also employed. The settings for the determination of the analytes were tabulated below.

Table 6 : GC- ECD operating conditions for the determination of pesticides

GC parameters	Conditions
Injection port	split
Purge time on	30 seconds
Injection port temperature	250^0C
Column head pressure	70kPA
Oven programme	Initial temperature :- 50^0C Initial time :- 3min Rate :- 30^0C/min Temperature :- 150^0C Rate :- 5^0C/min Final temperature :- 260^0C Final time :- 5 min

Experiments were also carried out using a HP 5890 Series II chromatograph from Helwett Packard (Avondale, PA, USA) was attached to a HP 5971 MS detector and the whole assembly was controlled by a computer. A 24m x 0.25mm I.D DB-5 methylphenylsilicone capillary column of 0.25 *um* film thickness supplied by J & W Scientific and helium carrier gas were also employed. The settings for the determination of the analytes were tabulated below.

Table 7 :- GC-MS operating conditions used for the separation of pesticides

GC Parameters	Conditions
Injection port	Splitless
Purge time on	30 seconds
Injection port temperature	250^0C
Column head pressure	70kPA
Oven programme	Initial temperature :- 50^0C Initial time :- 3 min Rate :- 30^0C/min Temperature :- 150^0C Final time :- 2 min Rate :- 5^0C/min Final temperature :- 260^0C Final time :- 5 min
MS Parameters	**Conditions**
Detector temperature	280^0C
Detector solvent delay	3 - 6 minutes
Helium carrier gas flowrate	1 ml/min
Detector mode	scan and selected ion monitoring (SIM)
Carrier gas pressure	6.35 psi
Carrier gas velocity	40.9 cm/sec
Timing for scan regime	start time / end time 9.500 – 10.095 scan for carbaryl 13.169 – 13.537 scan for simazine 13.563 – 13.867 scan for lindane 15.721 – 16.094 scan for chlorpyrifos-methyl 17.422 – 17.872 scan for chlorpyrifos
Ions monitored	Compounds – m/z values carbaryl – 89, 115, 144, 201 simazine – 44, 158, 173, 201 lindane – 109, 181, 219, 290 chlorpyrifos-methyl – 47, 79, 125, 286 chlorpyrifos – 97, 197, 258, 314

For these work, manual injections of 1μL of the extracts were used.

3.2.3 Sampling

The samples were collected in duplicate from the River Rother (pH 9.5) and were kept at 4^0C in the refrigerator for three days prior to extraction. Pre-treatment of the samples was carried out by filtering the water twice to remove any particulate that may be present. Then the samples were separated into four one hundred millilitre portions using 100 mL volumetric flasks where three of them were spiked with 10 mg/L of the pesticides standard solution. Similarly, this was done with five hundred millilitre samples. This was followed by solid-phase extraction (SPE) of the samples. Two samples containing 100 mL each of the spiked and non-spiked river water was also subjected to liquid-liquid extraction (LLE). This was carried out to obtain qualitative data for comparison with SPE.

3.2.4 Extraction Procedures

SPE cartridges were obtained from J.T Baker (Phillipsburg, NJ, USA). These were the 3 mL C_{18} (Octadecyl) cartridges. The cartridges were activated by drawing through 10mL acetonitrile, 10mL methanol and finally washed with 10mL Milli-Q water. The analytes of interest were then extracted by drawing through the samples at a slow to moderate rate under vacuum. The cartridges were then washed with Milli-Q water to remove any impurities and allowed to dry. The extractions were carried out using 3 x 1mL acetonitrile and were concentrated to 0.5 mL under stream of nitrogen gas.

The sample were each extracted using a 500 mL separating funnel containing the sample and 10 mL acetonitrile. The funnel was shaken and left to allow for the separation between the aqueous and the organic layers. This step was repeated twice. The organic layer containing the solvent was then recovered for the analysis. This extract was dried by passing it through a 2 cm diameter glass column containing sodium sulphate (Na_2SO_4) and subsequently reduce to approximately 2 mL under stream of nitrogen.

3.3 Analysis of Chloroanisoles

3.3.1 Reagents

Chloroanisoles used for the experiment were as listed below;

Group	Names	Molecular Weight
Mono-	2-chloroanisole	142
	3-chloroanisole	
	4-chloroanisole	
Di-	2,3-dichloroanisole	176
	2,4-dichloroanisole	
	2,5-dichloroanisole	
	3,5-dichloroanisole	
	3,4-dichloroanisole	
Tri-	2,3,4-trichloroanisole	210
	2,3,6-trichloroanisole	
Tetra-	2,3,4,5-tetrachloroanisole	244

All chloroanisoles used in this experiment were commercial products obtained from Lancaster (Morecambe, England) except for 2,3,4,5-tetra and 3,5-di chloroanisoles (Aldrich, Wilwaukee, USA). All standards had a purity of greater than 97% and were used without further purification. Standard solutions were prepared in methanol which was in-house re-distillate. Procedural blanks were analysed by gas chromatography with electron capture detector (ECD) before sample analyses. The stock standard solutions contained 100mg/litre of each compound and were

stored at 4^0C in the refrigerator. These standards were used to make working standard solutions by appropriate dilution.

3.3.2 Apparatus

An HP 5890 Series II chromatograph from Helwett Packard (Avondale, PA, USA) was attached to a HP 5971 MS detector and the whole assembly was controlled by a computer. A 24m x 0.25mm I.D DB-5 methylphenylsilicone capillary column of 0.25 *u*m film thickness supplied by J & W Scientific and helium carrier gas were employed. The settings for the determination of the analytes were shown in Table 8.

Table 8 : GC-MS operating conditions used for the separation of chloroanisoles

GC parameters	Conditions
Injection port	splitless
Purge time on	60 seconds
Injection port temperature	250^0C
Injection volume	1 L
Column head pressure	70kPA
Oven programme	Initial temperature : 50^0C Initial time : 4 min Rate : 5^0C/min Temperature : 180^0C Final time : 0 min Rate : 30^0C/min Final temperature : 280^0C Final time : 2 min
MS parameters	**Conditions**
Detector temperature	280^0C
Detector solvent delay	6 minutes
Helium carrier gas flowrate	1 ml/min
Detector mode	scan and SIM
Ions monitored	**Compounds** **m/z values** mono- 142, 127, 112, 99 di- 176, 161, 146, 133 tri- 210, 195, 167, 154 tetra- 246, 231, 203, 131 internal standard 266, 223, 75, 63

3.3.3 Sampling

The samples were collected and treated with the same methods as those of pesticides except for the tap water where it was collected directly from the tap into the volumetric flasks. They were collected in duplicate from the Sheffield-Tinsley Canal (under Pinfold Bridge, Staniforth Road), River Rother (about 3 miles south-west Junction 33 of M1) and from tap water in laboratory 830 (Owen Building, Sheffield Hallam University). The canal and river water were kept overnight at room temperature in 2.5 Litre amber coloured Winchester bottles prior to extraction. Pre-treatment of the samples was carried out by filtering the water twice using Whatman filter paper to remove any particulates that may be present. Then the samples were separated into four 100 mL volumetric flasks where three of them were spiked with 10 mg/L of each of the chloroanisole standard solutions. Similarly, the same procedure was applied to 1000 mL samples. The flasks were shaken for 5 minutes and left for half an hour before solid-phase extraction (SPE) was carried out on the samples.

Bond-Elut C_{18} cartridges, 3 mL volume tubes containing C_{18} octadecyl sorbent (J. T Baker) fitted with 75 mL reservoirs were pre-treated by passing 10 mL of acetone through each cartridge followed by 10 mL methanol and 10 ml Milli-Q water. The filtered samples were then aspirated through each cartridge at a flow-rate of approximately 6 ml/min.

Four samples were run simultaneously using a vacuum manifold. At no time during this operation were the cartridges allowed to aspirate to dryness. After the samples have been applied, the cartridges were further aspirated for 10-15 minutes to remove any residual water. The chloroanisoles were then eluted from the cartridges by passing 1 mL methanol through each cartridge under gravity. The extracts were carefully collected and to each 10 *u*L of the internal standard (2,4-dibromoanisole) was added prior to GC-MS analysis.

CHAPTER FOUR: Results and Discussions

4.0 Development Methods For Gas Chromatography

4.0.1 Effect Of Ramp Rate On Resolution

The various ramp rates were found to give large difference not only on the running times of the experiments but also to the resolution of each peak of the pesticides standard. The chromatograms at ramp rate of 5^0C min^{-1} gave the best resolution, clearly separating all the peaks. As the ramp rate was increased, the resolution decreased. It was found that at ramp rate of 30^0C min^{-1}, the pesticides standard was still clearly separated. Hence, this ramp rate was chosen for further experiment as it not only gave good resolution for the compounds but also reduced the run time of the experiment.

4.0.2 Effect Of Column Head Pressure

The different column head pressure used in the experiments were found to give insignificant effects on both resolution and run time. Hence, 70 kPA was chosen as it is the standard value used on the gas chromatograph.

4.0.3 Effect Of Detector Temperature On Resolution

In an electron capture detector, the sensitivity is very much related to the operating detector temperature. The lower limit at which the detector can be operated is set by the upper temperature of the column temperature programme. For any experiments, a suitable temperature that is high

enough to keep the detector clean without giving premature sample decomposition is required.

For the chosen detector temperatures used, it was found that the actual difference in the chromatogram resolution was difficult to discern and did not give significant effect on the run time. Hence, the temperature of 300^0C was chosen for the detector as this compromise between the detector upper limit of 345^0C and the column upper limit of 250^0C.

4.0.4 Effect Of Oven Temperatures

In the isothermal case, as expected, the run time of the chromatogram decreased as the temperature increased. The resolution of each pesticide was also decreased with temperature. However, for the standard mixture used, it was found that at 180^0C, the resolution was still clear for each component.

In the temperature programming, programme (B) was taking a longer time to complete the cycle. The initial ramp rate of $5^0C\ min^{-1}$ was found to separate each peak quite apart from one another resulting in high retention time different between the first and the last peak. With programme (A), even at initial ramp rate of $30^0C\ min^{-1}$, the peaks were still clearly resolved and appeared at a smaller interval between one another. Hence, programme (A) was chosen for used with the rest of the experiments as it gave good resolutions for the peaks at a lower run time.

4.1 Analysis of Pesticides

4.1.1 Stability of the Instrument

Prior to using the GC - MS , the instrument was checked for its stability. This was done by injecting mixture of pesticide standards in five consecutive runs. The peak areas obtained were averaged for each pesticide and the deviation (%) from the mean were recorded. The values obtained range from 0.30% for Carbaryl to 8.46% for Simazine. Therefore, this suggested that although the instrument might be stable for a certain compound, it might not be stable for another. In this case, although the value for Simazine was quite high compared to the other compounds, it still falls into an acceptable range.

4.1.2 Calibration of Standards

As in the case of other analytical instrumental methods, gas chromatography does not directly provide the concentrations of analytes in unknown samples. A calibration procedure is thus required. In this experiment , this was done by measuring signal intensities using a series of standard solutions of known concentration and plotting calibration graphs of

Area, A versus **Concentration, C**

In order to check whether the graphs are linear or otherwise, the product-moment correlation coefficient , r, was calculated for each standard.

product-moment correlation coefficient, r,

$$r = \frac{\Sigma_i \{(x_i - x)(y_i - y)\}}{\{[\Sigma_i (x_i - x)^2][\Sigma_i (y_i - y)^2]\}^{1/2}} \quad \text{(Eqn. 6)}$$

The constructed graphs can then be used in quantitative analysis to measure the amount of the analytes in an unknown samples.

The calibration standard solutions were prepared by diluting the pesticide standard individually in acetonitrile to various known concentration (100 mg/L to 0.01 mg/L) and injecting 1 *u*L of each series into the GC-MS. The calibration graph for each compound is shown in Appendix B - Figures 11 to 15.

4.1.3 Extraction Recoveries

As has been mentioned above, the recovery is calculated from the calibration graphs for each standard in the calibration mixture. It has been found that the recoveries for the samples subjected to solid-phase extraction were above 70% for all pesticides used in this experiment (refer to table 9) with percent mean standard deviation for each falls between ±3 and ±5 %.

Table 9 : Recoveries of Pesticides from River Rother

Compounds	Recovery %	Recovery %
	100 mL	**500 mL**
Carbaryl	226	90
Simazine	147	75
Lindane	183	133
Chlorpyrifos-methyl	108	74
Chlorpyrifos	93	75

However, the fact that less than 100% recovery recorded suggests some losses of analytes which could arise from incomplete elution from the cartridge in the final sample preparation stages. Acidification removes inorganic particulates and acid labile organic compounds from the water. Since no acidification was done prior to extraction, the other matters present in the samples might occlude in the SPE cartridge thus causing blockage. The target pesticides might then be not efficiently eluted. Another reason could be due to the fact that the C_{18} cartridge in the first place failed to retain the pesticides. In principle, the losses can be reduced if not totally avoided by using different solvent in the eluting step. Another possible explanation for the low recovery is the loss of the pesticides on the glassware used. The fact that concentration step was done to the extract using a stream of nitrogen gas might also contribute to the low recovery. During this step, it is possible that some volatile components are lost.

The high recoveries (> 100%) suggest that the pesticides used in this experiment may be found in the samples. In order to confirm this, the extracts were run using SCAN mode in the GC-MS for both spiked and non-spiked samples. (Refer to Appendix B, Figures 7 to 10, for illustrative chromatograms) The results from the library search for the unknown in the non-spiked samples showed that no pesticides used in the experiment were found. The high recoveries may be due to the presence of co-eluates in the samples. Library search was done at the particular retention time to determine the possible co-eluent. The results obtained was however rejected due to the unreliability of the percent library match which were very low. Another possibility was to carry out different extraction procedures on the samples. It is hoped that by doing this the presence of co-eluent can be totally eliminated or largely surpressed in one or two of the methods. This however was not carried out due to time limitation. Other sources of the high recoveries include column bleeding and change of detector sensitivity at the same time as the eluting compounds.

The exception for the high recovery, however, is for lindane which has been found in the sample. This result is confirmed by running the sample extract in both SCAN and SIM mode on the GC-MS and using the library search to identify the peak in the non-spiked samples. The retention time of this peak was then compared to that of lindane in the spiked samples. The observation was further confirmed by comparing the results obtained

by Al-Ahmadi [111] on the same samples which have been subjected to both liquid-liquid and solid-phase extractions and analysed using both GC-FID and GC-MS.

The presence of lindane in the River Rother might be derived from the agricultural sources which may be using lindane as a pest control agent. However, one possible source of lindane in this river is a tannery in Chesterfield situated just next the waterway.[112] Lindane is widely used to treat animal hides in order to preserve them from mould growth during transport from the Americas and subsequent storage prior to processing.

Also, the value obtained for individual pesticides suggests that the recovery change quite significantly with the increase in sample volumes. Comparing the two samples volumes, it was found that the pesticides recoveries were lower for each pesticide in the higher volume. This could be accounted for by the fact that the SPE cartridges have limited capacity to retain materials. Samples may contain various materials other than the analytes of interest. These materials may compete for the limited active sites on the catridges. At higher sample volumes, these active sites may have been fully occupied by these materials resulting in the loss of analytes of interest as they are eluted off instead of being retained by the cartridges.

4.1.4 Analytes losses through evaporation

The extracts eluted from the cartridge were concentrated to approximately 0.5 mL by using a stream of nitrogen at room temperature. This process itself as had been suggested, may give rise to major analyte losses.

4.1.5 Qualitative Comparison Between SPE and LLE

Comparing the two extraction methods, it was found that some of the peaks observed in the SPE extract were not observed in the LLE extract. The missing peaks were the simazine and the carbaryl. Hence, it can be concluded that in this work, the SPE method was preferred as it allowed for the extraction of all the pesticides used.

In the non-spiked sample of the LLE extract, a peak corresponding to lindane was also observed. This again suggested the presence of lindane in River Rother. The possible source had been discussed above.

4.2 Analysis of Chloroanisoles

4.2.1 Stability of the GC-MS

As in the case of pesticides, the stability of the instrument was checked by injecting a mixture of the chloroanisole standards in five consecutive runs. The results were then averaged and the deviation from the mean

were recorded. The calculated RSD (%) for each compound were as follows;

Compounds	RSD (%)
3-chloroanisole	0.79
4-chloroanisole	0.23
2-chloroanisole	0.67
3,5-dichloroanisole	0.20
3,4-dichloroanisole	0.50
2,4-dichloroanisole	0.56
2,3-dichloroanisole	0.62
2,5-dichloroanisole	0.20
2,3,4-trichloroanisole	0.17
2,3,6-trichloroanisole	3.32
2,3,4,5-tetrachloroanisole	0.13

Except for 2,3,6-trichloroanisole, the values for the other chloroanisoles were calculated to be between 0.13 to 0.79%. For, the 2,3,6-trichloroanisole, the slightly higher value suggested that the GC-MS was less stable for the compound. Other reasons could include baseline instability and the presence of co-eluent at around its elution time resulting in distortion of peak area. Overall, it could be said that the GC-MS was stable towards all chloroanisoles used in the work.

In order to check whether the GC-MS would also be stable towards the compounds on every other day, the same procedures as above were carried out on a daily basis. The retention times and peak areas for each compound was compared to check for any significant differences. Examples on some of the retention times and peak areas on a particular day were shown in Table 10 and 11;

Table 10 : Retention time - Peak Area of Chloroanisoles

Compounds	September, 23		September, 24	
	Retention time, min	Peak area	Retention time, min	Peak area
1,3- Cl.anisole	14.106	9428605	14.106	6412319
1,4- Cl.anisole	14.409	7250835	14.415	6677231
1,2- Cl.anisole	14.936	6505484	14.941	5510338
3,5-Cl.anisole	18.634	3240878	18.634	2891365
2,4-Cl.anisole	19.554	7555661	19.552	8710460
	19.770	18744617	19.769	18107141
	20.703	4960838	20.703	11372464
	22.006	3159819	22.005	2959331
	25.441	3029728	25.448	2674696

Table 11: Retention time - Peak area of Chloroanisoles

Compounds	October, 4		October, 5	
	Retention time, min	Peak area	Retention time, min	Peak area
1,3- Cl.anisole	14.112	5860989	14.087	5367056
1,4- Cl.anisole	14.430	6146817	14.396	6071626
1,2- Cl.anisole	14.960	5418131	14.926	4975702
	17.240	3165910	17.191	3008574
3,5-Cl.anisole	18.660	2837283	18.622	3300140
2,4-Cl.anisole	19.590	6495471	19.548	6045563
	19.810	14992692	19.765	15273162
	20.740	5092561	20.703	4287248
	22.030	2977830	21.996	2699255
	25.480	3204704	25.458	3487839

Comparing the results on 23 September and 24 September, it was found that the peak areas varied slightly for every compounds with the biggest difference was for 2,3-dichloroanisole. Similarly, the peak areas between the 4 October and 5 October were also slightly different. The difference between these areas could be due to the stability of the instrument towards each compound or simply because of the injection consistency of the operator. The latter was thought to be the most likely cause of this observation. This was because on both occasions, it was found that the

retention times for each compound coincide with one another and thus rejecting the possibility of instability of the instrument towards the compounds. This was further supported by the fact that the retention times on 24 September were similar to those on 4 October.

4.2.2 Extraction recoveries

The extraction recoveries of chloroanisoles were calculated as follows. For each chloroanisole, the ratio **(R1)** of the area of each component to the area of a known amount of internal standard was determined. A same amount of internal standard in methanol was added to the spiking solution and the ratio was calculated for each component in the spiking solution **(R2).** In order to calculate the yields, the ratios were compared and reported as a percentage (%). i.e

$$\text{\% Recovery} = \frac{R1}{R2} * 100\% \quad \text{(Eqn. 7)}$$

$$= \frac{C1}{A1} * \frac{A2}{C2} * 100\%$$

where C1 = area of the component

C2 = area of component in spiked sample

A1 = area of internal standard

A2 = area of internal standard in spiked sample

Tables 12 to 14 show the recovery of chloroanisoles using two different samples volumes. The relative standard deviation of all analytes are below ± 9% with the highest calculated for 3, 4-Dichloroanisole in tap water.

Table 12 :- Recoveries of Chloroanisoles from Sheffield Tinsley Canal

COMPOUNDS	MEAN RECOVERY (%) ± MEAN % STANDARD DEVIATION	
	100 mL	1000 mL
3-Chloroanisole	167.0 ± 3.1	168.5 ± 2.0
4-Chloroanisole	94.9 ± 3.8	126.6 ± 0.8
2-Chloroanisole	65.6 ± 3.4	60.2 ± 2.7
2, 5-Dichloroanisole	Not detected	
3, 5-Dichloroanisole	Not detected	
3, 4-Dichloroanisole	20.7 ± 1.5	22.3 ± 0.4
2, 4-Dichloroanisole	11.0 ± 1.9	12.2 ± 2.6
2, 3-Dichloroanisole	24.8 ± 1.2	16.5 ± 0.9
2, 3, 4-Trichloroanisole	Not detected	
2, 3, 6-Trichloroanisole	Not detected	
2, 3, 4, 5-Tetrachloroanisole	34.4 ± 0.4	Not detected

Table 13 :- Recoveries of Chloroanisoles from River Rother

COMPOUNDS	MEAN RECOVERY (%) ± MEAN % STANDARD DEVIATION	
	100 mL	1000 mL
3-Chloroanisole	31.6 ± 5.7	124.9 ± 1.6
4-Chloroanisole	30.5 ± 8.6	83.0 ± 2.2
2-Chloroanisole	29.7 ± 7.6	66.6 ± 0.9
2, 5-Dichloroanisole	Not detected	59.2 ± 1.2
3, 5-Dichloroanisole	81.6 ± 7.1	Not detected
3, 4-Dichloroanisole	10.5 ± 4.1	18.6 ± 1.9
2, 4-Dichloroanisole	5.6 ± 8.6	9.8 ± 1.3
2, 3-Dichloroanisole	30.2 ± 4.1	47.6 + 3.0
2, 3, 4-Trichloroanisole	109.2 ± 10.4	Not detected
2, 3, 6-Trichloroanisole	Not detected	31.0 ± 1.6
2, 3, 4, 5-Tetrachloroanisole	Not detected	11.5 ± 6.7

Table 14 :- Recoveries of Chloroanisoles from tap water

COMPOUNDS	MEAN RECOVERY (%) ± MEAN % STANDARD DEVIATION	
	100 mL	1000 mL
3-Chloroanisole	140.9 ± 5.7	176.8 ± 0.6
4-Chloroanisole	114.7 ± 2.2	155.2 ± 0.1
2-Chloroanisole	110.5 ± 5.9	116.2 ± 0.1
2, 5-Dichloroanisole	Not detected	Not detected
3, 5-Dichloroanisole	326.2 ± 0.9	Not detected
3, 4-Dichloroanisole	201.6 ± 8.9	151.1 ± 0.3
2, 4-Dichloroanisole	196.5 ± 7.2	162.8 ± 0.3
2, 3-Dichloroanisole	Not detected	Not detected
2, 3, 4-Trichloroanisole	Not detected	Not detected
2, 3, 6-Trichloroanisole	Not detected	Not detected
2, 3, 4, 5-Tetrachloroanisole	Not detected	Not detected

Comparing the samples from different sources, it was found that generally the recovery of chloroanisoles were highest for tap water and lowest for the River Rother. (refer Appendix B, Figures 18 to 24 for chromatograms) The low recovery in River Rother could be due to the fact that it contains high amount of organic matter which gives rise to competition for the active sites of the adsorbent in the cartridge between the chlorinated hydrocarbons and other hydrophobic compounds present in the samples.

The chloroanisole recovery in the canal was found to be similar for both sample volumes. On the contrary, the recovery showed a slight increase with volume in River Rother and tap water. In general, it was found that the recovery of almost all chloroanisoles from the different samples was below 90%. The reason for the low recovery could be due to either the loss of analytes which arise from incomplete elution from the cartridge in the final sample preparation stages or due to the fact that the C_{18} cartridge in the first place failed to retain the chloroanisoles. In principle, the losses could be avoided by increasing the solvent volume, using different solvent in the eluting step or by using SPE cartridge containing solid phase which could highly retain the chloroanisoles.

In the case of tap water, the high recovery values (> 100%) could be due to the presence of co-eluates. This co-eluate could either be the chloroanisole, itself, or other compounds in the water samples. The tap water used in this experiment may contain compounds which have similar properties as chloroanisoles. Therefore, like chloroanisoles, they are being retained in the cartridge and eluted by the solvent, which in this case is methanol. Perhaps, in order to reduce this problem, a different solvent which more preferentially elutes the chloroanisoles should be used in both the conditioning and eluting steps.

Also, the fact that chlorine might has been used in the treatment of tap water might contribute to the high recovery values. Chlorine has long

been used as disinfectant in the treatment of drinking water as a way to remove undesirable odours, colours, toxic organic compounds as well as reducing a number of potentially harmful organisms. Aqueous reactions of molecular chlorine have been reviewed by several authors[113]. When chlorine gas is bubbled into water, a rapid hydrolysis occurs resulting in the formation of hydrochloric (HCl) and hypochlorous (HOCl) acids. The HOCl can then proceed to react with other phenolic compounds in the water to produce malodrous and unpalatable chlorophenols. Other products of chlorination of phenolic compounds in drinking water include chloropicrin (Cl_3CNO_2)[114], chlorocresols and benzoquinones[113]. These compounds could have similar properties to chloroanisoles thus acting as co-eluents.

4.2.3 Analytes losses through concentration

In this experiment, the extract was eluted from the cartridges using 1 mL of methanol. The eluate was not concentrated to a lesser volume and thus no analytes losses should be attributed to this step.

4.2.4 Cartridge elution

In this experiment, it was observed that the tri- and tetra-chloroanisoles were either found in quite low recoveries or not at all in the elutes. This may be because of the small amount of solvent used were not enough to eluate the analytes. Hence, instead of 1 mL, perhaps the amount of methanol used to eluate the analytes should be increased.

On the other hand, the absence of these analytes could also be because of their strong retention by the Bond-Elut C18 . This could be confirmed by derivatizing the analytes before and after passage through the cartridge and eluting them in the solvent. In this case, further experiments should be done to find a suitable solvent phase which is capable of eluting the analytes retained in the cartridge. Another possibility is that the analytes were not at all retained by the cartridges. These analytes would therefore be expected to be in the water already passed through the SPE cartridge. In order to confirm this observation, this water should be analysed using a liquid-liquid extraction method. If the missing peaks of the analytes could be seen on the chromatogram after this procedure, then it could be stated that the wrong cartridges or wrong solvent have been used for the SPE. Hence, further experiments should be done to find a suitable cartridge that can retain the analytes.

CHAPTER FIVE - Conclusions And Suggestions For Future Work

5.0 CONCLUSIONS

5.0.1 Analysis of Pesticides

It was found from the experiments that the solid-phase extraction method used has resulted in some losses of the pesticides spiked into the samples. The various reasons for this observations have been discussed. Also, the samples collected from the River Rother have been found to contain a small amount of Lindane (an organochlorine pesticide). The likely sources for these findings have been discussed.

5.0.2 Analysis of Chloroanisoles

Samples from different sources have been analysed in order to compare the recovery in each and the possible factors which might affect the observation. It was found that recovery was highest in tap water and lowest in River Rother water.

The contaminated remains from a number of former factories producing aromatic compounds and chlorinated aromatic compounds from coal within the south of the Rother catchment might have been expected to give rise to phenol and chlorophenol contamination of the river. However no evidence of this was detected.

5.1 SUGGESTIONS FOR FUTURE WORK

So far, the experiments undertaken has progressed from analysing pesticides to chloroanisoles in different sources of water samples. In the future, the work on chloroanisoles can be further focused in analysing chloroanisoles in sludge samples. Chloroanisoles, as has been mentioned above are not naturally found in our environment but are products from the methylation of chlorophenols. Various workers have reported [115-118] the finding of various chlorophenols from different sources including water, soil and sludge. Also, biochemical research has been carried out in [119,120] in order to find which bacteria cause the biomethylation of chlorophenols to chloroanisoles and possibly chloroveratroles.

It is known [110] that the presence of chlorophenols in sludge results in some conversion to chloroanisoles. Therefore it is suggested that the change of the chlorophenols in spiked sludge samples is monitored to see whether they are unchanged, undergo dechlorination followed by degradation to chloroanisole and related compounds, or undergo direct degradation to chloroanisoles and related compounds. (refer to Appendix A) In order to determine this, not only the presence of chloroanisoles should be monitored but also the other possible products such as chloroveratroles, chloroguaiacols and chlorocatechol. The chlorophenols used for spiking the sludge can include all compounds in the group or limited to the few which are of environmental interest. These will include

pentachlorophenol (PCP) which is used as a precursor in many syntheses of biocides and 2,4-dichlorophenol which is closely related to 2,4- dichlorophenoxyacetic acid, a type of herbicide.

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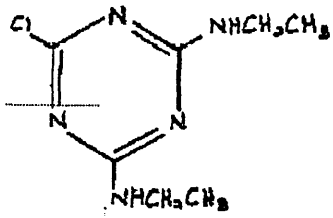
APPENDIX A

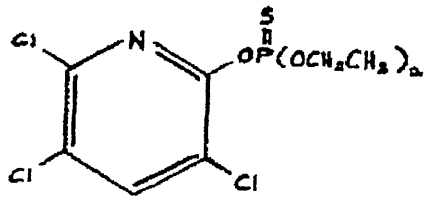
- STRUCTURES OF COMPOUNDS

- SCHEMATIC DIAGRAM OF GC-MS

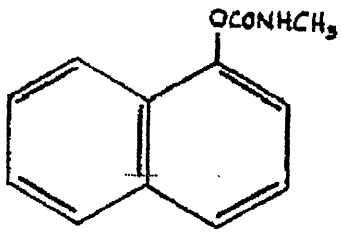
Figure 1 : Structures of Pesticides



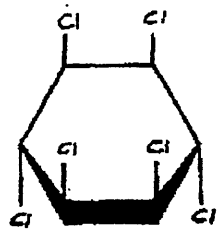
SIMAZINE



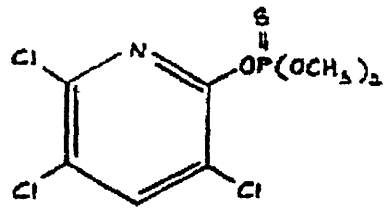
CHLORPYRIFOS



CARBARYL

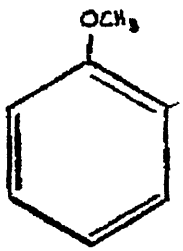


LINDANE

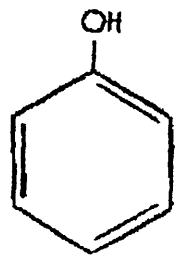


CHLORPYRIFOS-METHYL

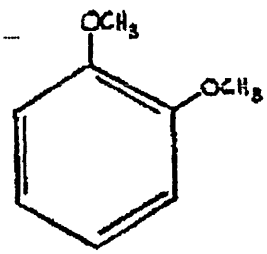
Figure 1a : Structural formulas for anisole and veratrole and the corresponding phenol, guaiacol and catechol from which they can be produced by biological methylation



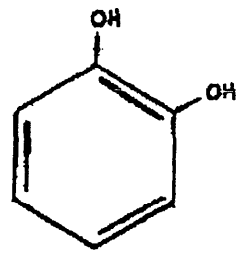
ANISOLE



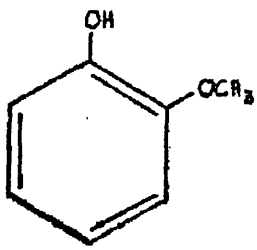
PHENOL



VERATROLE



CATECHOL



GUAIACOL

Figure 2 :- Schematic Diagram Of Gas Chromatography-Mass Spectrometry (GC-MS) [121]

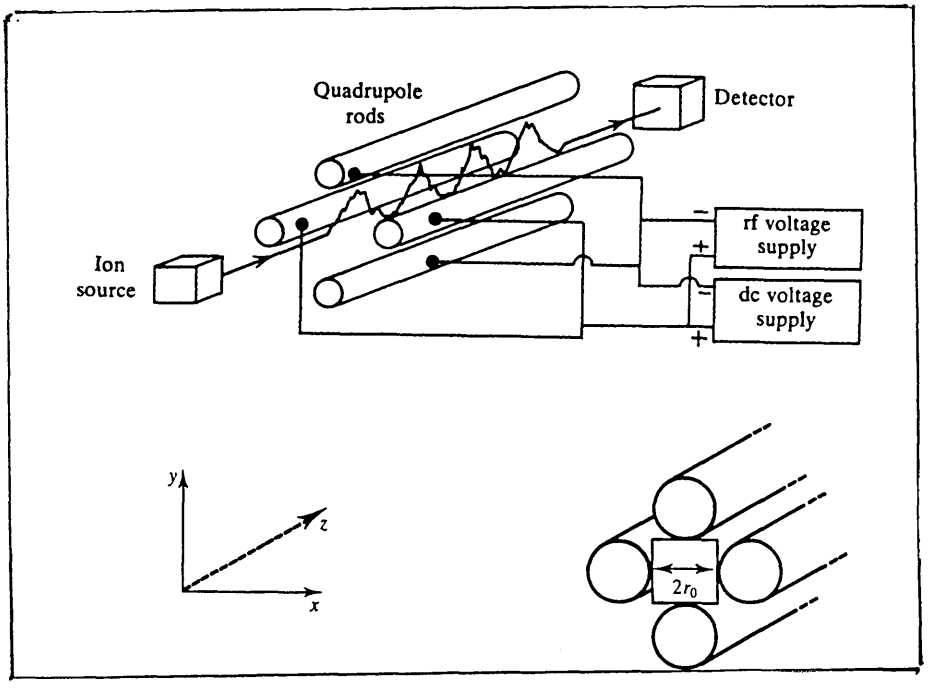


Figure 2a :- A Schematic Diagram Of The Direct Coupling Approach To Combined GC-MS

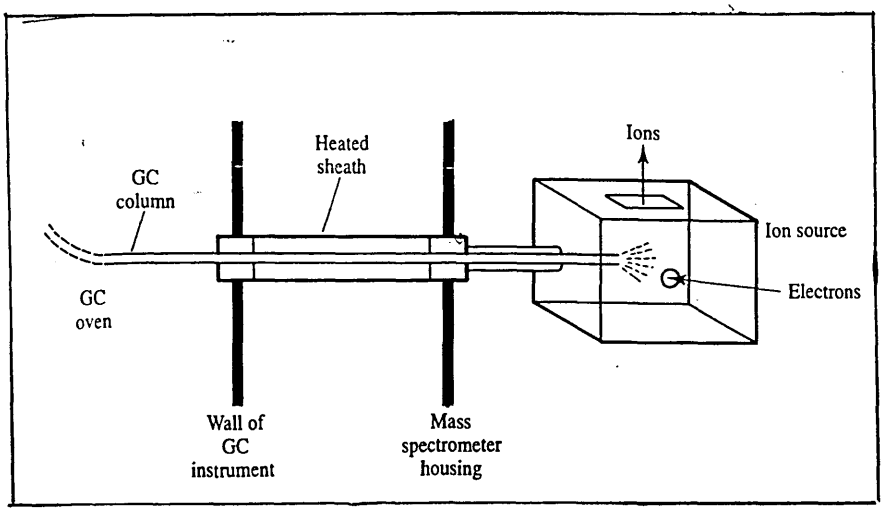
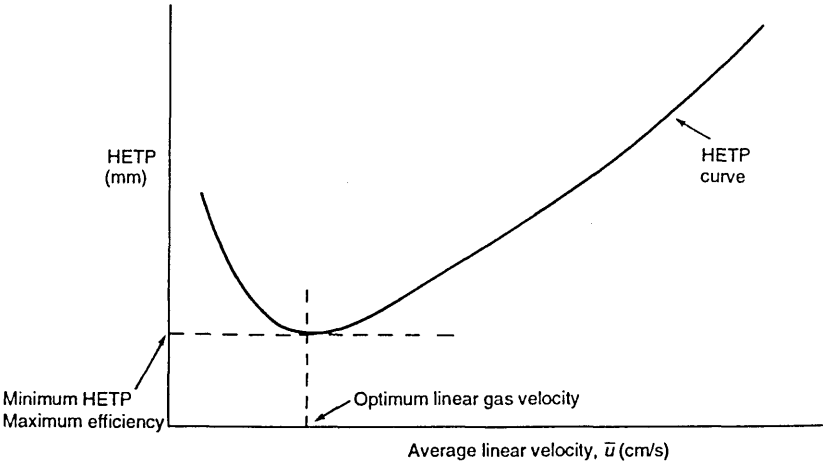
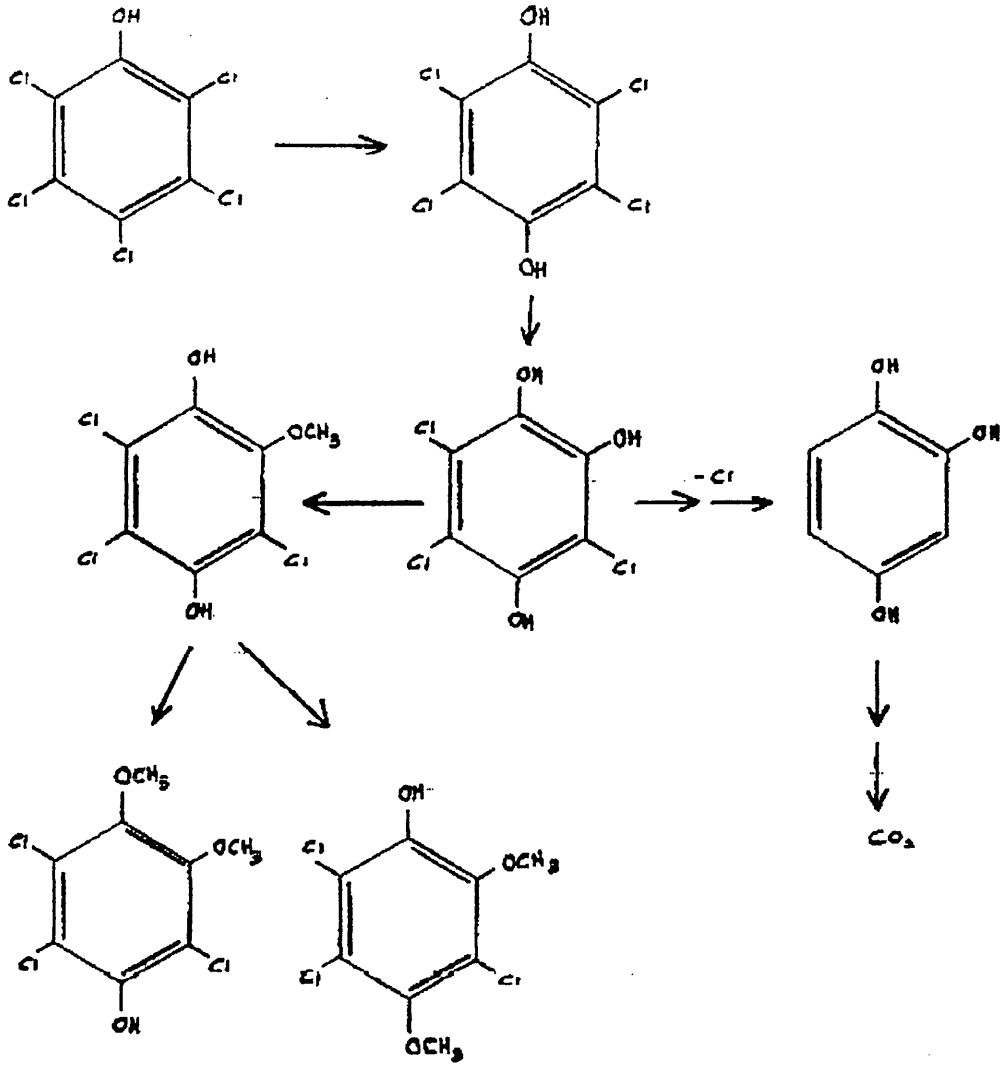


Figure 3 : Relationship between HETP and average linear gas velocity using van Deemter equation[16]



This diagram shows that a minimum value for the height equivalent to a theoretical plate, HETP exists at the optimim linear gas velocity. Below this velocity, HETP is strongly dependent on diffusion effects (the B term) and at higher flow rates on the mass transfer term, C.

Figure 4 : Possible biotransformation / degradation pathways for chlorophenols (demonstrated using pentachlorophenol or PCP)

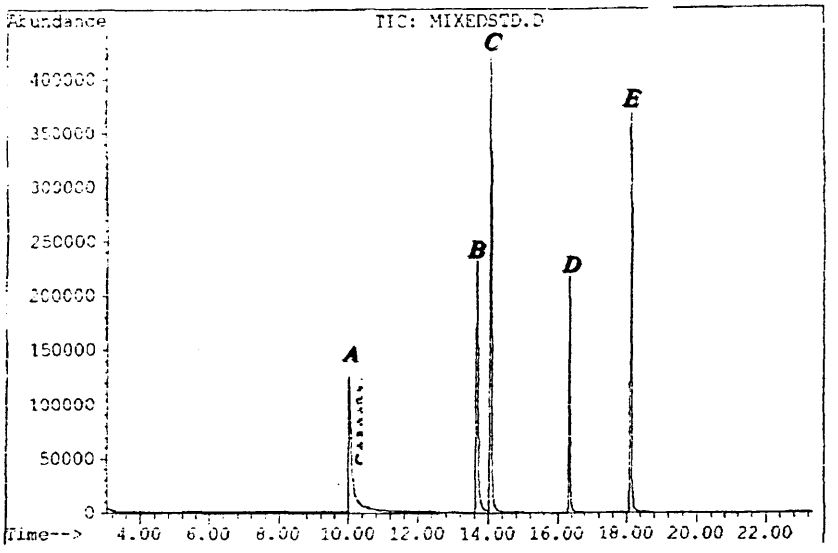


(Highly chlorinated phenols may be transformed by *O*-methylation of the hydroxylic group by certain enzymes present in both aerobic and anaerobic microorganisms. Phenols may be transformed into anisoles; phenols, guaiacol and catechol into veratrole etc.)

APPENDIX B

CHROMATOGRAMS OF STANDARDS AND SAMPLES

Figure 5 : Mixed Pesticides Standards on GC-MS



Note : A=Carbaryl, B=Simazine, C=Lindane, D=Chlorpyrifos-methyl, E=Chlorpyrifos

Figure 6 : Mixed Pesticides Standards on GC-ECD

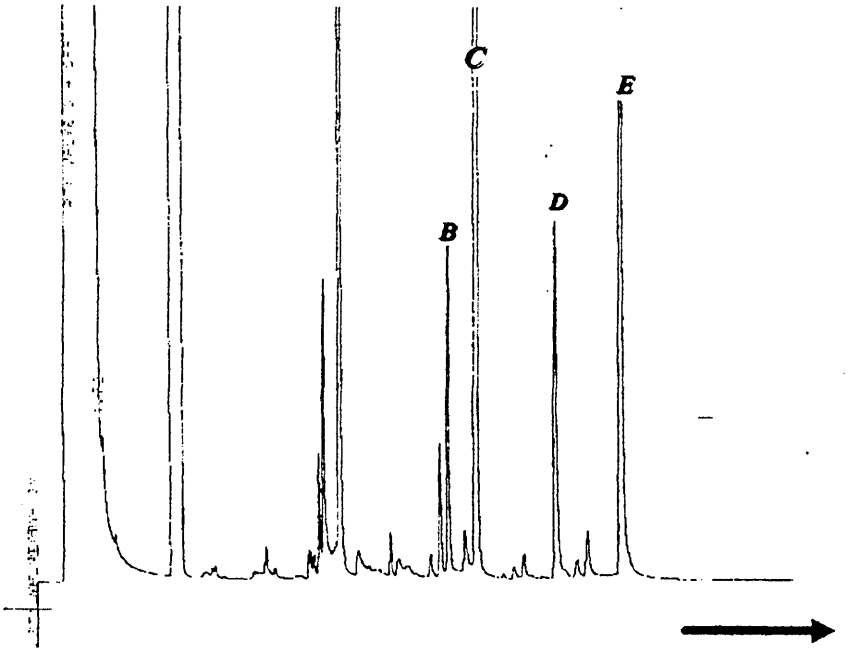


Figure 7 : Non-spiked River Rother on GC-MS

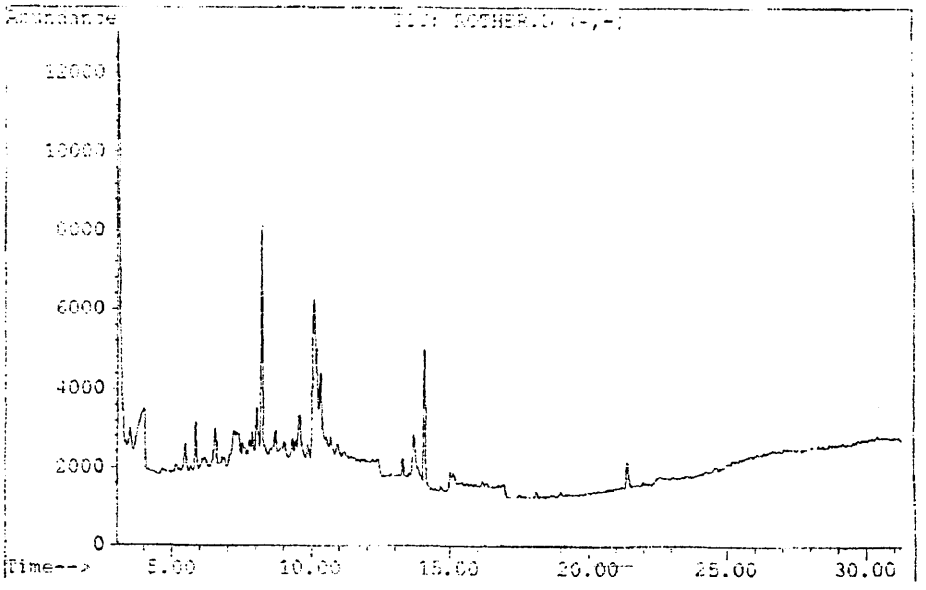
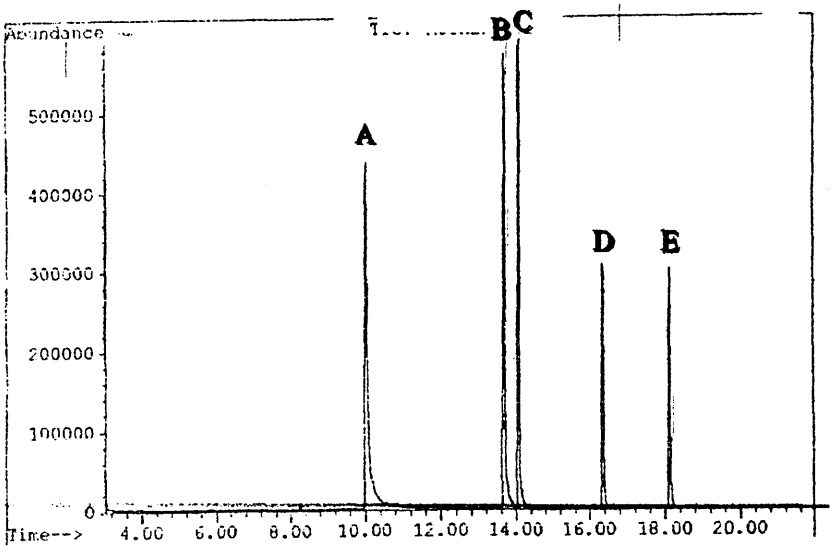


Figure 8 : Spiked River Rother on GC-MS



NB : A=Carbaryl, B=Simazine, C=Lindane, D=Chlorpyrifos-methyl, E=Chlorpyrifos

Figure 9 : Non-spiked River Rother

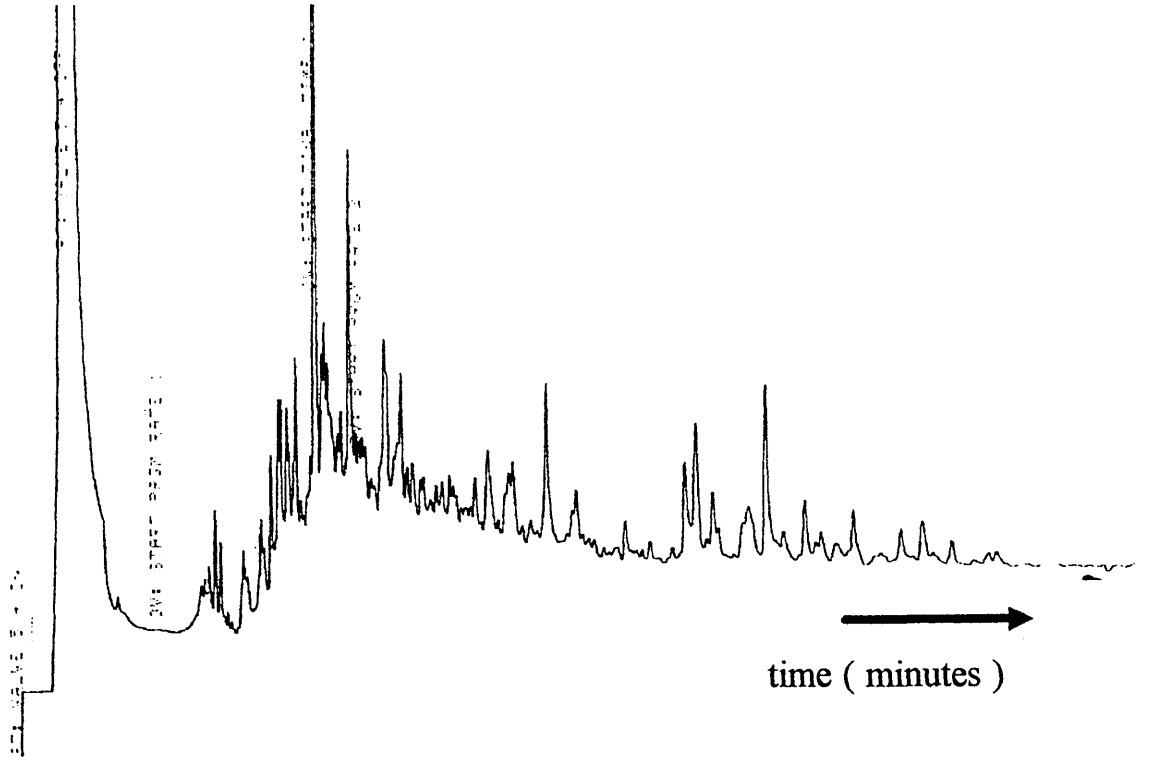


Figure 10 : Spiked River Rother

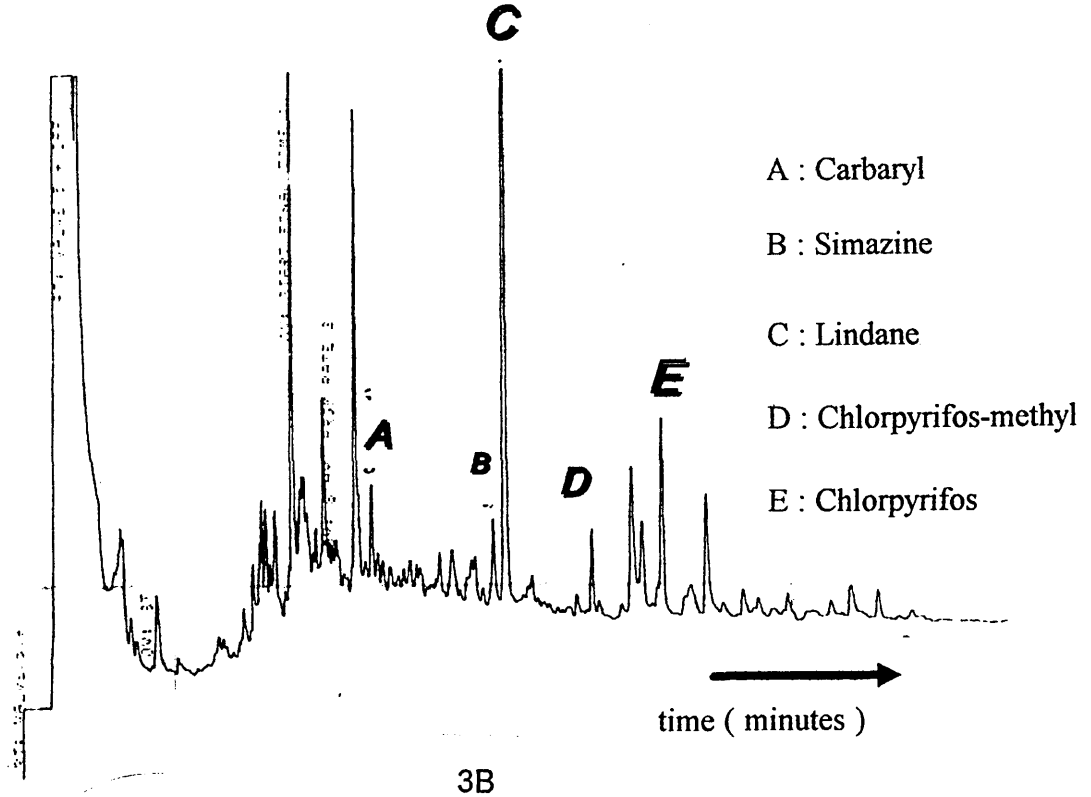


Figure 11 :- Calibration Graph Of Carbaryl Standard Solutions

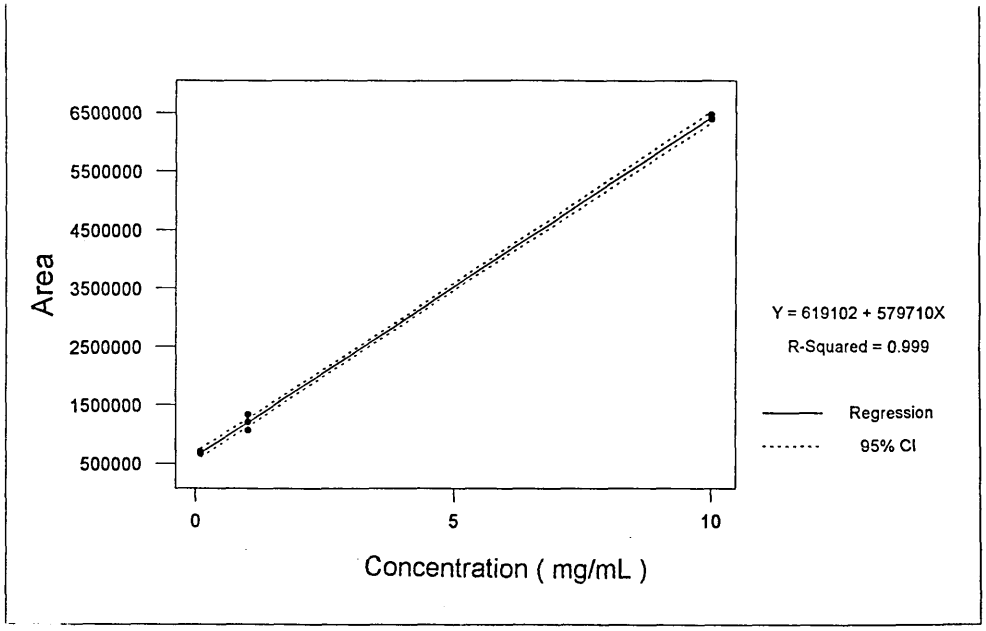


Figure 12 :- Calibration Graph Of Lindane Standard Solutions

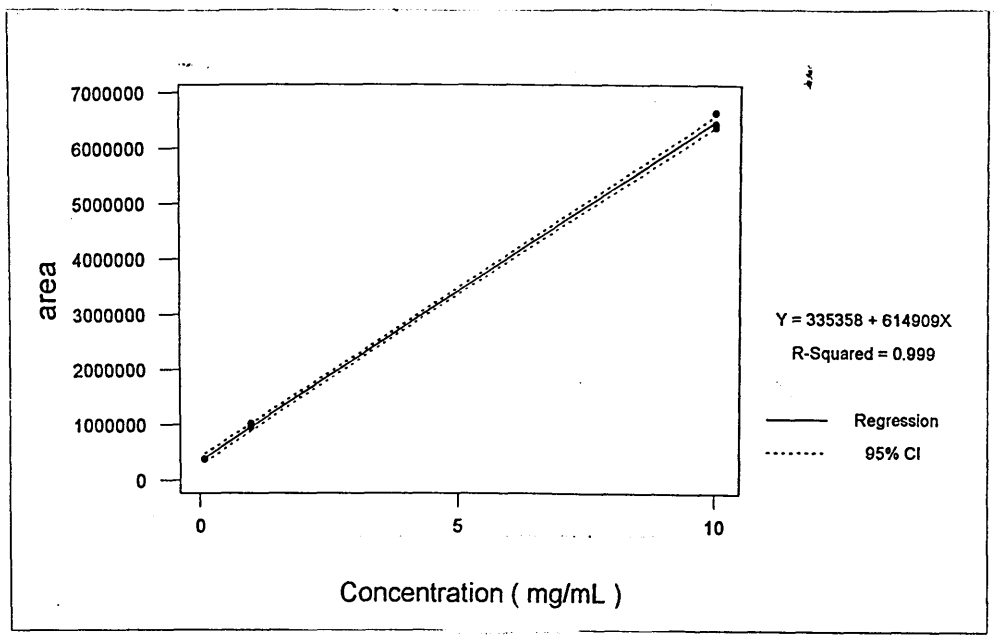


Figure 13 :- Calibration Graph of Simazine Standard Solutions

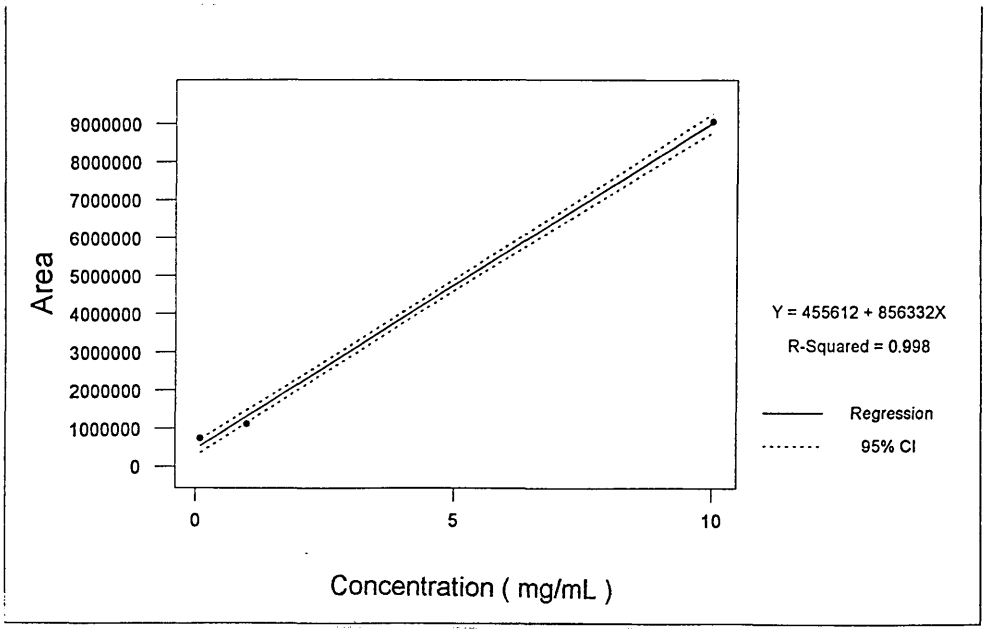


Figure 14 :- Calibration Graph Of Chlorpyrifos-methyl Standard Solutions

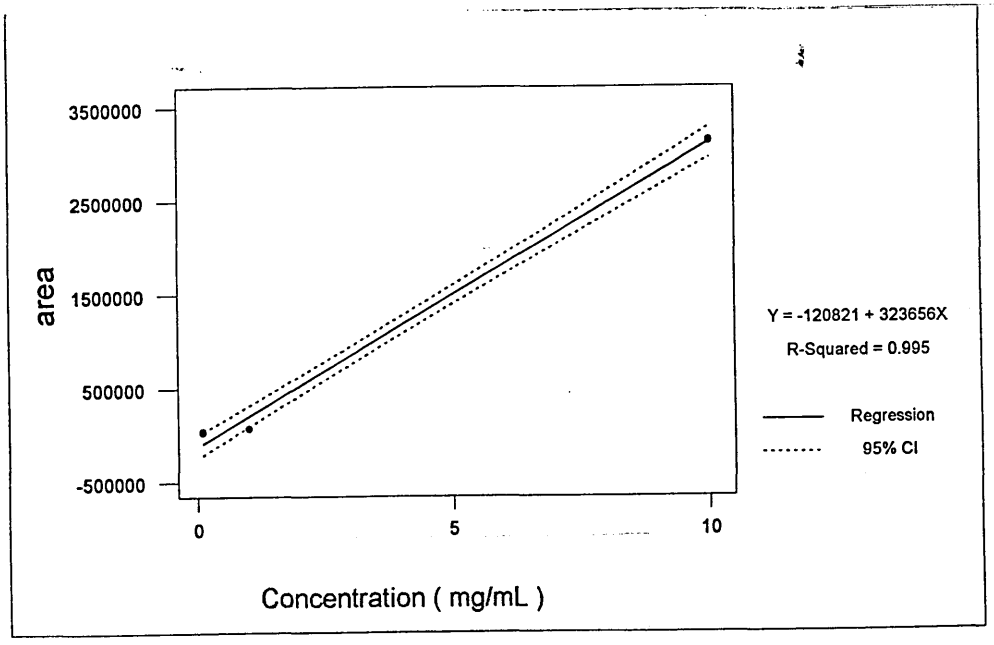


Figure 15 :- Calibration Graph Of Chlorpyrifos Standard Solutions

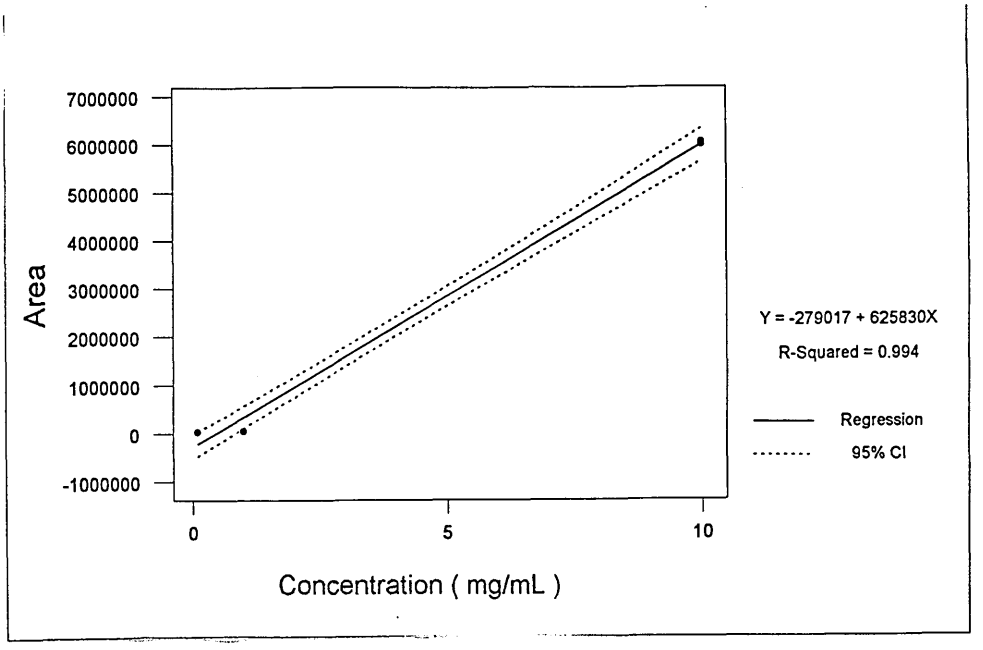
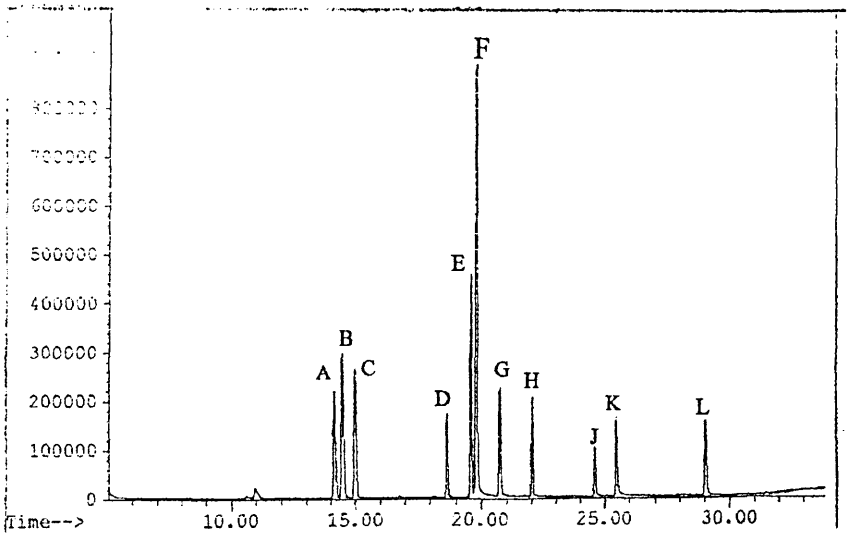


Figure 16 : Mixed Chloroanisoles Standards (SIM)



A=3-Chloroanisole; B=4-Chloroanisole; C=2-Chloroanisole; D=3,5-dichloroanisole; E=3,4-dichloroanisole; F=2,4-dichloroanisole; G=2,3-dichloroanisole; H=2,3,4-trichloroanisole; J=Internal standard; K=2,3,6-trichloroanisole; L=2,3,4,5-tetrachloroanisole

Figure 17 : Internal Standard (SIM)

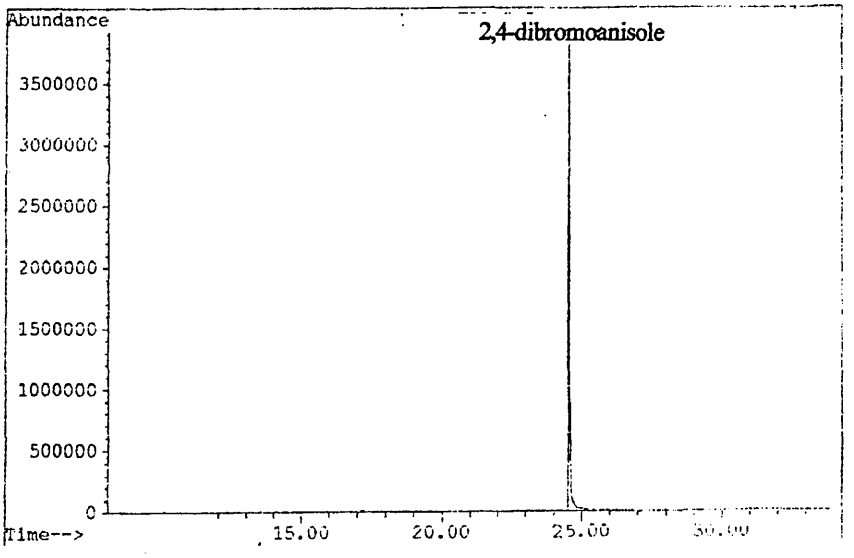


Figure 18 : Non-spiked Sheffield-Tinsley Canal (SCAN)

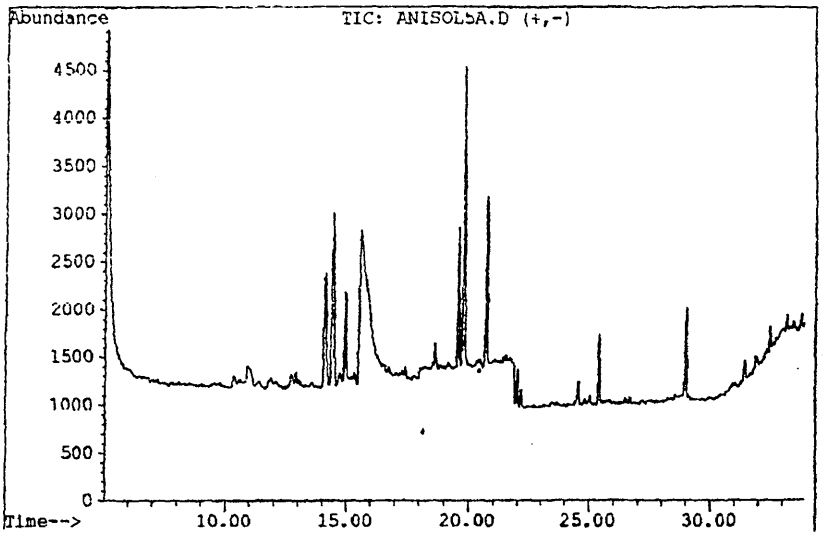
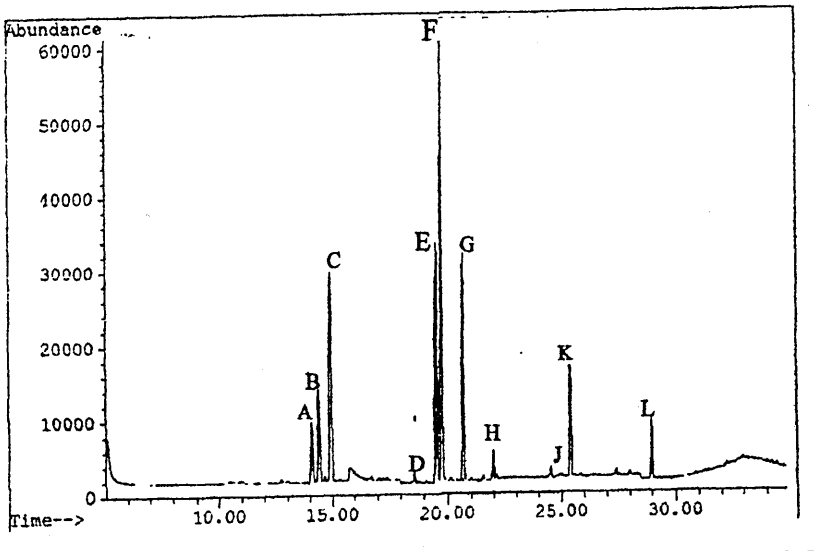


Figure 19 : Spiked Sheffield-Tinsley Canal (SIM)



A=3-chloroanisole; B=4-chloroanisole; C=2-chloroanisole; D=3,5-dichloroanisole; E=3,4-dichloroanisole; F=2,4-dichloroanisole; G=2,3-dichloroanisole; H=2,3,4-trichloroanisole; J=Internal standard; K=2,3,6-trichloroanisole; L=2,3,4,5-tetrachloroanisole

Figure 20 : Non-spiked River Rother (SCAN)

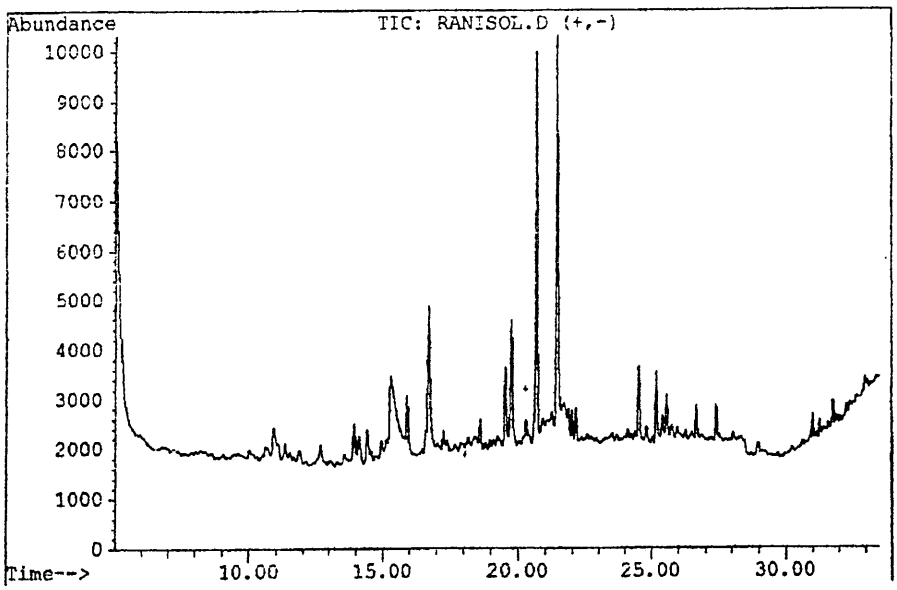


Figure 21 : Spiked River Rother (SCAN)

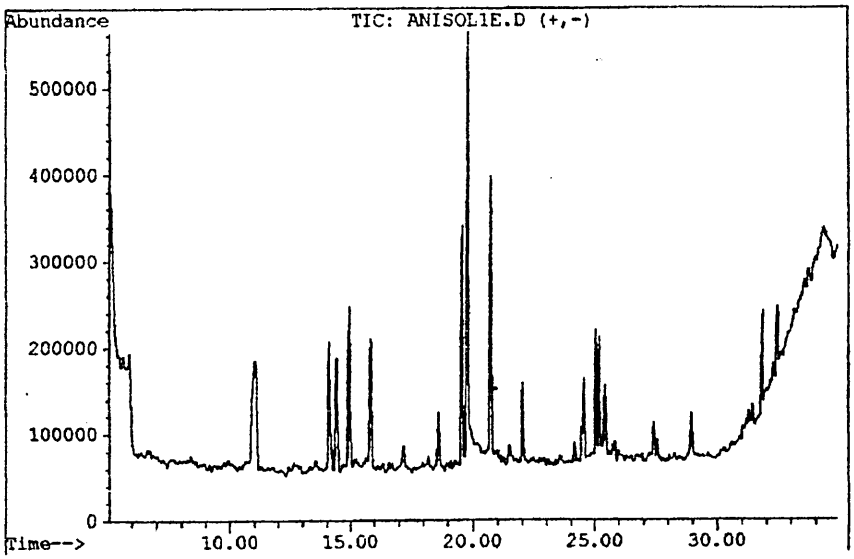


Figure 22 : Spiked River Rother (SIM)

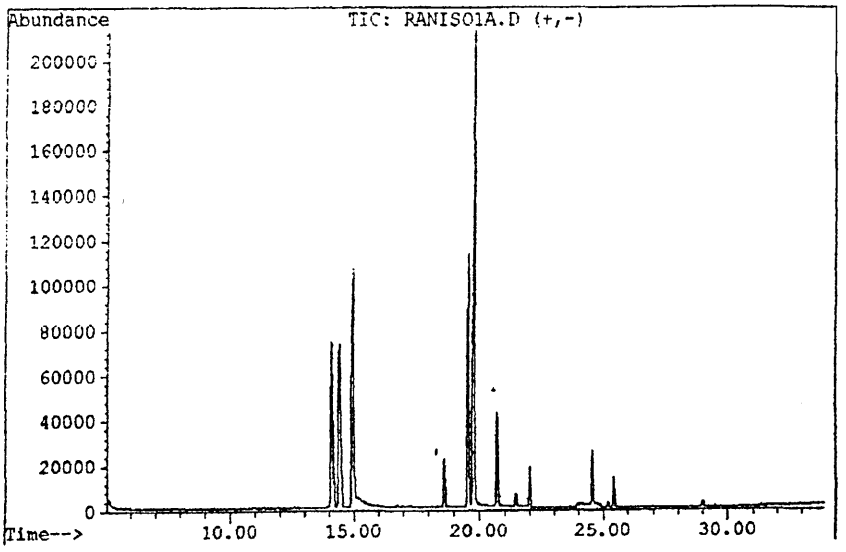


Fig 23 : Non-spiked tap water (SCAN)

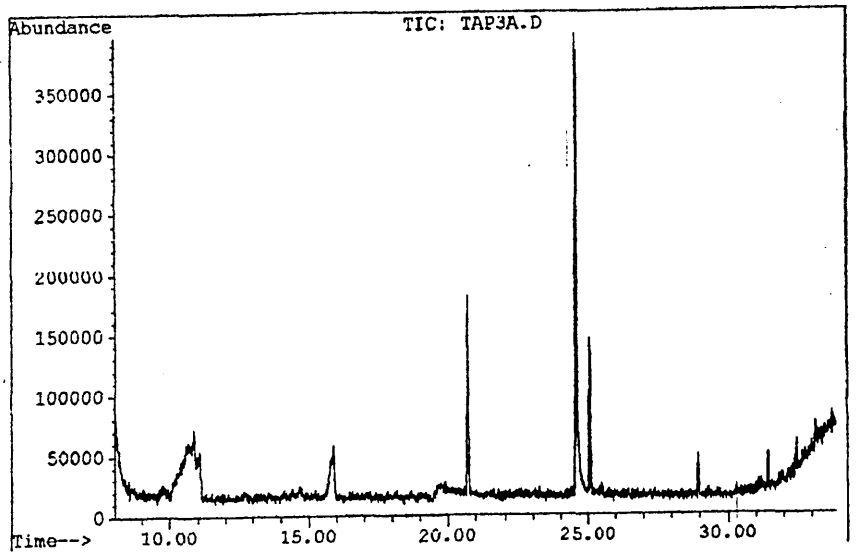


Figure 24 : Spiked tap water (SCAN)

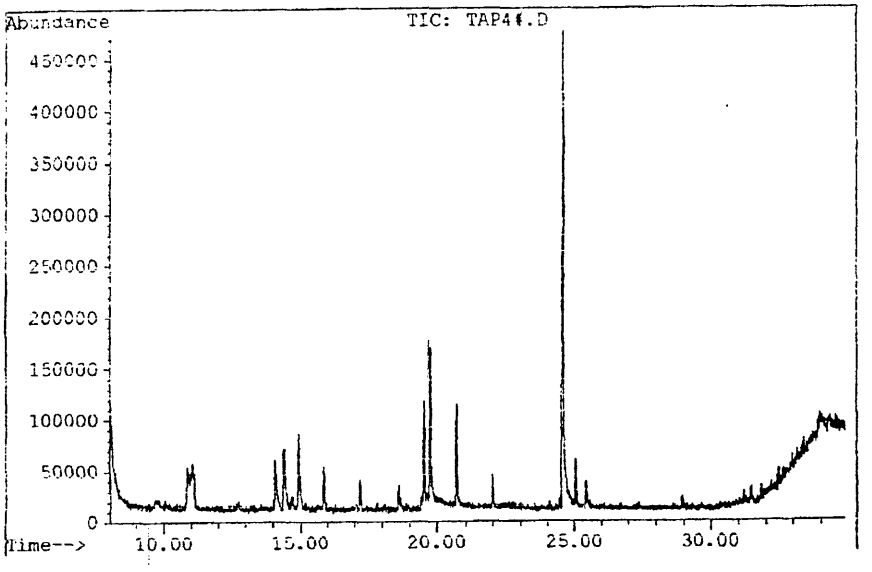
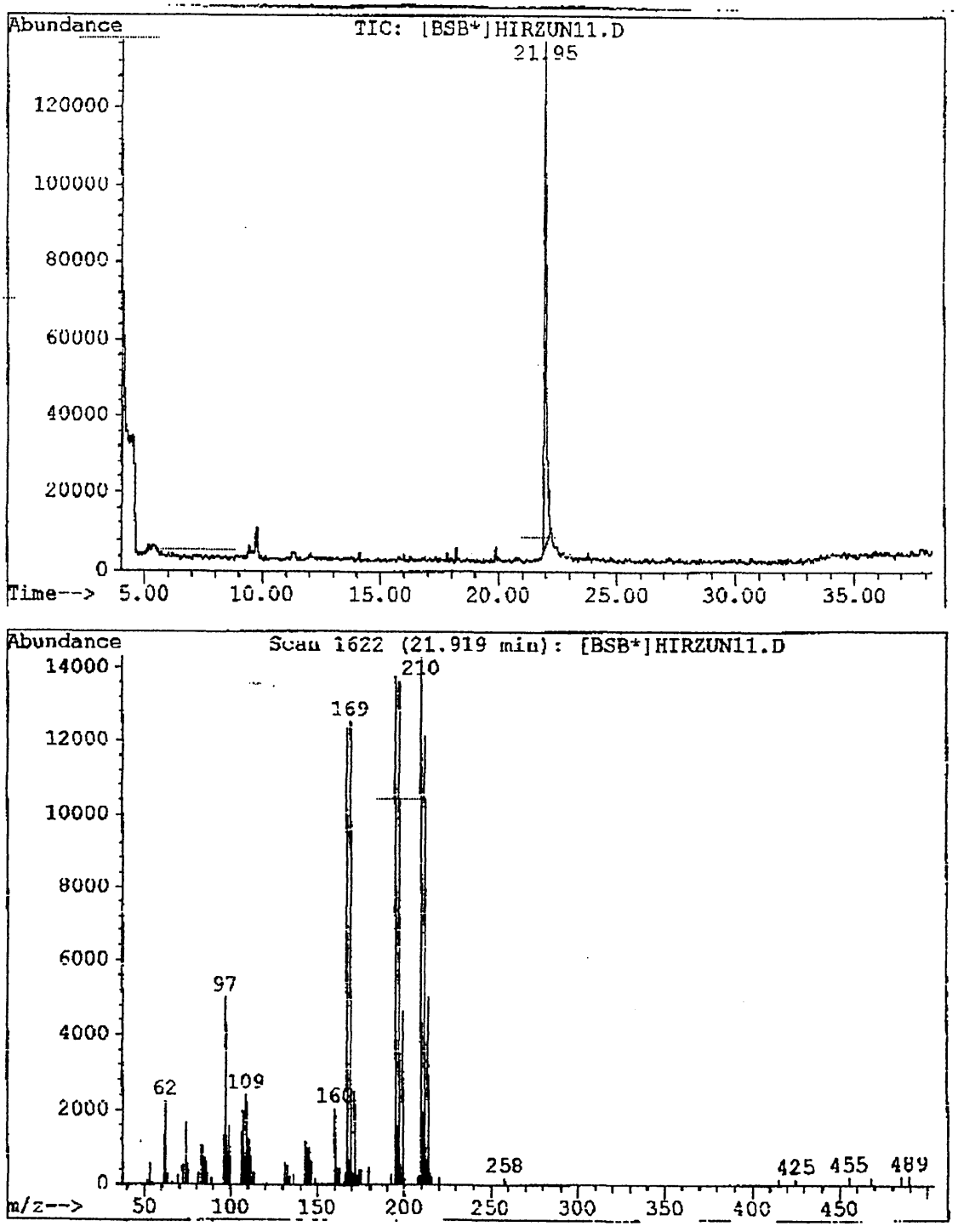


Figure 25 : Chromatogram and mass spectra of 2,3,4-trichloroanisole

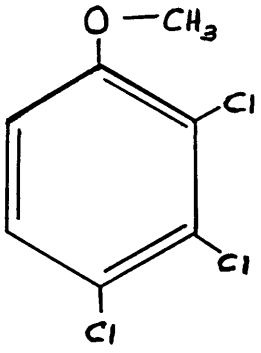


MASS SPECTRAL INTREPETATION

Example : Figure 25

In this work, the electron ionization method was used where the electron impact is expected to ionise the parent molecule. Using this method, the molecular ion is obtained and this is shown by the spectrum peak with greatest mass and usually at greatest abundance. From this, the molecular weight of the compound could be determined.

For known compound, 2,3,4-trichloroanisole;



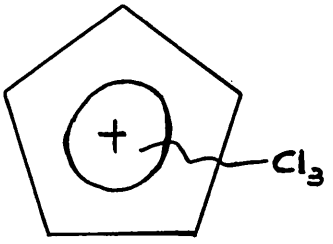
the possible calculated molecular weights are as follow

1 x Oxygen	1 x 16	16
7 x Carbon	7 x 12	84
5 x Hydrogen	5 x 1	5
3 x Chlorine	3 x 35 or 3 x 37	105 or 111
		210 or 216

Loss of either the $-CH_3$ (alkyl) or $-OCH_3$ (methoxy) groups attached to the benzene ring will result in the formation of smaller lighter ions.

If the compound lose the alkyl group

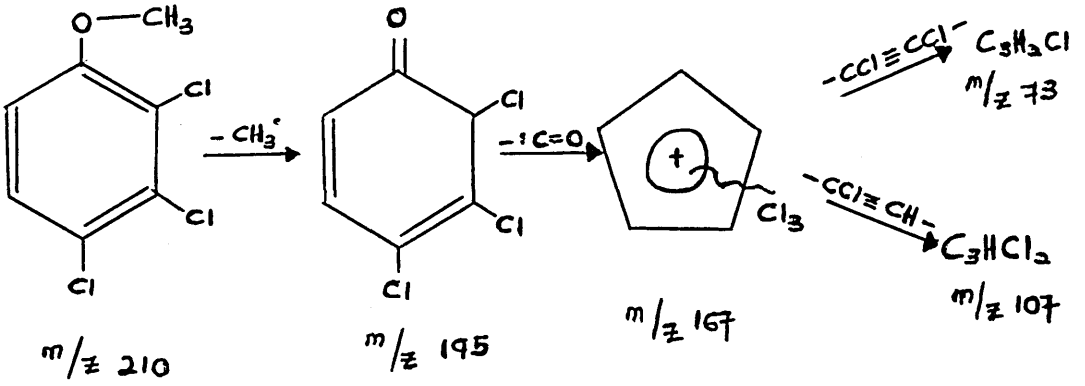
M-15 should give a peak at m/z 195 (this is clearly observed in the spectra). The peak at m/z 167 is a result of a further loss of -:CO (m=28) resulting in the formation of



Loss of [-CCl=CH-] (m=60) resulting in the appearance of peak at m/z 107 while loss of [-CCl=CCl-] (m=94) resulting in the appearance of peak at m/z 73.

On the other hand, if the parent compound loss the methoxy group -OCH_3, i.e M-31 should give rise to a peak at m/z 179. This was observed on the spectrum but at very low abundance.

Hence, it was concluded that the 2,3,4-trichloroanisole follows fragmentation pattern shown below;



APPENDIX C
HEALTH DATA

HEALTH AND SAFETY DATA

CHEMICALS	SIDE EFFECTS
ACETONE	Colourless mobile liquid with characteristic odour. Extremely flammable. Vapour/air mixture explosive. Can cause serious damage if splashed in eyes. Degreases skin, possibly causing dermatitis. Vapour narcotic in high concentration. Can react violently with oxidising agents.
METHANOL	Colourless volatile liquid with characteristic odour. Highly flammable. Vapour/air explosive mixture explosive. Toxic by ingestion. Damaging to eyes. High concentration of vapour may cause dizziness, stupor, cramps, and digestive disturbance. Lower level may cause headache and nausea. Chronic effects-damages tothe central nervous system.
ACETONITRILE	Colourless liquid with etherlike odour Vapour/air mixture explosive. Violent or explosive reaction with strong oxidizer. Causing nose and throat irritation at high concentration. Irritation to eyes. Can cause slight flushing of the face and a feeling of chest tightness when in contact with skin. Inhalation and shock.
CHLOROANISOLES	Colourless liquid or solid with chracteristic odour. Harmful by ingestion and inhalation. Irritating to eyes and skin.
PESTICIDES	Colourless or white solid with mercaptan odour. Toxic if swallowed. Irritating to eyes and skin. Carcinogenic.